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CASE STUDY

Clinical and Experimental Health Sciences

Self-care Agency in Pregnancy

Handan Ozcan[1], Ozlem Sahan[2], Mehmet Gunay[3], Hediye Simsek[3]

[1] University of Health Sciences, Faculty of Health Sciences, Department of Midwifery, Istanbul, Türkiye.
[2] Antalya Bilim University, Faculty of Health Sciences, Department of Midwifery, Antalya, Türkiye.
[3] Gumushane University, Faculty of Health Sciences, Department of Nursing, Gumushane, Türkiye.

Correspondence Author: Handan Ozcan
E-mail: hndnozcn@hotmail.com

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ABSTRACT

Objective: This study was conducted to evaluate the self-care agency of women during pregnancy.

Methods: A descriptive and cross-sectional design was used in the study. The population of the study consisted of women with pregnancy who presented to obstetrics outpatient clinics in Gumushane public hospital between January 30, 2018 and June 20, 2018, and the sample included women who accepted to voluntarily participate in the study (n=201). Data collection tools included a personal information form and the Self-Care Agency Scale. The data were analyzed on a statistical software package, and the level of significance was determined as $p<0.05$.

Results: : The mean age of the pregnant women who participated in the study was 27.50±4.84 (min.-max.=18-41), 72.5% of them had high school and higher education, and 54.8% was housewives. Profession, adequate and balanced nutrition, participation in social activities, and getting social support were found to create statistically significant differences in self-care agency scale scores ($p<0,05$). It was determined that more than half of the women with pregnancy had a very good level of self-care agency.

Conclusion: Many factors (increasing number of curettage, occupational groups, participation in social activities, adequate and balanced nutrition, and support from the family) affect the self-care agency of women during pregnancy. It is recommended that health professionals discuss and evaluate self-care issues in pregnancy in their in-service education programs.

Keywords: Pregnancy, self-care, self-care agency

1. INTRODUCTION

Pregnancy is a natural phenomenon, but every pregnancy poses a potential risk due to the emergence of psychological, physiological, and several social changes during pregnancy. It is a period in which the risk of morbidity and mortality is higher compared to other periods of life and may lead to a decrease in self-care agency (1,2). Self-care is defined by WHO as “the ability of individuals, families and communities to prevent disease, maintain health, promote health, and to cope with disability and illness with or without the support of a health-care provider” (3). Briefly, self-care refers to activities that individuals carry out to initiate and sustain daily living activities and well-being.

Many factors can affect self-care. Therefore, individuals may not be able to manage their self-care in some periods of life. In case of a deterioration of health and failure to meet care requirements during pregnancy, women with pregnancy may need complete or partial help while implementing self-care. At this point, nurses have important duties. Nurses make up an important professional group that supports those who are incompetent and in need of help to attend their medical care and self-care (4,5). Nurses should help women with pregnancy until they can handle their self-care and ensure that they can undertake and meet their self-care as soon as possible. Thus, nurses can be effective in improving the quality of life of women with pregnancy by promoting their general health, enhancing their self-care agency, and having them manage their self-care (5,6).

The support of relatives, especially of the spouse, is important and necessary for women with pregnancy to gain self-care agency. Some socio-demographic characteristics (education, age, income, occupation) and factors such as fatigue affect self-care agency in women with pregnancy (7). Fatigue can lead to the development of psychological problems in women with pregnancy (feeling tense, anxious, fearful, emotional, and worthless), incompetence in fulfilling the care of family members and the baby, decrease in social and sexual activities, or dissatisfaction with life, as well as adversely impacting their abilities, such as thinking, decision-making, and problem-solving (1,6,8).

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In recent years, the concept of self-care has come to the fore with the importance given to protecting, maintaining, and improving health as a primary healthcare philosophy rather than treating the disease. Self-care is a basic human need, and when it is not met, the situation results in a lack of care and deterioration of health (1,5,9). Therefore, this study was planned to determine and evaluate the self-care agency of women with pregnancy and the factors affecting it.

2. METHODS

2.1. Research Type

This study used a descriptive and cross-sectional design.

2.2. Place of the Research

The study was carried out in a public hospital in Gumushane, a province in the Eastern Black Sea region. The research was carried out between January 30, 2018 and June 20, 2018.

2.3. The Universe and the Sample of the Research

The population of the study consisted of women with pregnancy who presented to public hospital maternity outpatient clinics, and the sample included 201 women with pregnancy. To administer the Exercise of Self-Care Agency Scale, which is evaluated on a 5-point Likert-type scale, the sample size was calculated as 201 women with pregnancy who visited Training and Research Hospital within a year, based on ±3 standard deviation and a confidence interval of 95% (5% significance level). Inclusion criteria were being aged 18 or older, being in the 14-40th gestational week, speaking and understanding Turkish, having no cognitive problems, and accepting to participate in the study voluntarily. Women with pregnancy in the first trimester were excluded due to the difficulty of adaptation to pregnancy. Eight women with pregnancy refused to participate in the study. Verbal informed consents were obtained by making necessary explanations about the purpose of the research to the women. Each woman with pregnancy was given 20 minutes to complete the questionnaire.

2.4. Ethical Considerations

In order to conduct the research, necessary approval was obtained from the Ministry of Health Scientific Research Platform and the Scientific Research and Publication Ethics Committee of Gumushane University (Ethics committee number=95674917-044-E.9674). At the top of the questionnaire form, participants were provided information about the criteria of the Helsinki Declaration. The study was conducted with those who volunteered to participate.

2.5. Data Collection Tools

Data were collected using a personal information form and the Exercise of Self-Care Agency Scale.

The personal information form: This form was prepared by the researchers following a literature review (3,6,9). The form consisted of a total of 35 questions about the participants' socio-economic characteristics, obstetric history, and behaviors during pregnancy.

The Self-Care Agency Scale (SCAS): This scale was developed by Kearney and Fleischer in 1979. It aims to determine individuals' capacity and agency to handle their self-care. The validity and reliability study of the scale in Turkey was conducted by Nahcivan in 1993 (10). The scale, which focuses on the self-assessment of individuals' capacity to manage their self-care, consists of 35 items. Also, it has a 5-point Likert-type rating structure. Each statement on the scale is scored between 0 and 4 points. Cronbach's α internal consistency coefficient of the scale is 0.89 (10), and it was found as 0.85 in this study.

2.6. Statistical Analysis

The data obtained in the study were evaluated on the Statistical Package for the Social Sciences (SPSS-21) software package. The independent variables of the study are the socio-demographic characteristics of women with pregnancy, and the dependent variable is the Self-Care Agency Scale score. Descriptive data were presented as numbers and percentages. Kolmogorov-Smirnov analysis was performed to test the suitability of the scales for normal distribution. Results showed that none of the scales met the normality conditions in terms of their total scores. After it was determined that the scales contained parametric analysis conditions, Kruskal-Wallis and Mann-Whitney U tests were carried out for the variables. The level of statistical significance was accepted as $p<0.05$.

3. RESULTS

The mean age of the women with pregnancy participating in the study was 27.48±4.85 (min-max=18-41), the mean age of the spouses was 31.40±5.33 (min-max=20-48). Also, 72.5% of the women with pregnancy had high school or above education level, 55% were housewives, 45.7% were workers, and 86.6% of the spouses had a high school or above education level. Besides, 67.2% of the participants were found to live in a province, and 70.1% defined their income equal to expenses (Table 1).

When we look into the distribution of obstetric features of the women with pregnancy included in the study, 67.5% had one or two pregnancies, 60% had one delivery, 6% had curettage, and 14% had one or two miscarriages (Table 2). Also, 12.4% of women with pregnancy did not have a history of a risky pregnancy.

In the study, 81.9% of the women had a planned pregnancy, 7% got pregnant with fertility treatment, 96.5% went to regular health checks, 48% received training on pregnancy (36.5% from health personnel), and 68.9% had a regular sexual life. Also, 29.1% of the women were found to use complementary and alternative therapy during pregnancy, and 75.9% of them were observed to prefer herbal treatment (19.5% nausea, 10.5% vomiting, 4.0% anemia, 4.5% dizziness, 1% other complaints), 10.3% meditation, and 8.6% massage.

Also, 13.4% of the women with pregnancy were found to smoke, and 5.5% continued to smoke during pregnancy. Besides, 31% of the women stated that they felt calm, 47.5% happy, 26.5% anxious, and 3.5% upset. Moreover, 58.0% attended social activities, 87.9% had an adequate and balanced diet, 15.5% received dietician support, 77.0% brushed teeth every day, 76% slept 8 hours a day or more, 25% exercised regularly, and 91.4% had adequate family support.

The average score of the pregnant women from the Self-Care Agency Scale was 112.17±20.61 (min=51, max=136). Self-care of the women with pregnancy was at a good level.

When the factors affecting self-care agency in women with pregnancy was evaluated, it was found to be generally affected positively in women with pregnancy who worked as a civil servant, had an adequate and balanced diet, attended social activities, received social support from their family during pregnancy, and received help from someone in daily activities (Table 3, $p<0.05$).

On the other hand, the self-care agency was found to be higher in women with pregnancy who had a high income, had a core family type, did not have a risky pregnancy history, continued sexual life during pregnancy, had a planned pregnancy, and did not use complementary and alternative therapy during pregnancy, but no statistically significant differences were determined (Table 3, $p>0.05$).

Although there were no significant differences as a result of the analyses, self-care agency was found to be higher in women with pregnancy whose spouse had a high level of education, who did not smoke, went to regular pregnancy checks, did not have any education on pregnancy, did regular exercise, brushed teeth regularly, and had a chronic disorder.

Table 1. *Some socio-demographic characteristics of the women with pregnancy (n: 201).*

Education (the women)	**n**	**%**	**Education (the spouses)**	**n**	**%**
Elementary school	26	12.5	Elementary school	11	5.5
Middle school	30	15.0	Middle school	16	7.9
High school	59	29.5	High school	65	32.3
University	86	43.0	University	109	54.3
Job (the women)	**n**	**%**	**Job (the spouses)**	**n**	**%**
Education sector	12	5.9	Education sector	18	8.9
Health sector	43	21.3	Health sector	29	14.4
Civil servant	17	8.5	Civil servant	59	29.4
Worker	19	9.5	Worker	92	45.8
Housewife	110	54.8	Unemployed	3	1.5
Income	**n**	**%**	**Place of residence**	**n**	**%**
Income less than expenses	22	10.9	Village	13	6.4
Equal income and expenses	141	70.1	County	53	26.4
Income more than expenses	38	19.0	Province	135	67.2

Table 2. *The obstetric history of the women.*

Number of pregnancies (n:201)	**n**	**%**	**Number of deliveries (n:130)**	**n**	**%**
One	66	32.8	One	78	60.0
Two	70	34.9	Two	40	30.8
Three	35	17.4	Three	9	6.9
Four and more	30	14.9	Four and more	3	2.3
Number of miscarriages (n:201)	**n**	**%**	**Number of curettages (n: 201)**	**n**	**%**
Yes	28	13.9	Yes	13	6.5
No	173	86.1	No	188	93.5
Total	201	100.0	Total	201	100.0
Type of the previous delivery (n:130)	**n**	**%**	**History of risky pregnancies (n:201)**	**n**	**%**
Vaginal	80	61.5	Yes	24	11.9
Cesarean delivery	48	37.0	No	177	88.1
Vaginal/Cesarean delivery	2	1.5			

Table 3. *The distribution of the self-care agency scale scores of the women with pregnancy by some characteristics.*

Some Characteristics		n	Median (%95 CI)	Test Value
Level of education	Elementary school	26	106.00 (89.76-120.52)	
	Middle school	30	111.00 (85.36-118.92)	KW=2.916
	High school	59	101.00 (96.89-118.82)	p=0.405
	University	86	113.00 (107.25-117.44)	
Job	Education sector	12	129.50 (107.74-134.59)	
	Health sector	43	108.00 (103.32-115.81)	KW=12.990
	Civil servant	17	127.00 (111.85-131.03)	**p=0.01**
	Worker	19	102.00 (91.94-113.06)	
	Housewife	110	110.00 (88.41-111.16)	
Income Status	Income < expenses	22	107.50 (94.87-116.04)	KW=2.71
	Income = expenses	141	116.00 (109.02-116.09)	p=0.258
	Income > expenses	38	120.50 (109.59-122.14)	
Family type	Core family	178	116.00 (109.87-116.12)	U=1528.000
	Extended family	21	109.00 (97.87-116.99)	p=0.172
History of risky pregnancies	Yes	24	111.00 (97.61-116.56)	U=1763.500
	No	177	116.50 (109.98-116.22)	p=0.19
Continuation of sexual activity during pregnancy	Yes	135	115.50 (110.72-117.42)	U=3743.500
	No	61	111.44 (104.62-115.71)	p=0.309
Using complementary therapy during pregnancy	Yes	58	112.00 (103.92-114.99)	U=3620.000
	No	141	115.60 (110.89-117.61)	p=0.204
Planned pregnancy	Yes	163	116.00 (109.78-116.24)	U=2710.500
	No	36	113.00 (102.17-117.22)	p=0.474
Getting help in daily living activities	Yes	118	116.38 (111.00-118.73)	U=3752.500
	No	81	110.13 (104.24-113.36)	**p=0.010**
Participation in social events	Yes	116	118.53 (113.69-120.60)	U=3405.500
	No	84	107.00 (100.99-110.79)	**p=0.000**
Adequate support from the family	Yes	181	115.05 (110.73-116.68)	U=998.000
	No	17	101.03 (87.60-112.05)	**p=0.017**
Adequate and balanced nutrition	Yes	176	115.68 (11.42-117.36)	U=1242.000
	No	25	97.91 (86.98-107.72)	**p=0.001**

KW: Kruskal Wallis Test, U: Mann Whitney U Test, CI: Confidence Intervals

4. DISCUSSION

Pregnancy is a process in which the physiological and emotional states of women change. The process of pregnancy is critical in terms of the health of the mother and the newborn, as well as going through the whole process healthily. For this reason, it is very important that the pregnancy is planned, the woman with pregnancy goes to regular health checks, and she actively participates in her self-care (11). In our study, the majority of the participants had a planned pregnancy. Almost all of them were found to go to health checks regularly. Also, the vast majority of the participants had an adequate and balanced diet, slept and relaxed for an average of 8 hours or more a day, and brushed their teeth. In our study, the mean self-care agency scale score of the women with pregnancy was determined to be 112.17±20.61. The results of studies conducted in Turkey were found to be different from those of ours. For example, the mean self-care agency score was found to be 80.3±10.0 in Manisa province, 87.1±23.0 in a study conducted with 80 women with pregnancy in Erzurum province, and 92.0±18.9 in İstanbul province. In our study, the mean score on the self-care agency scale was higher compared to other studies. This difference was observed to be because the majority of the participants in other studies were elementary school graduates. Although there was no significant difference between educational status and self-care agency in the present study, the mean scores of those with high education levels were higher. Also, most of the women with pregnancy had a planned pregnancy, they paid attention to their diets, they went to regular health checks, they relaxed adequately during the day, and they were careful about their dental care, all of which showed that the women with pregnancy paid attention to their self-care. In some studies, no relationship was found between self-care agency and educational status. While some studies indicated that self-care agency increased in parallel to the increase in the level of education (12), others showed that the level of education had no effect or did not increase the self-care agency (1,13,14).

According to the results of studies conducted so far, self-care agency has been reported to be high among working women (6,12,14). In our study, self-care agency was found to be higher among women working as civil servants or those

working in the education sector. On the other hand, it was determined to be low among women working as workers and those working in the health sector.

In our study, although there was no significant difference between income status and self-care agency, self-care agency was observed to increase with the increase in income status. A review of the literature indicated that there were different relationships between self-care agency and income status. For example, Aktas (2015) stated that self-care decreased with the increase in income status, while Celik and Aksoy (2019) reported that self-care agency increased in parallel with the increase in income status (14,15).

In the present study, 77% of the women with pregnancy were found to brush their teeth every day, and self-care agency was higher among women who cared about dental care. Dental care also brings about important health consequences during pregnancy. Periodontal diseases pose risks, such as preterm birth and low birth weight (16,17). Tooth decay is reported to increase the risk of miscarriage by 15-20% before the 20th gestational week. Medical treatments are recommended especially before the 12th gestational week to reduce the teratogenicity effect and possible risks. Recommendations also include providing the women with pregnancy with oral hygiene education, giving them care support, monitoring them, and thus increasing the comfort of the woman, especially in this period (18). In their study conducted in Poland, Gaszynska et al. (2015) stated that 70% of the women with pregnancy had gingivitis. In the present study, it was noteworthy that approximately three out of four women with pregnancy paid attention to their dental care. It is also important to increase this rate (19).

Although there were no significant differences as a result of the analysis, self-care agency was higher in women who had a spouse with a high level of education, did not smoke, went to regular pregnancy controls, did not receive any training about pregnancy, exercised regularly, and had a chronic disease. In a study, it was recommended that women should be encouraged for physical activity for a healthy pregnancy (20). It was interesting that self-care agency was high in those who did not receive any training about pregnancy and that health care professionals had low self-care agency compared to other occupational groups. This suggested that individuals who were knowledgeable about health behaviors and pregnancy did not pay attention to these issues. However, there were contrasting results in the literature. Yılmaz and Beji (2010) determined that self-care agency was high among women with pregnancy who participated in pregnancy education (1). Ozcan and Beji (2015) reported that self-care agency scores of the women with pregnancy who received education before pregnancy were higher than those who did not receive any training (21). Tortumluoğlu et al. (2003) reported that the mean self-care agency scores of the women with pregnancy who went to regular health checks were higher than those who did not (22).

Providing individuals with information on self-care by encouraging them through effective communication enables them to participate in their self-care and strengthens the relationship between the person and the caregiver. Active involvement of the patient in healthcare often improves treatment outcomes and results in greater satisfaction (24-26).

5. CONCLUSION

High levels of self-care agency during pregnancy lead to a healthy and comfortable pregnancy. Exercising regularly, adequate and balanced nutrition, participation in social activities, and continuance of sexual activity are quite high among women with pregnancy who have high levels of self-care agency. It is very important that nurses participate in the process of promoting and sustaining self-care agency during pregnancy and support women with pregnancy. We recommend that in-service training programs on self-care should be organized and that the self-care status of women with pregnancy should be assessed especially during the prenatal period.

Limitations of the Study

Since the study was conducted in only one province, it cannot be generalized to all pregnant women.

Conflict of Interest

No conflict of interest was declared by the authors.

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Does the Presence of Metabolic Syndrome Alter Serum Uric Acid Concentrations, Pain, and Well-Being in Patient with Chronic Musculoskeletal Pain?

Seyda Toprak Celenay[1], Derya Ozer Kaya[2], Senem Sas[3]

[1] Ankara Yildirim Beyazit University, Health Sciences Faculty, Department of Physiotherapy and Rehabilitation, Ankara,Türkiye.
[2] Izmir Katip Celebi University, Health Sciences Faculty, Department of Physiotherapy and Rehabilitation, Izmir,Türkiye.
[3] Erciyes University, Medical Faculty, Department of Physical Medicine and Rehabilitation, Division of Rheumatology, Kayseri, Türkiye.

Correspondence Author: Seyda Toprak CELENAY
E-mail: sydtoprak@hotmail.com

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ABSTRACT

Objective: To compare serum uric acid concentrations, pain and well-being in patients having chronic musculoskeletal pain with and without MetS, and investigate cut-off values.

Methods: Patients having chronic musculoskeletal pain with (MetS group, n=48) and without MetS (control group, n=52) were included. The serum uric acid concentration, pain intensity, body composition, physical activity level, quality of life, and psychological status were evaluated by a uric acid blood test, Visual Analogue Scale, Bio-impedance Analyzer, International Physical Activity Questionnaire-7 (IPAQ-7), Nottingham Health Profile, and Hospital Anxiety and Depression Scale, respectively.

Results: Uric acid level, fat mass, waist/hip ratio were found higher in the MetS group in comparison to the control group ($P<0.05$). It was seen that patients in the MetS group had lower physical activity levels than those in the control group ($P<0.05$). The cut-off points of the uric acid level, fat mass, waist/hip ratio, and physical activity level for detecting MetS were found as 5.25 mg/dl, 37.50 kg, 0.91, and 247.25 METs-minutes/week, respectively.

Conclusion: Patients with MetS had a greater uric acid level, fat mass, waist/hip ratio, and a lower physical activity level than those without MetS. The increase of uric acid level, fat mass, and waist/hip ratio, and the decrease of physical activity may be critical for patients having musculoskeletal pain with MetS. These results should be considered for the management of these patients.

Keywords: Metabolic syndrome, pain, uric acid, body composition, quality of life

1. INTRODUCTION

The musculoskeletal pain and metabolic syndrome (MetS) with the related burden of the problems have become two recent global health challenges (1). Musculoskeletal pain is originated from musculoskeletal conditions such as joint problems, musculoskeletal injuries, or inflammatory diseases (2). Regional or widespread musculoskeletal pain is more common in patients with MetS, and the relations between MetS and musculoskeletal disorders were declared for intervertebral disk degeneration, knee osteoarthritis, shoulder adhesive capsulitis, neck pain, and tendinopathies (3-6). Musculoskeletal disorders trigger major inflammatory reactions and similarly, MetS are strongly associated with chronic low-grade inflammation or proinflammatory state. Systemic and chronic inflammation changes body chemistry over time, causes nociception in musculoskeletal tissues, leads to the expression of chronic disease, and prevents tissue healing (7,8).

MetS is a cluster of disorders with the combination of visceral obesity, hyperglycemia or insulin resistance, hypertension, and dyslipidemia (1). The NCEP ATP III criteria is used widely for diagnosis including measurements and laboratory tests. The level of serum uric acid, the final metabolism product of endogenous purine catabolism, was also assosiated with an increased risk for impaired MetS and oxidative stress (9). The oxidant-antioxidant paradox was declared for the serum uric acid levels (10). While the visceral fat area was declared to be the strongest contributor to elevated serum uric acid concentration, serum uric acid level increase was related to bone mineral density increase (11). The difference and impact of the serum uric acid concentration in musculoskeletal patients with and without MetS are questionable.

In addition to serum uric acid alterations, pain, fatigue, sleep disturbances, disability, physical inactivity, and impairments in quality of life (QoL) and psychological well-being are common in musculoskeletal disorders and MetS patients (12). Nowadays, more and more patients with musculoskeletal pain conditions have been consulting outpatient physical therapy clinics and seeking physiological and psychological well-being**.** The prevalence of MetS in these patients seems to be high, and it is a very well-known that the treatment of those patients with MetS is more difficult (13). However, to our

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knowledge, the clinical differences for physical activity habits, body composition, pain intensity, QoL, and psychological well-being, and laboratory features such as serum concentrations in patients having chronic musculoskeletal pain with and without MetS have not been researched up to now.

Thus, the current study aimed to compare serum uric acid concentrations, pain and well-being in patients having chronic musculoskeletal pain with and without MetS, and investigate cut-off values. The hypotheses of the current study were as below: 1. There are differences in serum uric acid level, pain intensity and well-being of patients having chronic musculoskeletal pain with MetS compared to those without MetS, 2. There would be cut-off values between the occurrence of serum uric acid level, pain intensity and well-being characteristics.

2. METHODS

2.1. Participants

One hundred and ten patients, referred to the department of physiotherapy and rehabilitation, were assessed. The inclusion criteria were to be a volunteer between 20 to 70 years of age with a chronic non-specific musculoskeletal pain disorder for more than six months. Exclusion criteria were the presence of cancer history, chronic heart failure, renal and/or liver dysfunction, pregnancy, alcohol consumption, and being unable to complete the assessment. Moreover, the volunteers using medications for pain control, anxiety, and depression were excluded.

The present study was planned by a case-control study design. The Ankara Yildirim Beyazit University's Ethics Committee approved the protocol of the present study (Approval number: 32) and conducted within the framework of the Helsinki Declaration principles. Written informed consent forms were provided to participants.

2.2. Assessments

All the patients included in the study as having a musculoskeletal disorder were assessed by the same physician (SS). The physician collected demographic and physical data, revealed the musculoskeletal disorder with the consideration of exclusion criteria. Moreover, the diagnosis of MetS was carried out by the examination and blood test results according to the diagnostic criteria guidelines of NCEP-ATP III (14). During examinations, blood pressure was measured with a sphygmomanometer (Erka, Perfect Aneroid, Germany) on the same arm (right) after having a 30-minutes rest. The weight, height, hip, and waist circumferences were measured with a meter. The blood samples were collected from the patients after one night of fasting. The following routine blood tests included: serum uric acid levels, glucose, high-density lipoprotein cholesterol (HDL-C), and triglyceride. After getting the exact MetS diagnosis the suitable and voluntary patients were assigned to the MetS and control groups. The flowchart of the cases was presented in Figure 1. The other assessments related to physical and psychological well-being were conducted by the same physiotherapist blinded to the group allocations (STC). Body composition, physical activity level, musculoskeletal pain complaints and general well being such as QoL and psychological distress were examined.

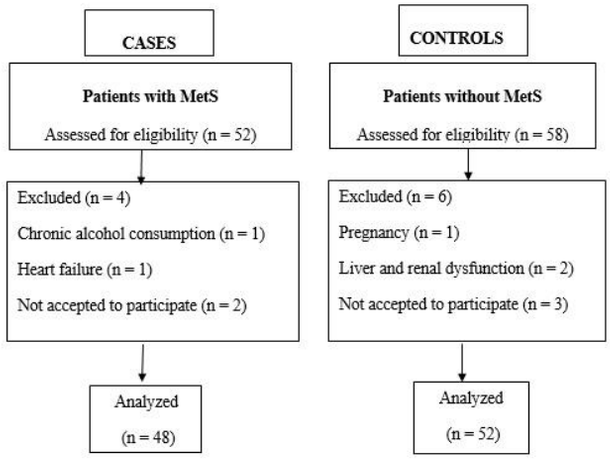

Figure 1. *The flowchart diagram for the participants.*

Body composition: The body composition was measured with Bio-impedance Analyzer (Bodystat®1500, Bodystat Ltd, Douglas, Isle of Man, UK). Patients' physical characteristics were entered into the analyzer. For the whole-body measurement, two electrodes were placed on the right hand and the other two electrodes were placed on the left foot. Within seconds by passing a safe signal at a low 400 µA and a frequency of 50 kHz through the body the results related to the body composition were displayed on a screen of the device and they were recorded (15).

Physical activity level: International Physical Activity Questionnaire-7 (IPAQ-7) Turkish version was used to assess the physical activity levels of the participants (16). The total score of the IPAQ-7 is obtained based on the duration and frequency of weekly vigorous and moderate-intensity physical activities and walking activities.

Musculoskeletal pain complaints: The musculoskeletal pain complaints were questioned. The patients were asked to mark their painful areas on a body diagram. Afterward, the pain intensity was questioned with the Visual Analog Scale (VAS) (17). This scale consists of a 10 cm horizontal line in length, in which "0" remarks "no pain" and "10" remarks "excruciating pain''.

Quality of life: Nottingham Health Profile (NHP) Turkish version was used to assess the QoL (18). The profile contains 38 items with 6 dimensions. The specific dimensions focus on pain, energy, emotional reactions, social isolation, sleep, and physical mobility. The scores of each part ranged from 0 (the best QoL) to 100 (the worst QoL). The total scores have been summed from all dimensions.

Psychological distress: Hospital Anxiety and Depression Scale (HADS), 14 items divided into anxiety (HADS-A) and depression (HADS-D) dimensions, was used for evaluating psychological distress. It is a valid and reliable questionnaire in Turkish (19).

The scores of each demension ranged from 0 to 21. The higher score indicated the deterioration of anxiety and depression.

2.3. Sample Size and Statistical Analyses

G*Power program (G*Power Version 3.0.10, Franz Faul, Universität Kiel, Germany) was used for the sample size calculation. For the pilot study, ten patients from each group were randomly recruited. According to the the IPAQ-7 scores of the pilot study, the sample with 90 participants (45 per group) was needed to obtain 80% power (d = 0.6 effect size and α = 0.05 type I error).

IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, New York: IBM Corp.) was used for the analyses. The normal distributions of the variables were assessed with visual and analytical methods. Descriptive data was demonstrated with mean ± standard deviation (SD) values, median, minimum (min), and maximum (max) values, frequency (n) and percentage (%). Independent samples t-test, Mann-Whitney U test, and Chi-square test were used to compare the group differences. The statistical significance level was accepted as $p<0.05$.

The cut-off values for predicting the presence of MetS were analyzed using the receiver operating characteristic (ROC) curves. The determined area under the curve (AUC) according to ROC graphics and 95% confidence intervals of the area were investigated. When a significant cut-off value was observed, the sensitivity and specificity were calculated. While evaluating the AUC, a 5% type-I error level was used to accept a statistically significant predictive value of the measurements.

3. RESULTS

One hundred patients completed the study. There was no difference between the groups in terms of the demographic and physical data ($P > 0.05$), except MetS components ($P < 0.05$) (Table 1).

Table 1. *Demographical characteristics of the groups.*

Characteristics	MetS group (n = 48)	Control group (n = 52)	P
Age (years, Mean ± SD)	57.58 ± 11.02	52.76 ± 13.39	0.054[a]
Gender (n, %)			
Female	35, 72.9	40, 76.9	0.644[c]
Male	13, 27.1	12, 23.1	
Smoking (n, %)			
No	46, 95.8	45, 86.5	0.105[c]
Yes	2, 4.2	7, 13.5	
Musculoskeletal pain localization (n, %)			
Neck	11, 22.9	13, 25.0	0.994[c]
Low back	26, 54.2	27, 51.9	
Hip	1, 2.1	1, 1.9	
Knee	8, 16.7	8, 15.4	
Shoulder	2, 4.2	3, 5.8	
MetS components			
Waist circumferences (cm, Mean ± SD)	108.01 0± 11.91	98.90 ± 12.82	< 0.001[a]*
Serum glucose (mg/dL, Mean ± SD)	115.50 ± 39.14	95.67 ± 19.86	0.002[a]*
Triglyceride (mg/dL, Median (Min; Max))	200.50 (95.0; 348.0)	115.00 (36.0; 392.0)	< 0.001[b]*
HDL-C (mg/dL, Mean ± SD)	38.46 ± 8.03	49.34 ± 14.86	< 0.001[a]*
SBP (mmHg, Mean ± SD)	126.77 ± 12.65	117.30 ± 12.38	< 0.001[a]*
DBP (mmHg, Mean ± SD)	77.91 ± 9.66	72.69 ± 8.42	0.005[a]*

**P < 0.05, SD: standart deviation, Min: minimum, Max: maximum, MetS: metabolic syndrome, HDL-C: high-density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure, [a]Independent sample t-test, [b]Mann Whitney U test, [c]Chi-square test.*

It was found that the uric acid level, fat mass, waist/hip ratio increased and the IPAQ-7 scores decreased in the MetS group in comparison to the control group ($P < 0.05$). No differences were shown for the other parameters regarding lean mass, water, BMI, pain intensity, QoL, HADS-A, and HADS-D scores between the groups ($P > 0.05$) (Table 2).

The uric acid level, fat mass, waist/hip ratio, and IPAQ-7 values were analyzed for cut-offs. According to the ROC analysis, the areas under the curve (AUC = 0.708, AUC = 0.677, AUC = 0.707, and AUC = 0.888) were significant for the uric acid, fat mass, waist/hip ratio and IPAQ-7 scores, respectively ($P < 0.001$, $P = 0.002$, $P < 0.001$ and $P = 0.008$, Table 3). The cut-off points of the uric acid, fat mass, waist/hip ratio, and IPAQ-7 scores were detected at 5.25 mg/dl, 37.50 kg, 0.91, and 247.25 METs-minutes/week, respectively. In the study, 64.60% sensitivity and 71.15% specificity were observed for ≥ 5.25 mg/dl of the uric acid, 33.30% sensitivity and 80.70% specificity were observed for ≥ 37.50 kg of the fat mass, and 64.60% sensitivity and 73.08% specificity were observed for ≥ 0.91 of the waist/hip ratio (Table 3, Figure 2a). Furthermore, 31.30% sensitivity and 82.69% specificity were observed for ≤ 247.25 METs-minutes/week of the IPAQ-7 scores (Table 3, Figure 2b).

Table 2. *Differences between Serum concentrations, physical and psychological well-being of groups'.*

Values	MetS group (n = 48)	Control group (n = 52)	P
Serum concentrations			
Uric acid (mg/dl, Mean ± SD)	5.70 ± 1.13	4.72 ± 1.30	< 0.001[a]*
Physical well-being			
Fat mass (kg, Median (Min; Max))	34.95 (20.0; 70.40)	30.65 (6.40; 60.10)	0.002[b]*
Lean mass (kg, Median (Min; Max))	46.05 (34.50; 71.50)	49.05 (34.40; 68.30)	0.440[b]
Water (lt, Median (Min; Max))	37.40 (29.70; 54.90)	36.85 (8.20; 49.30)	0.722[b]
Waist/hip ratio (Median (Min; Max))	0.97 (0.78; 1.20)	0.87 (0.70; 1.27)	< 0.001[b]*
BMI (kg/m², Median (Min; Max))	32.60 (24.73; 51.44)	31.79 (17.01; 45.79)	0.053[b]
IPAQ-7 (METs-minutes/week, Median (Min; Max))	396.0 (148.0;2376.0)	672.0 (148.5;2772.0)	0.008[b]*
Pain intensity (VAS, cm, Median (Min; Max))	5.70 (1.67;9.33)	5.0 (0.17; 9.57)	0.121[b]
NHP-energy (Median (Min; Max))	63.20 (0.0; 100.0)	63.20 (0.0; 100.0)	0.237[b]
NHP-pain (Median (Min; Max))	70.77 (0.0; 100.0)	53.63 (0.0; 100.0)	0.065[b]
NHP-emotional reactions (Median (Min; Max))	41.80 (0.0; 100.0)	42.47 (0.0; 100.0)	0.923[b]
NHP-sleep (Median (Min; Max))	39.16 (0.0; 100.0)	39.83 (0.0; 100.0)	0.960[b]
NHP-social isolation (Median (Min; Max))	22.01 (0.0; 100.0)	21.07 (0.0; 100.0)	0.868[b]
NHP-physical mobility (Median (Min; Max))	54.46 (0.0; 100.0)	43.35 (0.0; 100.0)	0.303[b]
NHP-total (Median (Min; Max))	302.23 (0.0; 567.14)	283.61 (22.42; 544.54)	0.381[b]
Psychological well-being			
Anxiety (Mean ± SD)	9.54 ± 4.67	8.40 ± 3.91	0.189[a]
Depression (Mean ± SD)	8.25 ± 4.78	7.78 ± 3.58	0.589[a]

**P < 0.05; SD: standard deviation, Min: minimum, Max: maximum, MetS: metabolic syndrome, IPAQ-7: International Physical Activity Questionnaire-7; VAS: Visual analog scale; NHP: Nottingham Health Profile, [a]Independent sample t-test, [b]Mann Whitney U test*

Table 3. *The Area Under the curve regarding uric acid, fat mass, waist/hip ratio and ipaq-7 values.*

Parameters	AUC ± SE	95 % Confidence Interval	p	Cut-off Point *	Sensitivity (%)	Specificity (%)
Uric acid (mg/dl)	0.708 ± 0.051	0.607 – 0.809	**< 0.001**	≥ 5.25	64.60	71.15
Fat mass (kg)	0.677 ± 0.053	0.573 – 0.782	**0.002**	≥ 37.50	33.30	80.77
Waist/hip ratio	0.707 ± 0.052	0.605 – 0.809	**< 0.001**	≥ 0.91	64.60	73.08
IPAQ-7 (METs-minutes/week)	0.653 ± 0.054	0.546 – 0.760	**0.008**	≤ 247.25	31.30	82.69

** It was detected to determine MetS according to youden index. IPAQ-7: International Physical Activity Questionnaire-7, SE: standard error, AUC: area under the curve, METs: metabolic equivalent*

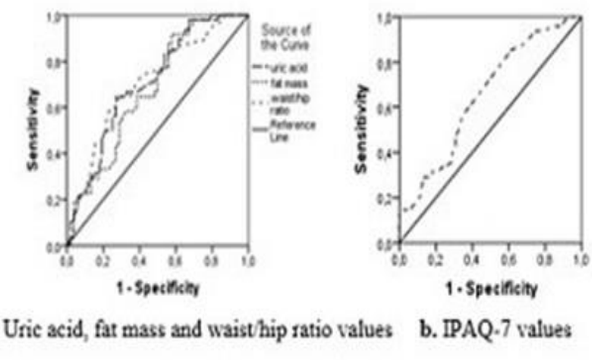

Figure 2a, b. *Receiver operating characteristic curve of uric acid, fat mass, waist/hip ratio and International Physical Activity Questionaire-7 (IPAQ-7) values.*

4. DISCUSSION

The present study yielded the following the results: (i) Patients with MetS showed greater uric acid level, fat mass, waist/hip ratio, and a lower physical activity level than those without MetS, (ii) no differences were detected for the other parameters of body composition, pain intensity, QoL, anxiety, and depression between the groups, (iii) the cut-off points for the uric acid, fat mass, waist/hip ratio and IPAQ-7 for detecting MetS were found as 5.25 mg/dl, 37.50 kg, 0.91 and 247.25 METs-minutes/week, respectively.

The serum uric acid is drawing increased attention as an inflammatory marker. Many studies reported higher serum uric acid concentration as a significant marker predicting the risk of developing cardiovascular diseases, stroke, cancer, and musculoskeletal pain (9,10,20,21). Afzal et al. (20) found that mean serum uric acid levels were higher in patients with chronic non-specific musculoskeletal pain in comparison to healthy controls, with 25% of patients showing hyperuricemia. They also declared that abnormalities of the uric acid profile may be an underlying biochemical abnormality in a significant number of patients. Furthermore, the increased serum uric acid level has also been reported to be associated with MetS and its components (21). However, the role of uric acid in the diagnosis of the MetS has not been established. Similar to

these studies, we also studied the uric acid level in patients having chronic musculoskeletal pain with and without MetS, and found increased uric acid levels in patients with MetS. These findings should be considered by clinicians to improve various symptoms of the patients having musculoskeletal pain with MetS.

MetS and musculoskeletal disorders may influence both physical and psychological well-being. Some parameters including physical inactivity, obesity, and depression are strong and independent predictors for the onset of an episode of intense and/or disabling musculoskeletal disorders; especially low back, neck, and knee pain, and these parameters have also been interrelated to the development of the MetS. Park et al. (22) reported that prevalence rates of MetS increased with BMI and physical inactivity. Lee et al. (23) put forward that increased physical activity levels were significantly correlated to decreased MetS. Similar to these studies, we also investigated physical well-being parameters including pain intensity, physical activity level, and body composition in patients having musculoskeletal pain with and without MetS, and found the increased fat mass and waist/hip ratio, and decreased physical activity in patients with MetS in comparison to those without MetS. There was no difference in parameters related to pain intensity. These findings should be considered for further studies to investigate the pain threshold and perception of the patients with MetS. Moreover, QoL is an important manifestation of both physical and psychological well-being. Several components of MetS and MetS-related adverse events have been associated with decreased QoL. Although different studies reported the impaired QoL in the MetS (24,25), a study by Vetter et al. (26) suggested that MetS itself was not related to impaired QoL. Therefore, the impact of MetS on QoL is less predictable and has not been clearly defined. In our study, no difference was shown between QoL and having MetS in patients with chronic musculoskeletal pain.

Besides, MetS has been found to be a significant predictor of some psychological disorders (27) and the reverse has also been reported (28). The evidence provided that depression was linked to MetS and its components (29). In contrast, according to a study by Hildrum et al. (30), no such association between depression and MetS was observed. Thus, the findings of these studies have been inconsistent regarding the association between psychologic status and MetS. We also compared the psychologic status in patients having musculoskeletal pain with and without MetS and put forward that no differences were detected for the anxiety and depression status between the groups. These results could be occurred due to the existence of chronic musculoskeletal pain.

Moreover, it may be substantial to determine the cut-off points of the uric acid, the fat mass, the waist/hip ratio, and the IPAQ-7 for the prevention and diagnosis of MetS. Thus, it was calculated these points for the uric acid (5.25 mg/dl), fat mass (37.50 kg), waist/hip ratio (0.91), and IPAQ-7 (247.25 METs-minutes/week) in this study. In literature, there were several studies related to the uric acid level, body composition values, and physical activity level in the MetS (21-23). However, there is no standard value for the uric acid, the fat mass, the waist/hip ratio, and the IPAQ-7 parameters. In health checkups, it may be beneficial to consider these values of the parameters.

The first limitation of the present study, different musculoskeletal pain patients included. More than 75 % of the individuals were non-specific low back or neck pain patients. The rest had joint problems. This should be taken into account. Secondly, body composition and general fat mass were presented. Especially, local fat mass related to the trunk, upper and lower extremities could be investigated in further studies due to the existence of MetS.

5. CONCLUSION

In conclusion, the current study demonstrated that patients with MetS had a greater uric acid level, fat mass, waist/hip ratio, and a lower physical activity level than those without MetS. Pain intensity, health profile characteristics, and psychological well-being did not differ in chronic musculoskeletal pain patients with and without MetS. Therefore, increased uric acid level, fat mass, and waist/hip ratio, and decreased physical activity level should be considered for the management of patients having musculoskeletal pain with MetS. Moreover, the increase of uric acid level, fat mass, and waist/hip ratio more than 5.25 mg/dl, 37.50 kg, 0.91 and the decrease of physical activity level more than 247.25 METs-minutes/week may be critical for MetS patients. The obscure relations need further attention during the treatment and healing processes of the patients.

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Clinical and Experimental Health Sciences

A Pilot Study on the Effect of Virgin Coconut Oil on Serum Lipid Profile and HS CRP Level Among Post-Acute Coronary Syndrome Patients: A Randomized Controlled Trial

Sharifah Shafinaz Sh Abdullah[1,2], Chong Mei Chan[2], Khatijah Lim Abdullah[3], Imran Zainal Abidin[4], Yap Bee Wah[5]

[1] University Teknologi MARA, Faculty of Health Sciences, Centre for Nursing Studies, Selangor, Malaysia.
[2] Nursing Department, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.
[3] Nursing Department, School of Medicine and Life Sciences, Sunway University, Selangor, Malaysia.
[4] Medical Department, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.
[5] University Teknologi MARA, Faculty of Computer and Mathematical Sciences, Selangor, Malaysia.

Correspondence Author: Sharifah Shafinaz SA
E-mail: shasya@uitm.edu.my

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ABSTRACT

Background: Virgin Coconut Oil (VCO) is beneficial for human health. Introducing VCO is cost-effective in Acute Coronary Syndrome (ACS) management compared to currently available medication. This study examines the influence of VCO on serum lipid profiles and hs CRP level among ACS patients.

Methods: A randomized single-blinded study was conducted from March 2018 to May 2018 among 70 ACS patients. The random sampling technique was used to allocate participants in the control and intervention groups respectively. Participants in Group A received one bottle containing 100 pieces of virgin coconut oil soft gels and were required to ingest 5 soft gels (5ml/5g) twice per day. Group B participants received routine treatment and statin. Changes in serum lipid profiles were identified using a paired t-test and an independent t-test.

Results: The 70 ACS patients were 51 years old and older and were mostly male. The total cholesterol level and Low-Density Lipoprotein Concentration (LDL-C) were reduced from baseline for 30 days. Most participants (68%) reported feeling better doing daily activities although some (22%) reported feeling worse from consuming virgin coconut oil due to its oily odour and slightly oily taste during eructation.

Conclusion: Virgin coconut oil was able to reduce serum lipid profile based on total cholesterol, triglyceride, and LDL-C through its consumption of 5 grams per day for 30 days. Virgin coconut oil may have a positive effect on cholesterol levels and other cardiovascular risk factors.

Keywords: Acute Coronary Syndrome, Cardiovascular Risk Factors, Serum Lipid Profile, Hs Crp Level, Virgin Coconut Oil, Medium Chain Triglyceride

1. INTRODUCTION

Acute coronary syndrome (ACS) occurs due to decreased blood flow in the coronary artery, which hinders the heart from functioning properly. It varies from non-ST-segment elevation myocardial infarction (NSTEMI) to ST-segment elevation myocardial infarction (STEMI) (1). The association between NSTEMI and unstable angina is strongly observed due to their similarity in clinical presentations and pathophysiology origins. NSTEMI is diagnosed by severe myocardial ischemia, evidenced by ECG and the presence of necrosis biomarkers in the circulation (1,2). ACS occurs in the intima, the inner layer of the coronary artery wall. The blood flow to the myocardium is reduced due to the partially or completely blocked arteries. The patient's condition will worsen from a myocardium infarction when the heart suffers damage or death from the lack of nutrients and oxygen.

In Malaysia, ACS has emerged as one of the widespread causes of fatality in recent years. According to the Ministry of Health Malaysia (MOH), cardiovascular disease is the number one cause of death since early 1980s (4). As mentioned by the World Health Organization (WHO), there are several strategies for cardiovascular prevention which reduce morbidity and mortality. Cardiovascular diseases are managed by adhering to these strategies, which are developed by considering the cost-effectiveness and innovativeness of healthcare (4). Introducing VCO is cost-effective in ACS management compared to currently available medication. VCO is a natural resource available in Malaysia which lowers lipid level sand glucose levels, based on results of animal studies (5,6,7,8). However, there are still limited studies on human testing. Further human studies are highly required to confirm the effects of VCO on ACS patients.

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Coconut oil is considered a food source and is used as complementary medicine. It is one of the primary natural products produced from coconut, used as a food ingredient and known as a functional food (9). Researchers have confirmed that there are significant benefits experienced by the intervention group (subjects who consume VCO as an intervention). Several studies on animals claimed that VCO has a valuable benefit on health such as lowering lipid and glucose levels, serving as anti-inflammation and analgesic agent, and increasing the effectiveness of hepatoprotective activity [5,8,10,11,12,13,14).

Even though there exist many animal studies which tested the various effects of VCO (10,15,16,17), similar human studies are still lacking. In addition, despite there being a large body of literature on VCO's ability to reduce serum lipids, only a few of these studies were performed in Malaysia. There is no clear evidence of VCO's effect on ACS patients in Malaysia. The volume of research on the benefit of VCO on ACS patients does not match the magnitude of the problem that is posed in Malaysia. This study, therefore, aims to investigate the impact of VCO on serum lipid profiles among ACS patients in Malaysia.

2. METHODS

This is a single-blind randomized controlled trial and equal randomization study with a ratio of 1:1 for parallel groups. The study was conducted in the medical cardiac ward in University Malaya Medical Centre (UMMC), Malaysia.

2.1. Participants

The targeted population was subjects admitted to the cardiology clinics who met the inclusion criteria and agreed to participate in this study. Patients who are aged 18 to 65, male or female, have ACS, at post-acute phase, and can understand Malay and English were included in this study. However, patients who are pregnant, have uncontrolled hypothyroidism, have a renal failure with creatinine >2mg/dL, and have liver failure were excluded from this study.

2.1.1. Sample Population

ACS consists of both STEMI and non-ST-segment elevation ACS (NSTE-ACS). It demonstrates the existence of anginal syndrome associated with electrocardiographic ST-segment elevation in two or more limbs of at least 0.1 mV or two or more precordial leads of at least 0.2 mV. The newly elevated and developed chest syndromes are explained through unstable angina pectoris irrespective of a significant increase in cardiac-specific troponin I values.

2.1.2. Sampling Method

Only subjects who met the eligibility requirements were randomized into the study. After consent was obtained, block randomization (1:1) and random number table were used for the allocation of treatment. The sample size for the study was calculated based on the formula by Sakpal, 2010 (18). Sample size estimation in a clinical trial with an alpha value of .05, power of 80%, and a clinically significant difference of 0.8 and standard deviation of 0.74 was calculated based on the result of the previous study by Thaw et al. (19)A lipid level change of 0.5 mmol/L was detected in a sample size of 40 subjects, 20 in each group with 5% level of significance and 80% power. Therefore, the sample size needed to be60 subjects (30 per group), considering a dropout rate of 10%.

2.2. Data collection and Randomization

The participants were recruited from the cardiac ward with the supervision and permission of the PPUM Cardiologist. Eligible subjects were given the subject information sheet and consent form. An explanation of the study procedure was given by the researcher. Participants were given sufficient time to decide whether to participate in this study. They were free to withdraw from the study at any time. Initial screening in the ward was performed by the researchers. A random sampling technique was used to assign participants to the control group and the intervention group.

Participants in Group A each received a bottle labelled A containing 100 pieces of VCO soft gels and were instructed to consume 5 capsules of VCO twice per day (0.5ml/capsule = 10 capsules/5 ml) for 30 days. They were also continuing routine treatment, including consuming statin. Group B remained on routine treatment and statin.

After 30 days, there followed up with all subjects for a blood test and anthropometry measurements in the ward. They were instructed to fast for at least 10 hours before the blood test. Group A was instructed to return the labelled bottles and the number of soft gels left was measured to monitor compliance. Participants who experienced any adverse effect would notify the medical officer and would receive appropriate treatment and be allowed to withdraw from the study (however, no adverse effect of VCO had been reported). This is a single-blind study where participants and medical laboratory technicians (outcome assessors) were not assigned the coded key. VCO soft gels were provided by local companies who helped in preparing, packing, and distributing them.

2.3. Preparation of VCO Soft Gel

VCO was extracted through the fermentation process (no heat was applied to retain its beneficial fatty acids). Each ml of VCO contains 0.46gm of lauric acid, C:12. Raw VCO from Company B was certified by UKM UNIPEQ. The local company has agreed to provide soft gels with 0.5ml of VCO (0.23gm of lauric acid) in each. In this study, all participants were supplied with 5ml of VCO with a total 1.15gm of lauric acid (C:12). Each VCO soft gel contained 0.23gm of lauric acid. The soft gel shells are a combination of gelatin, water, and opacifiers such as glycerin or sorbitol. The authors and the clinical supervisor (a cardiologist) inspected the bottles and were satisfied with the amount, appearance, and fatty acid compositions of the VCO soft gels. The researcher received the soft gels in boxes, which were kept

in the National Cardiovascular (NCVD) room located in Level 7, Menara Utama, UMMC. The NCVD room was locked throughout the experiment and the key was kept by the main author.

2.4. Ethical Considerations

The medical research ethics committee from the University of Malaya had provided ethical approval (reference number: 2017.528.5276, 17 July 2018). The study had been registered under ANZCTR with a unique number (ACTRN126.180.01736235).

2.5. Data Analysis

Each randomized group was summarized separately based on baseline characteristics. An intention-to-treat (ITT) analysis was used to fulfill primary outcomes, which included all randomized patients in the groups they were randomly assigned while disregarding anything that happens after randomization. A per-protocol (PP) analysis was used to fulfill secondary outcomes. Patients who reported >75% in the follow-up were included in the PP population.

3. RESULTS

The researcher and a research assistant had conducted the recruitment process following the CONSORT diagram (Figure 1). Only 71 out of 80 patients with ACS were invited and randomized for baseline evaluation. On the 30th day of follow-up, 61 patients attended; 10 patients were lost to follow-up and another two patients did not attend the follow-up due to disease progression. The total compliance was 86%.

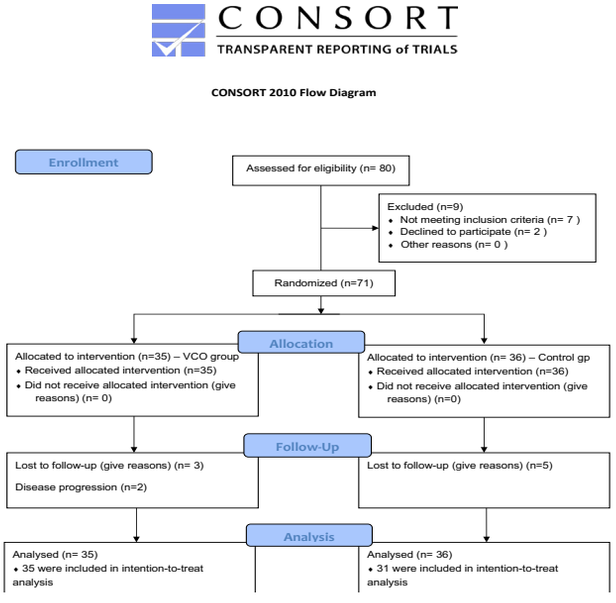

Figure 1. *CONSORT 2010 flow diagram*

Table 1 shows a description of the characteristics of the participants. The overall mean age was 50 and two-thirds of the participants were male. Mean changes for the serum lipid level on the 30th day of follow-up are shown in Table 2, for within each randomized group and comparison between groups. Total cholesterol (TC) concentrations were significantly reduced by 15.1%in the VCO group (95%CI – .19, 0.33, p< 0.001), low-density lipoproteins (LDL-C) and triglyceride concentrations also decreased from baseline to day 30 by 19.1% and 11.8% respectively. High-density lipoproteins concentration (HDL-C) showed no significant increase (but had reduced by 3.4%); however, there was a small increment from baseline to day 30. Serum lipid level of the control group did not show any statistically significant difference from baseline to day 30; however, there was a small clinical reduction in all lipid parameters. All participants showed no changes in serum Hs-CRP concentration.

Table 1. *Demographic and Clinical Characteristics of Participants*

Characteristic	VCO group (n =35) M± SD	Control group (n =35) M± SD	P-value t-test/ X^2/Fisher
Age (year)	50.5 ± 8.9	53.8±10.1	0.18[a]
Gender			
Male	24	23	
Female	11	12	0.10[b]
Race			
Malay	18	16	
Chinese	8	8	0.23[b]
Indian	9	11	
Education level			
Tertiary	17	16	
Secondary	18	19	0.24[b]
Marital status			
Married	30	32	0.61[c]
Single	5	3	
Diagnoses			
Unstable angina	14	16	
NSTEMI	11	10	0.92[b]
STEMI	5	5	
Medications			
Statin+ anticoagulant	5	5	
Statin + antihpt+anticougulant	13	13	0.98[b]
Statin+Antihpt+ Anti DMt+Anticoagulant	12	13	
Baseline Body Weight (kg)	77.5±14.7	74.1±10.5	0.2[a]
NUTRITICS (Nutrition analysis software)			
Total calories	2422 ± (956.9)	2367 ± (784.6)	0.91[a]
Total energy Kilojoules	9941± (4388)	14519 ± (2566)	0.15[a]
Protein %	17.8 ± (2.4)	17.8 ± (2.47)	0.91[a]
Carbohydrate %	56.9± (5.2)	50.5± (6.5)	0.45[a]
Saturated fat %	24.6 ± (5.0)	26.6 ± (4.6)	0.10[a]
Alcohol %	0.6 ± (1.5)	1.1 ± (2.0)	0.72[a]
Level of activities			
• None – little or no regular exercise	0	0	
• Light – 1-3 days per week	14	13	0.92[x]
• Moderate – 1-3 days light week or 5 days week hard	11	12	
• Very active – 6 days week hard	6	6	

[a] *Independent t test;* [b] *Chi Square test;* [c] *Fisher exact test*

Table 2. *Comparison between VCO group and Control group for baseline data and Day 30 (post intervention)*

Clinical parameter	VCO group (n=30)				Control group (n = 31)				Comparison between two groups	
Serum lipid profile	Baseline (Day 0)	Post intervention (Day 30)	t (df)	*p	Baseline (Day 0)	Post intervention (Day 30)	t (df)	*p	t (df)	**p
Total Cholesterol	4.66±1.47	3.94 ± 0.86	3.06 (34)	0.00	4.30±1.13	4.23±1.14	-.55 (35)	0.32	-1.5(69)	0.58
Triglyceride	1.61±0.96	1.42 ± 0.74	2.10 (34)	0.04	1.81±0.89	1.78±1.13	0.15 (35)	0.87	-0.79 (69)	0.87
HDL-C	1.17 ±0.43	1.13 ±0.28	-15 (34)	0.88	1.14±0.60	1.07±0.25	-.74 (35)	0.46	0.87(69)	0.46
LDL-C	2.61 ±1.21	2.11 ±0.71	2.67 (34)	0.01	2.61±0.83	2.47±1.07	1.02 (35)	0.46	-2.1(69)	0.31
HS CRP	2.63 ± 4.2	5.48±18.8	-.90(34)	0.34	1.92±4.6	2.4±5.1	-1.19 (35)	0.24	0.93 (69)	0.35

**Paired t test; ** Independent t test*

Over 30 days, self-reported compliance was high among 87% of the patients in the intervention group, similarly in the statin group (86%). For feedback, 68% of the participants reported feeling better in doing daily activities and 22% reported feeling worse in consuming virgin coconut oil due to oily odour and slightly oily taste during eructation.10% of the patients had reported stomach discomfort during the first two days of VCO consumption but symptom relief on day three. The dietary intake levels were presented using the nutrition analysis software (NUTRITICS) at baseline across the intervention group. Total fat intake, protein, and carbohydrate did not differ among the intervention group. Most participants reported no changes in regular physical activity. The regular physical activity increased by approximately 18% and 16% for patients in the VCO group and control group respectively.

The VCO fatty acid composition used in the intervention is presented in Figure 2. Lauric acid C12:0 (48%) was the major element found in 94% of the saturated fatty acid, followed by myristic acid C14:0 (19%), and palmitic acid C16:0 (9%).

Fatty acid profile		Concentration (%)
C6	Caproic	2.215
C8	Caprylic	12.984
C10	Capric	6.806
C11	Undecanoic	0.028
C12	Lauric	47.280
C13	Tridecanoic	0.030
C14	Myristic	15.803
C15	Pentadecanoic	0.006
C16	Palmitic	6.688
C16:1	Heptadecanoic	0.011
C17	Stearic	0.011
C18	Oleic	1.481
C18: 1n9c	Elaidic	5.073
C18: 1n9t	Linoleic	0.231
C18: 2n6c	Linolelaidic	1.168
C18: 3n6g	γ Linolenic	0.045
C18: 3n3a	α Linolenic	0.007
C20	Cis-11-Eicosenoic	0.039
C20: 1n9	Behenic	0.039
C22	Cis-13,16-Docisadienoic	0.006
C24	Lignoceric	0.020

Figure 2. *Fatty acid composition of VCO (30)*

4. DISCUSSION

In this trial, middle-aged men and women with ACS who were admitted in the cardiology unit in UUMC were randomly assigned to consume 5ml (5grams) of VCO per day while continuing routine treatment and medication for 30 days. The control group would only continue routine treatment and medication. Significant changes were reported in triglyceride and LDL-C concentrations, as well as total cholesterol between the two groups at the end of the trial. VCO consumption also resulted in statistically significant differences in TC, triglyceride, and LDL-C. However, it did not significantly raise HDL-C concentration, though

there was a small increment in pre – and post-means. All lipid parameters showed no statistically significant increase in the control group.

The results agreed with the recent study by Khaw et al. (19), who found that VCO was able to reduce LDL-C and increase HDL-C. However, this study showed no changes in HDL-C. Several previous studies have reported that coconut oil was not favoured in lowering lipid level (20,21). These studies have reported their findings on the effect of coconut oil instead of VCO. There is a lot of difference between regular coconut oil and VCO. The process of extraction is different, and the end product also contains different compositions of fatty acids. In general, the process of extracting coconut oil uses the bleaching and deodorizing processes where chemical agents are added, which makes the oil lose beneficial fatty acid. On the other hand, VCO can be extracted using either the heat-free process of fermentation, cold pressing, or through minimal heat exposure, processes which retain all-important fatty acids that are beneficial to health. The process of extraction plays an important role in why some coconut oil studies seem to have harmful effects on health, particularly on lipid profiles.

In addition, this study showed VCO consumption having a good effect on the lipid profile at only 5ml, containing 1.3grams of lauric acid, compared to previous studies that used 30ml (24grams of lauric acid) [20]. In this study, 1.15grams of lauric acid had significantly reduced the lipid level. The VCO compositions are listed in Figure 2. They contain fatty acid at more than 50%, an ingredient which played a role in lowering

lipid level. As reported by several previous studies, lauric acid contributes to lowering the lipid level.

The transmission of lauric acid is direct to the liver in the form of energy and other metabolites (23,24). Ketone bodies are a type of these metabolites that can be used by extra hepatic tissues in the form of energy. This shows that synthetic MCT oil and dietary fats might have different physiological effects. Due to different experimental doses, feeding periods, and experimental designs, there are inconsistent findings of lauric acid observed on serum cholesterol levels.

There are several different ways in which lauric acid can emerge which moderate serum cholesterol levels. One of the most highly oxidized substance is lauric acid, which contributes minimally to fat accumulation and obesity. Half of the fatty acids in coconut oil are lauric acid. Long-chain fatty acids are predominantly used for allowing the triglyceride structure to be consumed rapidly. Lauric acid is transported directly to the liver using the portal vein (23) Lauric acid is not found in phospholipids, but it might be present minimally in chylomicrons (24)

Across the mitochondrial membrane, lauric acid is rapidly transported by passive diffusion. It is rapidly metabolized in a number of ways in the liver. Two acyl-CoA dehydrogenase enzymes oxidize lauric acid (25). It can metabolize ketone bodies, which are important energy sources in the body for extra hepatic organs (23,24,25,26,27). It is contrary to the study by Vijayakumar et al.(22) who found no difference in blood lipid levels among 200 participants. They reported difficulty in interpreting findings as all participants were on statin therapy. In the current study, the statin group showed some reduction in lipid level; however, there was no statistically significant outcome observed. The statin functions to inhibit Hydroxy-Methyl-Glutaryl-CoA reductase (HMGCR), which acts as the main enzyme of the cholesterol biosynthesis pathway (28,29). This function can lead to several side effects on the human body.

There were several confounding factors like eating out, duration of consumption, and physical activities which could not be assessed accurately since the study was conducted on free-living subjects (Vijayakumar et al. 2016a). The difference in population, research methodology and VCO dosage are the main factors that contribute to the different findings across VCO studies.

The strength of this study is the use of randomized design with a high completion rate (85%) and self-reported dietary compliance over 30 days. However, it was a short-term trial of 30 days of intervention, which is the major limitation. There lacks an examination throughout a longer duration of intervention. The findings of the study are not generalizable for the wider population.

5. CONCLUSION

In this trial, VCO with rich lauric acid appears to have a beneficial effect on serum lipid levels. The actions of lauric acid as MCT in reducing serum lipid and its numerous benefits have been reported in the previous two years and the current study. This study focuses on the individual with coronary problems already treated with a statin. Intervention groups received 5ml of VCO (1.13grams of lauric acid) in soft gel form and would continue on a statin for 30 days whereas the control group would continue solely on a statin. Baseline and 30th-dayfollow-up were recorded and compared. The findings revealed that the group consuming VCO showed a reduction of TC by 15.1% (95%CI – .19, 0.33, $p < 0.001$), LDL-C and triglyceride concentrations also decreased from baseline to day 30 by 19.1% and 11.8% respectively. HDL-C concentration showed an insignificant increase in the control group.

MCT is advantageous for CHD risk, which implies that different dietary sources of fats can be reflected from observational trials. VCO as a natural product can reduce cholesterol parameters better compared to statin. Therefore, medical personnel should consider this natural product as one of the front-line treatments for cardiovascular disease patients as well as a method of prevention for CVD. The existing dietary suggestions cannot be changed by the findings of this study, such as reducing saturated fat intake, but they call for further clarification to develop a scientific relationship between health and dietary fats.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Clinical and Experimental Health Sciences

Investigation of the Cultural Competence Levels of Diabetes Nurses

Derya Cinar[1], Nermin Olgun[2], Aynur Koyuncu[2]

[1] Izmir Bakircay University, Faculty of Health Sciences, Nursing Department, Izmir, Türkiye.
[2] Hasan Kalyoncu University Health Sciences of Faculty, Nursing Department, Gaziantep, Türkiye.

Correspondence Author: Derya Cinar
E-mail: derya.cinar@bakircay.edu.tr
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ABSTRACT

Objective: It is important for nurses to have cultural competence in order to keep up with the cultural diversity brought by globalization. In this study, we aim to examine the cultural competence levels of diabetes nurses in diabetes education and care in accordance with the cultural characteristics of individuals with diabetes.

Methods: This study was conducted in a cross-sectional descriptive type with 183 diabetes nurses between September 15 and December 15, 2020 in Turkey. The data were collected using the Introductory Information Form and the Nurse Cultural Competence Scale.

Results: We determine that almost all of the diabetes nurses had cultural competence above the average. We find that having a postgraduate degree from diabetes nurses, receiving cultural education before, and having a diabetes nurse experience of 6 years or more affected the cultural competence levels ($p<0.05$).

Conclusion: This research shows that diabetes nurses in Turkey have cultural competence in providing care to individuals with diabetes from different cultures, and that education and experience affect this situation. It is predicted that diabetes nurses will contribute to the self-management of individuals with diabetes by implementing strategies to improve their cultural competence.

Keywords: Diabetes, nursing, cultural competence

1. INTRODUCTION

In the historical process, the phenomenon of migration has gained a different speed and dimension with globalization (1,2). According to the 2020 World Migration Report 2020 of the International Organization for Migration (IOM), it has been reported that the global international migrant population reached 272 million in 2019. This rate is approximately 6 million in Turkey and the majority of the immigrants come from countries such as Syria, Iraq, Afghanistan, Iran, and Somalia (3,4). The coming together of different cultures has led to an increase in cultural diversity (1,2). It has been reported that cultural diversity is a factor affecting the incidence of the disease (2). In the United States, the incidence of coronary heart disease, stroke, diabetes, asthma, and cancer has been reported to be higher in the minority population (5). In this respect, it has become important to consider cultural diversity in health care. In the context of health care, appreciating, accepting, and respecting the values, beliefs, and preferences of different cultures is defined as cultural competence (2). Cultural competence requires an understanding of producing solutions to reduce cultural differences and providing culturally competent care (2,5). Individuals' cultural norms and values significantly affect their understanding of health and illness, lifestyle, and behavior (6). The literature suggests that among the difficulties that health professionals face in culture-specific care, patients experience communication barriers related to their linguistic and cultural backgrounds (7-9). The cultural competence of health care professionals reduces racial and ethnic differences and improves patient care quality, patient satisfaction, and patient care outcomes (10).

In this context, nurses should develop cultural competence skills to have cultural sensitivity and competence (1,11,12). Studies conducted predicted that with the increase in cultural diversity, patient expectations will also increase, and therefore, the cultural competence levels of nurses should be increased. For this purpose, it is recommended to plan training programs that will improve cultural competence during the nursing education process and post-graduate in-service training. These studies suggest that the training given

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about cultural competence is an effective strategy, and the level of cultural competence increases in the training groups (10,13,14).

Diabetes is one of the urgent public health problems of the last century and has become a pandemic due to its global effects (15). It has been reported that type 2 diabetes is more common in individuals of Asian and African American descent, and the incidence of retinopathy, one of the microvascular complications of diabetes, is higher in African Americans (55.7%) (6). In a study conducted in Mexican Americans, it was shown that culturally competent diabetes self-management education decreased the HbA1c and fasting blood glucose levels of individuals with diabetes and increased their knowledge about diabetes (16).

As far as is known, although studies are examining the cultural competence of nurses and nursing students in the literature, no studies are examining the cultural competence levels of diabetes nurses (2,5,10,17,18). Due to the increasing cultural diversity with globalization, it is obvious that there is a need for data on the cultural competence of diabetes nurses who care for individuals with diabetes from different cultures. In this context, our research is the first and original study examining the cultural competence of diabetes nurses in Turkey. The aim of this study is to determine the cultural competence levels of diabetes nurses who care for individuals with diabetes.

2. METHODS

2.1. Study Design, Setting, and Sample

This is a cross-sectional descriptive study conducted with diabetes nurses in Turkey between September 15 and December 15, 2020. The population of the study consisted of 724 nurses working as diabetes nurses in Turkey and actively registered with the Diabetes Nursing Association. Diabetes Nursing Association is the only organization in Turkey where diabetes nurses are actively registered. A convenience sampling was used, and 183 (25.3%) diabetes nurses who answered the questionnaire and responded to the inclusion criteria constituted the sample of the study. Caring for individuals with diabetes, being 18 years of age and older, being actively registered with the Diabetes Nursing Association, and volunteering to participate in the research were determined as the inclusion criteria of the research.

2.2. Ethical Considerations

The non-interventional research ethics committee of a state university in Turkey was approved by the ethics committee (decision no: 2021/61) and was conducted following the Declaration of Helsinki. Written permission was obtained from the Diabetes Nursing Association, where diabetes nurses are registered in Turkey, for data collection. Before answering the online questionnaire, the participants were provided to read and approve the informed consent text explaining the purpose and rationale of the study.

2.3. Data Collection

The data were collected by sharing the online questionnaire form created with Google Forms by the researchers through the Diabetes Nursing Association between September 15 and December 15, 2020, to diabetes nurses via mail and SMS. The data of the study were obtained by using the Introductory Information Form and the Nurse Cultural Competence Scale (NCCS-T).

2.4. Instruments

Introductory Information Form: This is an electronic form consisting of 17 questions developed by researchers in line with the literature (1,2,11,17-19). The form consists of questions that include some socio-demographic characteristics of the participants (age, gender, education level, working year) and introductory information about cultural care (such as having previously received cultural education, caring for people with diabetes from different cultures, difficulties in providing care).

Nurse Cultural Competence Scale (NCCS-T): The scale was developed by Perng and Watson in 2012 (20). The Turkish adaptation of the scale (NCCS-T) was done by Gözüm et al. (2016) (11). The scale, consisting of a total of 20 items, was designed to evaluate the cultural competence level of nurses. The scale consists of three sub-dimensions: cultural knowledge (7-35 points), cultural skills (11-55 points), and cultural sensitivity (2-10 points). The lowest score that can be obtained from the scale is 20 and the highest score is 100. A high total score indicates that the individual has a high level of cultural competence.The Cronbach's alpha coefficient of the NCCS-T, which was adapted into Turkish, was found to be 0.96 (20). In this study, the Cronbach's alpha coefficient of the NCCS-T was calculated as 0.93.

2.5. Statistical Analysis

Statistical analyzes were reported using the SPSS 26.0 statistical software. In descriptive analyses, number (n), percent (%), mean±standard deviation, and median (minimum-maximum) values are presented. In comparative group analysis; Student's t-test and Mann-Whitney U test were used in paired groups. By checking the conformity of the continuous variables to the normal distribution in the groups, analyzes were performed using the Parametric Student's t-test in cases where the normal distribution was provided, and the Mann Whitney U test, which is a nonparametric test, in cases where it was not. Multiple linear regression analysis was performed to determine the effect of various independent variables on the cultural competence level score of diabetes nurses. All statistics were evaluated at the $p<0.05$ significance level.

2.6. Limitations of the Study

In this study, the presence of participants from geographically different regions of Turkey increased the possibility of representing different regions. The fact that only 25% of the diabetes nurses actively working in Turkey constituted the sample of the study shows that the research findings cannot be generalized to the population. The other limitations of this study are that nurses were not questioned about the rate of individuals with diabetes from different cultures they care for and their ability to speak a language other than Turkish.

This study shows that diabetes nurses in Turkey have cultural competence in caring for people with diabetes from different cultures and that education and experience affect this situation. Cultural competencies of diabetes nurses will contribute to diabetes management.

3. RESULTS

3.1. Sociodemographic Characteristics of Diabetes Nurses

Data including some introductory characteristics and cultural care-related characteristics of diabetes nurses are shown in Table 1. When the sociodemographic characteristics of the diabetes nurses included in the study were examined; it was determined that 99.5% were women, 50.3% were between the ages of 25-41, 76.5% were married and 48.6% were living in western Turkey. According to the professional characteristics of the nurses, 67.2% of them have vocational education at the undergraduate level, 53.0% have less than 20 years of professional experience, 53.5% have worked as a diabetes nurse for 6 years or more, and 89.6% have diabetes nurse certification determined.

When the characteristics of the participants regarding cultural care are examined; they reported that 29.5% of them had previously received a cultural education, 100% of them stated that the culture-specific care would affect the adherence of the diabetic individual to treatment, they routinely evaluated the health/disease perception of individuals with diabetes, they observed their traditional and ethnic practices, and they always encountered individuals from different cultures. Diabetes nurses stated that 63.9% of them had language problems and 60.1% of them had communication problems, in the order of frequency as the difficulties they encountered while giving care to individuals from different cultures. It was determined that the participants learned information about the cultural structures of individuals from different cultures, respectively, from family/friends and relatives by 85.8%, and by information sources such as social media and internet by 78.7%. When the participants were asked whether they felt competent while providing cultural care while providing education and care to individuals with diabetes and their families from different cultures; 88.5% reported that they thought they had cultural competence (Table 1).

Table 1. *Some introductory characteristics of diabetes nurses and information about culture-specific care (n=183).*

Variables	X̄ ±SD	Median (Min-Max)
Age (year)	40.77±6.69	41 (25-59)
Working duration as a nurse (year)	19.13±8.16	20 (1-40)
Working duration as a diabetes nurse (year)	7.36±5.49	6 (1-25)
	n	**%**
Cultural education status		
Yes	54	29.5
No	129	70.5
Willingness to receive cultural education		
Yes	175	95.6
No	8	4.4
The effect of culture-specific care on patient compliance		
Yes	183	100
No	-	-
Evaluation of the patient's perception of health/illness		
Yes	183	100
No	-	-
Evaluation of the traditional/ethnic practices of the person with diabetes		
Yes	183	100
No	-	-
Encounter with patients from different cultures		
Yes	183	100
No	-	-
The state of feeling culturally competent in oneself		
Yes	162	88.5
No	21	11.5
Challenges they face in culture-specific care*		
Expectations of physiological care needs	57	31.1
Expectations of psychological care needs	73	39.9
Their expectations regarding their spiritual needs	24	13.1
Culture-specific expectations	66	36.1
Communication problem	110	60.1
Language problem	117	63.9
Information sources where patients from different cultures learn about their cultural structures		
Family/relative/friend	157	85.8
Pre/post graduation trainings	54	29.5
individual experiences	78	42.6
Travel experiences	58	31.7
Social media/internet	144	78.7
Other	6	3.3
Thinking that you have cultural competence		
Yes	162	88.5
No	21	11.5

** Multiple options are marked.*

3.2. Some İntroductory Characteristics of Diabetes Nurses and Their Status Towards Cultural Care, and the Mean Scores of NCCS-T

It was determined that 89.6% of the diabetes nurses had a cultural competence level above the average (60 points and above). It was determined that 100% of the participants had cultural knowledge (14 points and above), cultural skills (27 points and above), and cultural sensitivity (4 points and above) levels above the average. When the total and sub-dimension scores of NCCS-T were examined; a total score of 71.65±9.86 points in NCCS-T, cultural knowledge was calculated as 24.34±3.92 points, the cultural skill was calculated as 40.01±5.63 points, and cultural sensitivity was calculated as 7.29±1.17 points (Table 2).

Table 2. *Distribution of diabetes nurses' total score and sub-dimension score averages of NCCS-T (n=183).*

NCCS-T	X̄ ±SD	Median (Min-Max)
Cultural knowledge	24.34±3.92	25 (14-35)
Cultural skills	40.01±5.63	41 (27-55)
Cultural sensitivity	7.29±1.17	8 (4-10)
NCCS-T Total point	71.65±9.86	72 (47-100)

It was determined that diabetes nurses with a graduate degree had a significantly higher mean score in the total score of the NCCS-T, cultural knowledge, and cultural skills sub-dimension than the nurses with a bachelor's degree ($p<0.05$). Nurses with 6 or more years of experience as diabetes nurses were found to have a significantly higher mean score in the NCCS-T total score, cultural knowledge, and cultural skills sub-dimension than nurses with less than 6 years of experience ($p<0.05$). The diabetes nurses' total score, cultural knowledge, and cultural skill sub-dimension mean scores were significantly higher than the nurses who received previous training on cultural care ($p<0.05$). Those who think that they have cultural competence while providing education and care to individuals with diabetes and their families from different cultures have a significantly higher mean score of NCCS-T total score, cultural knowledge, cultural skills, and cultural sensitivity sub-dimensions than the nurses who think that they do not have cultural competence ($p<0.05$). (Table 3).

In the multiple regression model, among the factors that significantly affect the diabetes nurses' total and sub-dimension scores of NCCS-T; having a graduate degree, having 6 or more years of working experience as a diabetes nurse, no previous cultural education, and having 20 years or more professional experience. These variables explain 9% of the cultural competence level of diabetes nurses (Table 4).

Table 3. *Distribution of some introductory characteristics of diabetes nurses and their total score and sub-dimension score averages in NCCS-T (n=183).*

Variables	NCCS-T Total X̄ ± SD	Cultural knowledge X̄ ± SD	Cultural skills X̄ ± SD	Cultural sensitivity X̄ ± SD
Age groups				
25-41 years	72.03±9.48	24.57± 3.84	40.15±5.40	7.30±1.24
42 years and older	71.27±10.27	24.12±4.01	39.87±5.87	7.28±1.10
	t=.519 p=0.605	t=.783 p=0.435	t=.327 p=0.744	t=.107 p=0.915
Education status				
Undergraduate	70.18±9.15	23.74±3.65	39.12±5.29	7.32±1.09
Graduate	74.66±10.63	25.58±4.20	41.85±5.90	7.23±1.34
	t=-2.944 **p=0.004**	t=-3.033 **p=0.003**	t=-3.149 **p=0.002**	t=.495 p=0.621
Professional experience period				
1-19 years	72.20±9.53	24.63±3.93	40.22±5.44	7.36±1.26
20 years and above	71.16±10.16	24.09±3.92	39.83±5.81	7.23±1.09
	t=.714 p=0.476	t=.939 p=0.349	t=.461 p=0.645	t=.707 p=0.481
Working duration as a diabetes nurse				
Under 6 years	70.04±8.92	23.81±3.72	39.08±5.06	7.16±1.01
6 years and above	73.05±10.45	24.81±4.05	40.82±5.98	7.40±1.29
	t=-2.073 **p=0.040**	t=-1.734 p=0.085	t=-2.108 **p=0.036**	t=-1.399 p=0.163
Prior cultural education				
Yes	74.55±10.19	25.55±3.73	41.53±5.79	7.46±1.17
No	70.44±9.49	23.84±3.91	39.37±5.46	7.22±1.17
	t=2.614 **p=0.010**	t=2.733 **p=0.007**	t=2.393 **p=0.018**	t=1250 p=0.213
Thinking that you have cultural competence				
Yes	72.64±9.48	24.72±3.77	40.56±5.44	7.36±1.17
No	64.04±9.58	21.47±4.02	35.80±5.40	6.76±1.04
	t=3.901 **p=0.000**	t=3.683 **p=0.000**	t=3.766 **p=0.000**	t=2.231 **p=0.027**

t= Independent Samples t test; p<0.05

Table 4. *The effect of some introductory characteristics of diabetes nurses on the total and sub-dimension scores of NCCS-T (n=183).*

Variables	B	*SE*	*Beta*	*t*	p
NCCS-T Total					
Costant	70.633	4.429		15.946	0.000
Education status	4.525	1.494	0.216	3.028	0.003
Working experience as a diabetes nurse	3.220	1.518	0.163	2.121	0.035
Prior education status	-4.045	1.544	-0.188	-2.620	0.010
Professional experience period	-1.983	1.512	-0.101	-1.311	0.191
Cultural knowledge					
Costant	24.438	1.762		13.866	0.000
Education status	1.853	0.595	0.222	3.116	0.002
Working experience as a diabetes nurse	1.100	0.604	0.140	1.820	0.070
Prior education status	-1.720	0.614	-0.200	-2.800	0.006
Professional experience period	-0.853	0.602	-0.109	-1.417	0.158
Cultural skill					
Costant	38.535	2.533		15.216	0.000
Education status	2.772	0.854	0.232	3.244	0.001
Working experience as a diabetes nurse	1.797	0.868	0.160	2.071	0.040
Prior education status	-2.122	0.883	-0.172	-2.404	0.017
Professional experience period	-0.877	0.865	-0.078	-1.014	0.312
Cultural sensitivity					
Costant	7.693	0.553		13.915	0.000
Education status	-0.109	0.186	-0.044	-0.584	0.560
Working experience as a diabetes nurse	0.315	0.189	0.134	1.665	0.098
Prior education status	-0.196	0.193	-0.076	-1.020	0.309
Professional experience period	-0.263	0.189	-0.112	-1.394	0.165

NCCS-T total: F=5.620, p=0.000, R2=0.112, Adjusted R2=0.092
Cultural knowledge: F=5.733, p=0.000, R2=0.114, Adjusted R2=0.094
Cultural skill: F=5.518, p=0.000, R2=0.110, Adjusted R2=0.090
Cultural sensitivity: F=1.301, p=0.271, R2=0.28, Adjusted R2=0.0076

4. DISCUSSION

It is an inevitable fact that more people with diabetes will need care in the future due to the increase in the incidence rate of diabetes all over the world and Turkey. Nurses need to have cultural competence in order to keep up with the cultural diversity brought by globalization. According to the research findings, it was determined that the majority of diabetes nurses (89.6%) had a cultural competence level above the average. In addition, it was determined that the cultural knowledge, cultural skills, and cultural sensitivity levels of all participants (100%) were above the average. These results are promising and show that diabetes nurses have developed cultural competencies in the care of individuals with diabetes. It is thought that Turkey's becoming a preferred country for health tourism for Asian, European, and Arab countries, the number of tourists receiving health care services of half a million (551,747) and the immigrant population exceeding 6 million may be effective in the development of the cultural competencies of diabetes nurses (4,21).

There is no study in the literature examining the cultural competence levels of diabetes nurses. In the literature, it has been stated that nurses' competence to understand different cultures, recognize and respect different beliefs and cultural differences, and provide care in accordance with the patient's culture and expectations will improve patient care outcomes (22). It is predicted that diabetes nurses have cultural awareness and sensitivity, and the development of cultural knowledge and skill competencies will contribute to the care of individuals with diabetes.

It has been emphasized that the insufficient level of cultural knowledge, skills, and sensitivity of nurses may cause a decrease in the quality of patient care and inequalities in health services (10). However, considering the increasing cultural diversity, it is a fact that it is not possible for nurses to recognize and have knowledge of all existing different ethnic, cultural, religions, and beliefs [19]. For this reason, it is thought that there is a need for evidence-based interventions to improve cultural competence, to develop institutional procedures, and to develop objective strategies that will enable the identification of the cultural values of patients receiving care.

In our study, nurses' educational status, diabetes nursing experience time, receiving the status of cultural educationand professional experience time affected cultural competence level of diabetes nurses. It was found that the cultural competence levels of those who worked as diabetes nurses for 6 years or more were higher than those who had less than 6 years of experience ($p<0.05$). Diabetes is a disease that requires individual approaches according to individual needs and preferences. For effective and effective diabetes care, it is important for diabetes nurses to consider the cultural differences of individuals and to be culturally competent (6). In this direction, during the care and education of individuals with diabetes, all of the nurses participating in our study; it is predicted that they reach cultural competence with their awareness that culture-specific care affects patient compliance and their experience of routinely evaluating the patient's health/illness perception and traditional/ethnic practices. It is thought that as the experience of caring for the person with diabetes increases, the cultural competence levels also increase.

According to the findings of the study, it was determined that the cultural competence levels of the participants with postgraduate education were higher than the nurses with a bachelor's degree ($p<0.05$). It is seen that as the education level of nurses increases, their cultural competence levels also increase. In addition, it was determined that approximately one-third of the nurses (29.5%) had previous education about culture, which increased their cultural competence levels. Similar to our study findings, one-third of the participants (33%) (7) in a study and a small number of participants (15%) [8] in another study did not receive any training on cultural issues, and nurses were more willing to receive training than

physicians. reported (8). In our study, it was determined that the majority of nurses (95.6%) were willing to receive training on cultural care. These results show that nurses have experiences of caring for patients from different cultures, demands for cultural care, and educational needs related to cultural competence.

In the literature, it has been determined that those who have educational experience in improving the cultural competence of nurses have a higher level of cultural competence (10). Studies with nurses and nursing students have shown that the educational initiative increases the level of cultural competence (8,10,17,22-24). Skill-based courses given to health professionals via the internet to improve cultural competence and simulation-based training in the nursing and medical education curriculum are among the educational strategies that are frequently applied (14). It is emphasized that cultural competence training given to nurses will increase their cultural knowledge, skills, awareness, and sensitivity (10,19).

The fact that Turkey has a wide range in terms of cultural diversity causes nurses to meet and care for individuals from different cultures (1,25). It was determined that all of the diabetes nurses included in our study encountered and gave care to individuals from different cultures. Participants stated that they encountered language and communication problems most frequently when caring for individuals with diabetes from different cultures. Studies have reported that the majority of health professionals have difficulties in communicating with individuals from different cultures (7-9,26). It has been stated that this situation may be caused by the lack of a common language with the patients, the lack of written materials in different languages, the lack of a sufficient number of translators, the lack of information about the health-disease perception and living conditions of individuals from different cultures (8). In addition to providing intercultural communication training to health professionals in overcoming communication barriers, cultural competence measures such as the availability of professional translators in clinical settings and the preparation of information brochures translated into different languages are recommended (7-10).

The majority of diabetes nurses (88.5%) stated that they have cultural competence in the care of individuals with diabetes and their families from different cultures. In some studies, it has been reported that nurses and nursing students do not consider themselves competent in providing care to individuals from different cultures and they think that their competencies about different cultures are at a low level (9,22). These results, which differed from our study findings, suggested that this may be due to differences between the study sample population and the patient groups receiving cultural care.

5. CONCLUSIONS

This study shows that diabetes nurses in Turkey have cultural competence in caring for people with diabetes from different cultures and that education and experience affect this situation. Cultural competencies of diabetes nurses will contribute to diabetes management.

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Conflict of interest statement

The authors declared no conflict of interest.

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Clinical and Experimental Health Sciences

The Dietary, Serum and Urine Analysis of Boron and Micronutrients in Postmenopausal Women

Aysegul Gulbahar[1], Gaye Ozgur Cakal[2], Sevim Dincer Cengiz[3], Gamze Sinem Caglar[3]

[1] Izmir Katip Celebi University, Ataturk Teaching and Research Hospital, Department of Obstetrics and Gynecology, Izmir, Türkiye.
[2] Ankara University, Institute of Nuclear Sciences, Ankara, Türkiye.
[3] Ufuk University Hospital, Department of Obstetrics and Gynecology, Ankara, Türkiye.

Correspondence Author: Gaye Ozgur Cakal
E-mail: gcakal@ankara.edu.tr
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ABSTRACT

Objective: Boron is a nutritionally important trace element that interacts with other micronutrients. Boron plays a critical role in bone mineralization and metabolism. In the present study, the association between boron and micronutrients related to bone metabolism was analysed in postmenopausal women.

Methods: In a prospective cohort study in 40 postmenopausal women 24-hour urine and blood samples were collected for sodium, phosphorus, calcium, magnesium, and boron. Daily food consumption, bone mineral density, and Fracture risk assessment tool scores were recorded.

Results: The mean age was 53.2 ± 5.9 years. Dietary habits revealed insufficient dietary fiber and excessive dietary sodium. The serum and urine boron levels were 26.80 µg/L and 21.22 µg/day, respectively. Urine boron levels were lower in the osteoporosis group ($p = 0.66$). A negative correlation between urine Na and boron was detected ($p < 0.001$). Urinary Na and Ca are negatively correlated with Fracture risk assessment tool scores ($p = 0.010$, $p = 0.019$, respectively).

Conclusion: The low urinary boron levels in our participants might be due to increased Na excretion due to excessive consumption of Na. Therefore, consulting postmenopausal women about their dietary habits is of concern. Further understanding of the role of boron in bone metabolism will help to accomplish new treatment strategies for osteoporosis and standardization of boron supplementation.

Keywords: Boron, Mineral Metabolism, Menopause, Bone

1. INTRODUCTION

Boron is a nutritionally important trace element and daily boron intake differs according to personal food consumption. Vegetables and fruits are the primary sources of boron, whereas the highest amount of boron can be found in leafy greens. Absorption of boron can easily happen in the gastrointestinal tract and excretion mostly happens with the urine. Despite high amounts of boron intake, there is no significant change in plasma levels, as plasma boron levels are generally kept steady-state by homeostatic mechanisms.

Accumulating evidence in the literature indicates that boron plays a role in mineral and estrogen metabolism (1). In menopausal women, boron deprivation might be associated with menopausal symptoms and bone health. Therefore, research about the effect of boron supplementation on menopausal women has been of interest (2). In the study of Nielsen and Penland et al., women experiencing discomforts associated with menopause were given boron supplementation (3). The patients in this study did not have symptoms of relief in terms of hot flashes and night sweats, but serum 17b-estradiol concentrations were increased (3). As a result of their study, the authors concluded that boron possibly affects hormone processes in humans at the cell membrane level (3).

The major impact of boron in human metabolism is on bone health. The distribution of boron in bone is greater than the other tissues. The previously documented relationships of boron with micronutrients like vitamin D, calcium (Ca), magnesium (Mg), and phosphorus (P) indicate a strongly possible role of boron on bone health (4,5). Boron, directly or through the mechanism of other minerals and micronutrients can affect the development of bone formation. In animal models, boron has an effect on the enhancement of cell maturation and growth for long bones (4,5). Postmenopausal women who received boron supplementation have higher Ca absorption and lower urinary magnesium excretion (6,7). Considering evidences about the role of boron on bone health gathered from in vivo and vitro studies, this study was designed to explore the boron levels and other

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micronutrients like Mg, Ca, P, and vitamin D, related to bone metabolism in postmenopausal women.

2. METHODS

Our study was prospectively carried out in a university-affiliated hospital. All patients were informed and consents were collected from all participants. Approval was obtained from local ethical committee (200620117/06.2017). Candidates were selected from the women who admitted to gynecology clinic fulfilling the inclusion criteria during the study period.

Inclusion criteria was being in menopause that was defined as at least one year no menstrual bleeding, serum follicle-stimulating hormone (FSH) level >40 mIU/mL, serum estradiol (E2) level <25 pg/mL. All of the participants of the study were over 40 years of age and all were nonsmokers. Weight (kg) / height (m^2) was used to calculate the body mass index (BMI). The biochemical blood analyses of the participants, including serum creatinine levels, were normal. Patients' glomerular filtration rate (GFR) were measured by the modification of diet in renal disease (MDRD) equation (8).

Exclusion criteria were patients diagnosed with hypogonadism, hyperparathyroidism, thyroid dysfunction, chronic liver disease, chronic renal disease, malabsorption, malnutrition, diabetes mellitus, pregnancy, Cushing syndrome or any other diseases which can cause secondary osteoporosis or under osteoporosis treatment or receiving hormone therapy. Furthermore, patients, having any history of antiepileptic, corticosteroid, anticoagulant drug use, alcohol consumption and/or GFR <60 ml/min/1.73 m^2, were excluded from the study.

2.1. Serum markers

Biochemical measurements were taken at least 12 hours overnight fasting. FSH, E2, alkaline phosphatase (ALP), parathyroid hormone (PTH), calcitonin, 25 hydroxyvitamin D3 (25-OH D3), serum electrolyte and mineral levels (Ca, P, Zinc (Zn), Mg, Sodium (Na)) were measured. Having vitamin D level less than 20 ng/ml was defined as deficiency.

2.2. Serum and urine boron analysis

Non-heparinized tubes were used to collect blood samples for boron from the antecubital vein. To estimate the dietary boron intake, 24-hour urine samples after the first urine in the morning were collected. Boron levels were measured using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS, 7500 cx, Agilent Technologies, Inc.). For the boron analyses by ICP-MS, the intra-assay coefficient of the variation was 1.66% and inter-assay coefficient of variation was 3.26%.

2.3. Bone mineral density measurement

Bone mineral density (BMD), expressed in g/cm^2, was measured at the lumbar spine (L1-L4) and femoral neck, the greater trochanter, Ward's triangle. Dual Energy X-ray Absorptiometry (DEXA, GE Healthcare DPX Duo) was used to measure BMD. Femoral neck BMD was measured perpendicular to the femoral midline at the narrowest point of the femoral neck. Spine BMD was found from the mean of four lumbar vertebrae (L1-L4) and measured in the anteroposterior view. The same radiology technician administered all the bone scans to eliminate discrepancies. Definitions of osteoporosis based on BMD measurements provided by The World Health Organization (WHO) were used to define normal, osteopenia and osteoporosis groups (9).

2.4. Fracture risk assessment tool (FRAX)

While evaluating the 10-year probability of hip fracture risk and major fracture risk, fracture risk assessment tool (FRAX®) was used. FRAX integrates age, gender, height, weight, risk factors and BMD of femoral neck in g/cm^2 to calculate the risk as a percent (10,11).

2.5. 24-hour urine analysis

24-hour urine samples were collected. The first urine in the morning was discarded and the urine on that day was collected for 24-hours including the first urine of the following day. Na, P, Ca and Mg levels were measured in 24-hour urine samples.

2.6. Dietary analysis

In order to evaluate food consumption frequency, 54 kinds of food and dietary habits were recorded. The researcher with an interviewer filled in the form. Individual food records were filled in by the patients participating in the study for determining personal food consumption (twice in the weekdays and once in the weekend, 3 days in total). According to Dietary Reference Intake (DRI) dietary fiber and Na levels were categorized as sufficient, insufficient and excessive consumption (12-14).

IBM Statistical Package for the Social Sciences (SPSS) version 11.5 was used for the statistical analysis. The Shapiro-Wilk test was used to evaluate the distribution of continuous variables. Descriptive statistics for continuous variables were presented as mean ± standard deviation or median (minimum-maximum) and for categorical variables were shown as the number of patients and percentage (%).

The significance of the difference based on the averages was assessed by Student's T test if two independent groups were present and one-way analysis of variance (One-Way ANOVA) if more groups were present. Likewise, the significance of the difference based on the median was analyzed by the Mann-Whitney U test when two independent groups were present and the Kruskal-Wallis test when more groups were present. If one-way analysis of variance or Kruskal-Wallis test statistical results were considered important, post hoc Tukey HSD or Conover non-parametric multiple comparison

tests were used to determine the conditions leading to the difference. Nominal variables were evaluated using Pearson's chi-square test. $p < 0.05$ was considered to be statistically significant.

3. RESULTS

The data from forty menopausal women were eligible at the end of the study period. The mean age and menopause age of the patients were 53.2 ± 5.9 and 48.7 ± 3.54 years, respectively. The median value for menopause duration was 3.0 years (ranging from 1 to 19 years). Levels of FSH, E2, ALP, PTH, calcitonin, and 25-OH D3 were 60 (40 – 177) mIU/mL, 15 (5-25) mIU/mL, 77±22 U/L, 47 (26-119) pg/mL, 3.5±0.7 pg/mL, 15(5-78) mg/L, respectively. The serum levels of Ca, P, Zn, Mg, Na were within normal ranges.

Among the participants of this study 70% (n=28) had vitamin D deficiency. No significant difference was observed in serum parameters when the patients with vitamin D deficiency were compared to cases with normal vitamin D results, except PTH. The PTH levels were significantly higher in cases with vitamin D deficiency when compared to cases with normal Vitamin vitamin D levels (61 vs 45 pg/ml, p = 0.043, respectively).

Results of 24-hour urine analysis for Na, P, Ca, and Mg were 107 (23-278) mEq/day, 718 ± 220 mg/day, 139±76 mg/day, 95±27 mg/day, respectively. The urinary Na excretion was significantly higher in cases with vitamin D deficiency than in cases with normal vitamin D levels (114 vs 64 mEq/day, p = 0.004, respectively).

According to DRI the adequacy of diet; all patients have insufficient consumption of dietary fiber (16.27 g/d) and excessive consumption of dietary Na (5120.79 mg/d). The daily consumption of Ca, Mg, P, Na, K, Zn, and Cu were compared between osteoporosis, osteopenia and normal BMD groups. No significant difference was found between the groups regarding the consumption of these micronutrients.

The median value for 24-hours urine boron levels and serum boron were 21.22 µg/day (0.02122 ppm, range: 3.65-56.11 µg/day) and 26.80 µg/L (0.0268 ppm, range: 8.80-66.40 µg/L), respectively. Neither serum boron levels nor urinary boron levels were found to be correlated with any of the serum parameters, including E2 (p = 0.38, p = 0.46, respectively).

Among the 40 patients, 12 (30%) had osteoporosis, 13 (32.5%) had osteopenia and 15 (37.5%) had normal BMD according to WHO criteria. The patients with osteoporosis tend to be older (p = 0.009). The BMI of the groups were similar in osteoporosis, osteopenia and normal BMD groups(p = 0.33). In addition, osteoporosis group's age and duration of menopause were significantly higher when compared with osteopenia and normal BMD groups (p = 0.027 and p = 0.026, respectively) (Table 1).

Table 1. *Comparison of age, menopause age and duration of menopause in the study group.*

	Osteoporosis (n=12) (%30)	Osteopenia (n=13) (%32.5)	Normal BMD (n=15) (%37.5)	p
Age (year) (mean±SD)	57.4±6.9	51.8±2.8	51.0±5.5	0.009*
BMI (kg/m2) (mean±SD)	29.1± 3.8	29.1±5.3	31.5± 5.0	0.33
Menopause age (year) (mean±SD)	50.6±3.8	48.9±2.8	47.0±3.2	0.027*
Duration of menopause (year) (median, min-max)	5.0(3.0-19.0)	3.0(1.0-5.00)	2.0(1.0-8.0)	0.026*

BMD: Bone mineral density, SD:Standart deviation.

**p values <0.05*

When osteoporosis, osteopenia and normal BMD groups were compared, although not significant, osteoporosis group's urine boron levels were lower when compared to osteopenia and normal BMD groups (p = 0.66) (Table 2).

Table 2*. Comparison of serum boron level and urine boron level in the study group.*

(median, min-max)	Osteoporosis (n=12) (%30)	Osteopenia (n=13) (%32.5)	Normal BMD (n=15) (%37.5)	p
Serum boron level (µg/L)	28.15 (14.80-45.30)	27.30 (11.90-66.40)	26.50 (8.80-40.80)	0.66
Urine boron level (µg/L)	16.835 (8.17-56.11)	27.00 (3.66-48.88)	21.66 (4.20-52.20)	0.834

BMD: Bone mineral density.

**p values <0.05*

Based on the results, there were no statistical significance between BMD parameters (Lumbar and Femur T scores) and serum or urine boron levels (p = 0.35, p = 0.60; p = 0.6, p = 0.41, respectively).

According to FRAX scores, 10-year probabilities for major osteoporotic fracture risks of all participants were lower than the defined threshold (20%). Only one case had ten-year probability of hip fracture risk (>3%) in the study group. The correlation analysis of 24-hour urine results of elements with FRAX scores showed that 24-hour urinary excretion of Na was negatively correlated with major osteoporotic fracture risk scores and also with hip fracture risk scores (r = – 0.401, p = 0.010 and r = – 0.0336, p = 0.034; respectively). The 24-hour Ca excretion was negatively associated with FRAX major osteoporotic fracture risk scores (r = – 0.370, p = 0.019).

In the correlation analysis of serum electrolyte and mineral levels, a negative correlation between serum magnesium level, serum boron levels and 24-hour urine boron levels were found statistically significant. Furthermore, a positive correlation between serum zinc level and serum boron levels and 24-hour urine boron levels were determined (Table 3).

Table 3. *The results of the correlation analysis of serum and urine boron with serum electrolytes, minerals and vitamin D.*

	Serum Boron Level		Urine Boron Level	
	r	p	r	p
Calcium	-0.061	0.71	-0.093	0.56
Phosphor	0.076	0.64	0.099	0.54
Zinc	0.534	<0.001*	0.365	0.020*
Magnesium	-0.349	0.027*	-0.353	0.026*
Sodium	0.149	0.36	-0.091	0.58
PTH	-0.117	-0.47	-0.301	0.058
Calcitonin	-0.069	0.67	-0.040	0.81
ALP	-0.017	0.91	0.009	0.95
25-OH-D3	-0.139	0.39	-0.033	0.84

ALP: alkaline phosphatase, PTH: parathyroid hormone, 25-OH-D3: 25-Hydroxyvitamin D3
**p values <0.05*

In the correlation analysis of 24-hour urine electrolytes (Na, Ca, P, Mg), with serum boron and 24-hour urine boron levels, a negative correlation between 24-hour urine Na and 24-hour urine boron level was found ($r = -0.528$, $p < 0.001$). Additionally, a positive correlation between 24-hour urine phosphorus and 24-hour urine boron level were also detected ($r = 0.545$, $p < 0.001$) (Table 4).

Table 4. *The results of the correlation analysis of serum and urine boron with 24-hour urine parameters.*

	Serum Level Boron		Urine Boron Level	
	r	p	r	p
24-hour urine Na (mEq/day)	-0.103	0.53	-0.53	<0.001*
24-hour urine Ca (mg/day)	-0.035	0.83	0.095	0.56
24-hour urine P (mg/day)	0.107	0.51	0.54	<0.001*
24-hour urine Mg (mg/day)	0.119	0.46	0.30	0.061

**p values <0.05*

4. DISCUSSION

The previously reported reference range for median urine boron value was 647 (282-2072) µg/L (15). In our study, the mean urinary boron excretion was much less than this value (21.22 µg daily (range, 3.65-56.11 µg/day)). Our findings revealed that urinary excretion of boron was negatively correlated with Na excretion. The low urinary boron levels in our participants might be due to increased Na excretion related to excessive consumption of Na. Therefore, consulting postmenopausal women about their dietary habits is of concern.

Although the underlying mechanism for skeletal mineralization has not fully been understood yet, it can be stated that an alkaline environment increases bone density and prevents osteoporosis that makes it a prerequisite for bone mineralization (16). Na(+)/H(+) exchanger proteins play an important role in intracellular ion homeostasis in osteoblasts (17). The kidney buffers the acidic medium and the excretion of Na with Na / K – ATPase pump helps to organize the structure of the bone mineral medium and avoid bone resorption by converting acidic environment to alkaline medium (18). In this context, the hypothesis that urinary Na excretion was negatively associated with bone mass and the risk of osteoporosis was tested by Park et al. (19). The authors reported that in premenopausal and postmenopausal women, urinary Na excretion was negatively related to bone mineral content (BMC) and bone mineral density (BMD) (20). In our study, the negative correlation between urinary NA excretion and FRAX scores supported this data. Moreover, the food records of our patients showed excessive consumption of dietary Na. We can conclude that excessive consumption of salt could be a potential risk factor for bone fracture, as suggested by others (20).

In bone mineralization, calcium phosphate crystals secure bone hardness. The gut, bone, and kidney hold important transport mechanisms for P and Ca, which are regulated by PTH and 25-OH D3. P and Ca metabolism are intimately interrelated (21). Moreover, Mg determines the metabolic fate of Ca and its deficiency causes urinary excretion and accumulation of Ca in soft tissues (18,22). Accordingly, clinically neither can be considered in isolation. The interest in this study was boron and there was in vivo and in vitro evidence that boron was interrelated with these mechanisms at different stages. Boron supplementation was associated with increased Ca absorption and lower urinary Mg excretion (6,7). In animal nutrition models, the presence of moderate amounts of vitamin D in boron and Mg containing diets showed that in a magnesium-deficient diet but supplemented with boron, the animals were able to maintain an adequate rate of growth, independent of vitamin D presence (4,5). In chickens, a diet deficient in vitamin D but supplemented with boron shows increased 25-OH D3 levels (6). Our results, with a study group of which 70% suffering from vitamin D deficiency, showed a positive correlation between urinary P excretion and urinary boron excretion. Moreover, serum levels of Mg were positively correlated with serum and urine levels of boron. More information about boron helps better understanding of mechanisms regarding bone metabolism.

The limited number of participants and lacking dietary boron intake were limitations of this study. In the comparison of healthy and osteopenia and osteoporosis groups in terms of serum boron level, with an error rate of 5% the power of our study is 32%. The low number of participants also disabled us to draw strong conclusions. In conclusion, large population-based studies are needed to confirm the relation between micronutrients and boron in postmenopausal women.

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Conflicts of interest

The authors report no conflict of interest.

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Clinical and Experimental Health Sciences

Morphological and Biochemical Investigation of the Healing Effects of Exercise on High Fat Diet Induced Kidney and Bladder Damage

Merve Acikel Elmas[1], Ozlem Bingol Ozakpinar[2], Meltem Kolgazi[3], Goksel Sener[4], Feriha Ercan[5]

[1] Acibadem University, School of Medicine, Department of Histology and Embryology, Istanbul, Türkiye.
[2] Marmara University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Türkiye.
[3] Acibadem University, School of Medicine, Department of Physiology, Istanbul, Türkiye.
[4] Fenerbahce University, Vocational School of Health Services, Istanbul, Türkiye.
[5] Marmara University, School of Medicine, Department of Histology and Embryology, Istanbul, Türkiye.

Correspondence Author: Feriha Ercan
E-mail: fercan@marmara.edu.tr
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ABSTRACT

Objective: The aim of this study was to evaluate the ameliorative effects of swimming training on renal and bladder damage caused by a high-fat diet (HFD) using morphological and biochemical measurements.

Methods: Sprague Dawley rats were fed either standard chow (CONT, 6% fat) or HFD (45% fat) for 18 weeks, these rats were divided into two subgroups at the last 6 weeks of the experiment. The exercise groups (CONT+EXC, HFD+EXC) were trained daily swimming sessions (1 h per day for 5 days/week) during the last 6 weeks. Kidney and bladder samples were prepared for light and electron microscopic examination at the end of experiment. Malondialdehyde, glutathione, interleukin-6, and tumor necrosis factor-α were measured by biochemically.

Results: Regular morphology of the renal cortex and bladder mucosa was observed in the CONT and CONT +EXC groups. Degenerated renal corpuscles and proximal tubules in the kidney and degenerated urothelium with leaky tight junctions and mast cell increase in the bladder mucosa were observed in the HFD group. Ameliorated renal cortex and bladder mucosa were observed in the HFD+EXC group. In addition, malondialdehyde, glutathione, interleukin-6, and tumor necrosis factor-α levels were also consistent with the histological findings.

Conclusion: HFD-induced renal and bladder damage may be related to increased oxidative damage. It was observed that the histological damage and altered oxidative stress parameters could be reversed by swimming training, and it is thought that moderate swimming exercise may play a role in regulating oxidative stress.

Keywords: High fat diet, exercise, kidney, bladder

1. INTRODUCTION

Obesity is one of the major epidemic problems in western countries (1). Diabetes and metabolic syndrome, cardiovascular disease, hypertension, non-alcohol-related liver disease, erectile dysfunction, glomerulonephritis, overactive bladder syndrome as well as obesity are important risk factors that cause the pathogenesis of urogenital tract (2). Oxidative stress is observed with an increase in reactive oxygen species (ROS). Additionally, several studies have shown that ROS is elevated in obese individuals (3). Obesity-induced oxidative stress has an important role in the pathogenesis of urogenital system disorders (4). There is a tendency for sodium to remain in obesity-associated hypertension in both human and animal models. So, this abnormality in sodium homeostasis, lead to the development of hypertension. Natriuretic and anti-natriuretic factors induce these failures. Since the renin angiotensin system (RAS) is the main regulator of sodium and water homeostasis, this can cause abnormalities, especially in the kidneys and other organs of the urogenital system (5). The most well-known abnormality of obesity affecting the kidney is obesity-associated glomerulopathy. It is characterized by glomerulomegaly and may be accompanied by glomerulosis lesions (5, 6). Bladder dysfunctions caused by obesity and/or diabetes (impaired bladder sensation and detrusor contractility) generate significant stress, resulting in increased bladder capacity, limiting normal daily activities, and lowering quality of life. (6). Recent studies have shown that symptoms such as restlessness (62%), incomplete emptying of the bladder (45%) is common in obesity-related urogenital problems. Metabolic syndrome lead to malfunction of bladder in animal models and obese patients because of the chronic inflammation (6). Obesity includes complex problems of metabolic changes

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such as chronic inflammation, and altered lipid metabolism (5). Metabolic alterations in the bladder tissue regarding obesity could be related to urothelial abnormality, and detrusor and autonomous nervous system overactivity (6).

Exercise is a non-pharmacological intervention to prevent or treat the symptoms of obesity-related diseases. Experimental studies have shown that moderate hypertension associated with obesity is reduced by exercise (7). Studies have shown that regular exercise has positive effects on obesity-related health parameters, even without weight loss (8, 9). It is also stated that regular exercise leads to a reduction in adipose tissue, thus reducing the negative effects of obesity on health parameters (9, 10). Swimming exercise was ameliorated morphology of adipose tissue and pancreatic islets of obese animals (11). In addition, several studies have shown strong associations between physical activity levels and circulating leptin and obesity gene expression in adipose tissue (12, 13). Experimental studies have shown that altered lifestyle factors such as exercise affect ROS levels in rats fed a high-fat diet (HFD) (3, 7). Exercise also has a positive effect on RAS by decreasing cardiac angiotensin-converting enzyme in healthy rats (2). Thus, exercise is an effective way to reduce oxidative stress without pharmacological treatment. The aim of this study was to investigate the ameliorative effect of exercise on renal and bladder tissue morphology and oxidative stress markers in HFD-induced obesity using histological, ultrastructural, and biochemical methods.

2. METHODS

2.1. Animals and Experimental Design

Male Sprague Dawley rats (8 weeks old, 200–300 g) were kept in normal plastic cages with free access to food and water. These animals were housed in a room (21 °C) and light–dark cycle (12:12 h). The Animal Care and Ethical Committee for Experimental Animals at Marmara University endorsed the experimental protocols (date: 15.08.2016, protocol code: 089.206.mar).

2.2. Experimental Design

Sprague Dawley male rats (n = 5 in each group) were randomly divided into two main groups according to diet type as standard chow (control: CONT, 6% fat) or HFD (45% fat). After 12 weeks these groups were divided into two subgroups as CONT+EXC and HFD+EXC. During the last 6 weeks of the experiment, these exercise groups maintained their diet for 18 weeks and were trained in daily swimming sessions (60 min/day, 5 days/week). Swimming exercise was done in a plastic tank (60 cm × 150 cm × 45 cm) with warm water at 32 ± 1 °C for 1 hour of 5 days a week. The weight of rats was measured periodically for weekly. At the end of the 18 weeks, ketamine and xylazine were injected intraperitoneally to euthanize the animals. Kidney and bladder samples were processed for histological, ultrastructural and biochemical evaluations.

2.3. Light Microscopic Preparation

Kidney and bladder samples were fixed in 10% neutral buffered formalin solution for light microscopic investigations. These samples were dehydrated with alcohol, cleared with xylene and incubated in paraffin. Paraffin sections were stained with hematoxylin and eosin (H&E) and with periodic acid Schiff (PAS) for histological evaluation. Stained sections were examined under a photomicroscope (Olympus BX51, Tokyo, Japan).

2.4. Transmission Electron Microscopic Preparation

The kidney samples were fixed with 2.5 % glutaraldehyde in phosphate buffered solution (PBS; 0.1 M, pH 7.2), then post-fixed in 1% osmium tetroxide (OsO_4) in PBS (0.1 M, pH 7.2), dehydrated in increasing concentration of ethyl alcohol series and embedded in Epon 812 (Fluka, Sigma–Aldrich Chemical, Steinheim, Switzerland). The urinary bladder samples were fixed in 2.5% glutaraldehyde in PBS (0.13 M and pH 7.4), these tissue samples were en bloc stained with ruthenium red and postfixed with OsO_4 (ratio used 1 part of stock ruthenium red solution: 4 parts of 1% OsO4) for 1 hour. Then dehydrated with alcohol series and embedded in Epon 812 resin (14). Toluidine blue (TB) was used to stain semithin sections, which were then examined under a light microscope. Uranyl acetate and lead citrate were used to contrast ultrathin sections. Ultrathin sections were evaluated under a transmission electron microscope (JEOL 1200 EXII, Tokyo, Japan).

2.5. Measurement of Malondialdehyde and Glutathione Levels

The thiobarbituric acid reactive compounds were determined using a spectrophotometric technique to evaluate tissue malondialdehyde (MDA) levels as an indicator of lipid peroxidation (15). Glutathione (GSH) levels in kidney and bladder samples were determined by the method of Ellman (16). Results of MDA and GSH level were expressed as nmol/g.

2.6. Measurement of Interleukin-6 and Tumor Necrosis Factor – α Levels

Tissue interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels were measured with commercial rat TNF-α and rat IL-6 ELISA kits (Bioassay Technology Laboratory). Results were expressed as pg/mL.

2.7. Statistical Analysis

One-way analysis of variance was used to assess the data, followed by Tukey's multiple comparison tests. Prism 6.0 GraphPad software was used for statistical analysis (San Diego, CA, USA). $P<0.05$ was used as the statistical significance level.

3. RESULTS

3.1. Body Weight of Experimental Animals

The body weight of rats in the HFD (P<0.01) and HFD+EXC (P<0.001) groups was significantly higher than the CONT group in 12 weeks. The body weight of HFD (P<0.01) and HFD+EXC (P<0.01) groups was also significantly higher than the CONT group in 18 weeks (Table 1).

Table 1. *Body weight of experimental groups at the begining (1st week), before the exercise training (12th week) and at the end (18th week) of the study. **P < 0.001 and **P < 0.01 vs CONT group. Values are given as mean ± SEM.*

	0.day	12 Week (day 84)	18 Week (day 126)
CONT	224.5±16.5	291.7±6.35	321.4±4.6
CONT+EXC	231.7±6.9	325.9±14.8	356±21.7
HFD	221.1±11.7	353.8±11.7**	384.2±5.4**
HFD+EXC	245.8±13.8	380.4±14.2***	396.1±13**

3.2. Histopathological Results

Normal morphology of renal corpuscles and tubules was noticed in the kidney samples of the CONT and CONT+EXC groups. Degenerated renal corpuscles with dilatation and cellular debris in Bowman space, mild degeneration of tubule epithelium with decrease in PAS positive staining at the apical surface and the presence of cellular debris in the tubule lumen were noticed in the HFD group. Mild glomerular congestion and degeneration of tubule epithelium with PAS positive staining at the apical surface were observed in the HFD+EXC group (Figure 1).

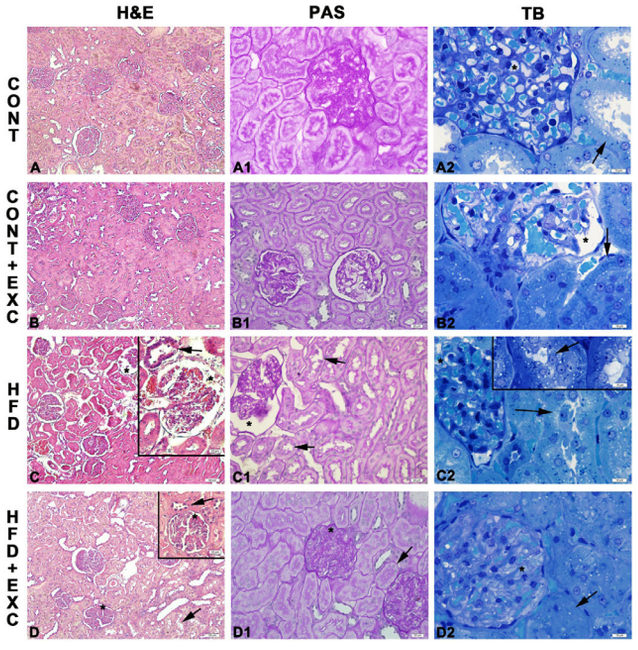

Figure 1. *Representative photomicrographs of kidney samples in the experimental groups. Regular renal corpuscles and tubules are seen in the CONT (A, A2) and CONT+EXC (B, B2) groups. Degenerated renal corpuscles with glomerular congestion and dilated Bowman's space with cellular debris (*), degenerated proximal tubules (arrow) are seen in the HFD group (C, C2). Quite normal morphology in renal corpuscles and decreased degenareted tubules (arrow) are seen in the HFD+EXC group (D, D2). Regular PAS positive stained glomerular (*) and tubular (arrow) basement membranes and apical cytoplasm of proximal tubules are seen in the CONT (A1), CONT+EXC (B1) and HFD+EXC (D1) groups. Degenerated renal corpuscle with dilated Bowman's space (*) and degenerated proximal tubules with decrease of PAS positive staining (arrow) are seen in the HFD group (C1). A-D: H&E staining, A1-D1: PAS staining; A2-D2: TB staining. Scale bars: A-D: 50 µm; insets in C and D and A1-D1: 20 µm; A2-D2: 10 µm.*

Normal bladder morphology with urothelium and a few numbers of mast cells in mucosa were recognized in CONT and CONT+EXC groups. Degeneration in urothelium with decrease of PAS positive staining in apical luminal surface and increase of mast cells in mucosa and migration of mast cells in urothelium were seen in the HFD group. Quite regular urothelium with increase of PAS positive staining in apical luminal surface and a few numbers of granulated mast cells in mucosa were seen in HFD+EXC group (Figure 2).

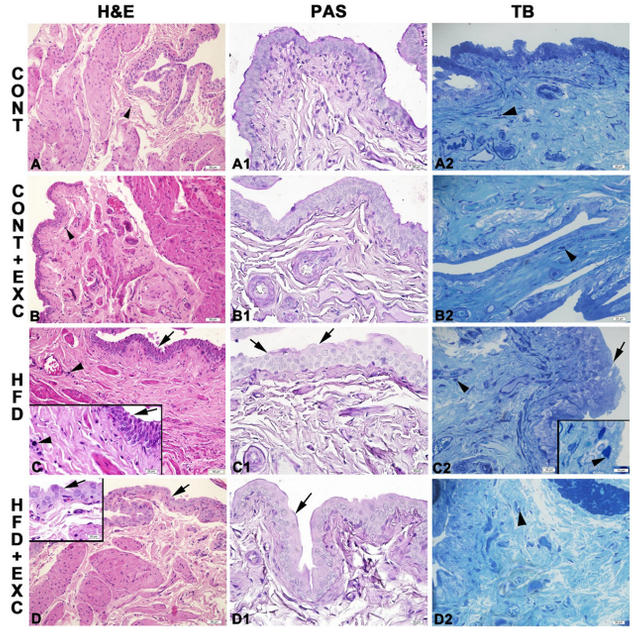

Figure 2. *Representative photomicrographs of bladder samples in the experimental groups. Regular mucosa with urothelium and a few numbers of mast cells (arrowhead) are seen in the CONT (A) and CONT+EXC (B) groups. Degenerated urothelium (arrow) and increase of mast cells (arrowhead) are seen in the HFD (C and C2) group. Quite regular mucosa with urothelium (arrow) is seen in the HFD+EXC (D) group. Regular urothelium with PAS positive stained apical luminal surface (arrow) are seen in the CONT (A1), CONT+EXC (B1) and HFD+EXC (D1) groups. Degenerated urothelium with decrease of PAS positive staining (arrow) in apical luminal surface (arrow) are seen in the HFD group (C1). Regular mucosa with a few number of mast cells (arrowhead) are seen in the CONT (A2), CONT+EXC (B2) and HFD+EXC (D2) groups. Increased mast cells (arrowhead) in mucosa and migrated mast cells (arrowhead) in urothelium (inset) are seen in the HFD group (C2). A-D: H&E staining, A1-D1: PAS staining; A2-D2: TB staining. Scale bars: A-D: 50 µm; insets in C and D, A1-D1 and A2-D2: 20 µm; inset in C2: 10 µm.*

3.3. Ultrastructural Results

Regular proximal tubules with apical microvillus structures and regular pedicels, glomerular capillaries and basement membrane were observed in CONT and CONT+EXC groups. Degenerated proximal tubules with desquamation of microvillus structures, irregular capillary endothelium of glomerulus were seen in the HFD group. Quite regular proximal tubules with microvilli and pedicels, glomerular capillaries and basement membrane were observed in the HFD+EXC group (Figure 3).

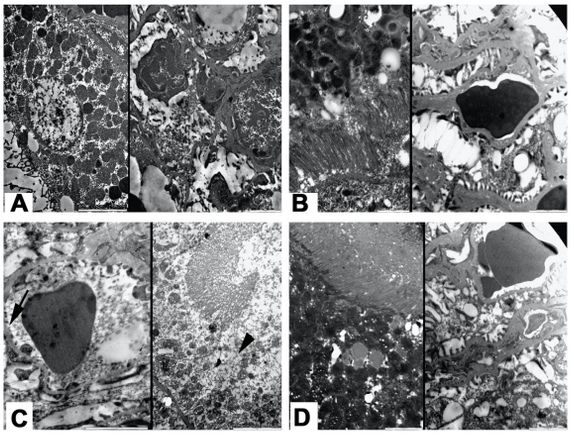

Figure 3. *Representative electronmicrographs of kidney samples in the experimental groups. Regular ultrastructure of proximal tubules, pedicels, glomerular capillaries and basement membrane are seen in the CONT (A), CONT+EXC (B) and HFD+EXC (D) groups. Degeneration in glomerular capillary endothelium (arrow) and degenerated proximal tubule cell with loss of microvilli (arrow head) are seen in the HFD group (D).*

Regular apical urothelial cells with impermeable tight junctions were observed in CONT and CONT+EXC groups. Degenerated apical urothelial cells with penetration of ruthenium red into the intercellular space were observed in the HFD group. Quite regular apical urothelial cells and impermeable tight junctions were observed in the HFD+EXC group (Figure 4).

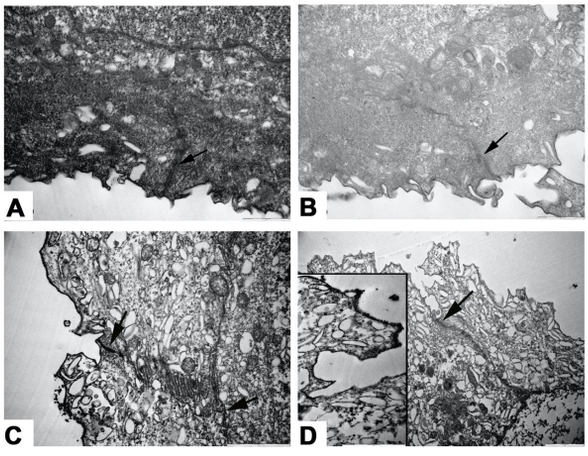

Figure 4. *Representative ruthenium red stained electronmicrographs of urothelium in the experimental groups. Regular ultrastructure of apical urothelial cells with impermeable tight junctions (arrow) are seen in the the CONT (A), CONT+EXC (B) and HFD+EXC (D) groups. Penetration of ruthenium red into the intercellular area (arrow) are seen in the HFD group (C).*

3.4. Biochemical Results

In kidney samples, MDA level was significantly higher (P<0.001) in the HFD group than the CONT and CONT+EXC groups. MDA level was reduced in the HFD+EXC group (P<0.001) compared to the HFD group. GSH level was significantly reduced in the HFD group compared to the CONT (P<0.05) and CONT+EXC (P<0.01) groups. Although statistically not significant, GSH level was increased in the HFD+EXC group compared to the HFD group. The HFD group had higher levels of renal IL-6 and TNF-α than the CONT (P<0.001) and CONT+EXC (P<0.01) groups. When compared to the HFD group, the HFD+EXC group had lower IL-6 (P<0.01) and TNF-α (P<0.001) levels (Figure 5).

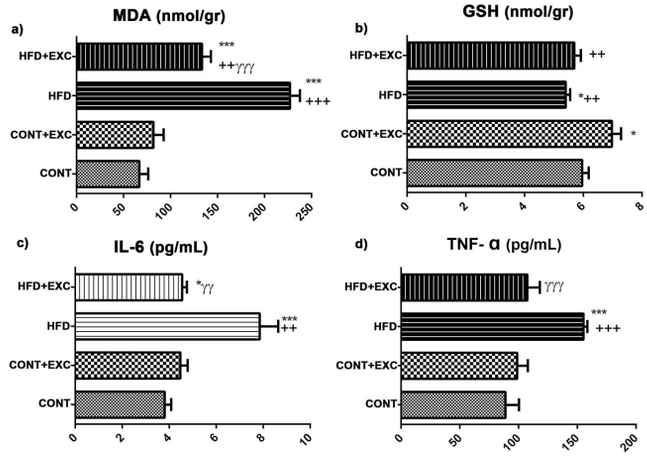

Figure 5. *MDA, GSH, IL-6 and TNF-α levels of kidney samples in the experimental groups. *P<0.05 and ***P<0.001 compared to CONT group; ++P<0.01 and +++P<0.001 compared to CONT+EXC group; γγP<0.01 and γγγP<0.001 compared to HFD group.*

In the bladder samples, MDA level was significantly higher (P<0.001) in the HFD group than the CONT and CONT+EXC groups. The HFD+EXC group had lower MDA levels (P<0.01) than the HFD group. GSH levels in the HFD group were significantly lower than the CONT (P<0.05) and CONT+EXC (P<0.01) groups. However, when compared to the HFD group, this value was higher in the HFD+EXC group (P<0.05). When compared to the CONT and CONT+EXC groups, the HFD group had a higher level of IL-6 (P<0.01). When compared to the HFD group, the HFD+EXC group (P<0.05) had a lower IL-6 level (Figure 6).

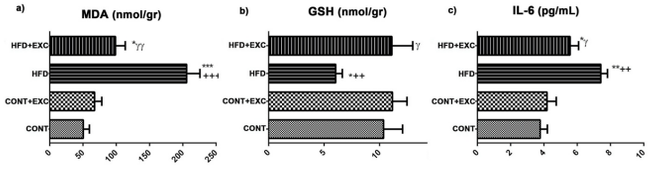

Figure 6. *MDA, GSH and IL-6 levels of bladder samples in the experimental groups. *P<0.05, **P<0.01 and ***P<0.001 compared to CONT group; ++P<0.01 and +++P<0.001 compared to CONT+EXC group; γP<0.05 and γγP<0.01 compared to HFD group.*

4. DISCUSSION

The findings of this study demonstrated that the weight of HFD and HFD+EXC groups higher than the CONT group. Glomerular and tubular morphological damage in kidney, and urothelial damage, increased mast cells in bladder mucosa were found in the HFD group. MDA, IL-6 and TNF-α levels in kidney and MDA and IL-6 levels in bladder were increased and GSH level was decreased in both tissues in the HFD group. However, all these histopathological and oxidative damage parameters were ameliorated in both kidney and bladder tissues in the HFD+EXC group.

Long-term HFD feeding may result in obesity and affect renal lipid metabolism in mice (17, 18). Chronic hyperglycemia and hyperlipidemia regarding obesity effects excess nutrient influx to the kidney (19). Altered lipid metabolism finally causes renal injury, glomerulosclerosis, interstitial fibrosis and albuminuria (17). Sun et al. (2020) reported that HFD induced hyperglycemia and hyperlipidemia and kidney cells imposed upon excess amount of nutrients (19). HFD fed mice showed degeneration in the proximal tubules because of the massive ROS production. Physical capacity, lipid metabolism, and oxidative status substantially improve with exercise (20, 21). Coelho et al (2010) showed that physical activity ameliorates oxidative stress parameters by lowering oxidant production (22). Park et al (2013) showed that regular exercise protects renal injury by reducing oxidative stress (23). Parallel to the previous studies tubular degeneration and altered renal corpuscles morphology were seen in the present study. These tubular and renal corpuscle degenerations might be associated with the increase of oxidative stress parameters in the HFD fed rats. It was observed that HFD induced renal degeneration was ameliorated by the moderate swimming exercise by inhibiting of oxidative stress generation.

Gasbarro et al. (2010) reported that HFD induced bladder dysfunction in the sense of obstruction rather than overactive bladder (24). Bladder fibrosis observed in HFD animals because of the bladder dysfunction (25). ROS are modify protein function by carbonylation in HFD (6). Oberbach et al. (2013) showed that carbonylated proteins were increased in HFD induced urinary bladder degeneration (5). Bladder overactivity is observed in HFD fed rats (26). However, if mice are fed with HFD more than 8 months, they develop voiding dysfunction and reduced urinary tract fibrosis. (27). Inflammation also reported in the fructose diet fed rat bladder (8). Obesity has been linked to increased TNF-α expression in adipose tissue and serum levels (28). But, the relation between the TNF-α and bladder dysfunction has rarely been reported (26). Fan et al. (2014) have been shown that impaired HFD mouse contractility could be ameliorated by TNF-α antogonist treatment (26). Morphological alterations especially for urethelium and the smooth muscle degeneration have been observed in hyperlipidemic rats and fructose fed rats (8, 26). Experimental studies showed that obesity alters blood flow of the bladder and so that causes bladder dysfunction. Exercise increases blood flow of the bladder and so that heals bladder function (29). Exercised animals have increased bladder capacity and larger volumes (7). Additionally, oxidative stress related mast cell degranulation is observed in HFD fed rats (30). Proinflammatory activity also induces mast cell degranulation (31). Parallel with the previous studies, urothelium degeneration, mast cell degranulation in mucosa and increase of MDA and IL-6 level in bladder samples were observed in this study. Moderate swimming exercise improved these morphological and biochemical changes in HFD fed rats by the regulation of oxidative stress generation.

HFD-induced obesity accelerates the production of ROS and oxidative stress. Glutathione S-transferase and MDA levels are important to evaluate lipid peroxidation index and GSH concentration. GSH is important to protect tissues from oxidative damage (3). MDA is an oxidative cell parameter and a product of lipid peroxidation in the cell membrane (32, 33). Increased blood plasma MDA and decreased GSH levels were observed in HFD induced obese rats (3). Moreover, exercise has been reduced MDA level and increased GSH level in heart and aorta tissues in HFD induced obese rats (34). Exercise might be effected for preventing obesity-induced oxidative stress in HFD fed rats by increasing antioxidant enzyme activity and decreasing lipid peroxidation (3). Pro-inflammatory cytokine IL-6 level in kidney was increased in the HFD fed rats (35). This increase in kidney samples might be regarding harmful effects of chronic inflammation and this effect may elevate the lipotoxicity development (35). It was shown that association exists between increased renal lipid accumulation and proinflammatory mediators IL-6 and TNF-α (36). Renal lipotoxicity is also related with glomerulonephritis and proteinuria development (37). TNF-α is also higher in bladder tissue of hyperlipidemic rats. In the present study, it has been observed that IL-6 and TNF – α levels were increased in HFD fed rats comparing to the standard chow fed rats. These parameters were decreased in moderate swimming exercise treated HFD fed rats.

5. CONCLUSION

Our findings showed increase of oxidative and morphological damage in both kidney and bladder in rats fed a high-fat diet. It was observed that the histological damage and altered oxidative stress parameters improved with swimming exercise. Moderate swimming exercise may play a role in the regulation of oxidant/antioxidant balance in obesity induced kidney and bladder damage.

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Clinical and Experimental Health Sciences

Evaluation of D-Dimer and Neutrophil/Lymphocyte Ratios of COVID-19 Patients Whom Applied to Karapınar State Hospital

Saadet Kader[1], Turan Akdag[2], Levent Sariyildiz[3], Zeynep Ozel[4]

[1] Karapinar State Hospital, Biochemistry Laboratory, Konya, Türkiye.
[2] Necmettin Erbakan University, Meram Vocational School, Konya, Türkiye.
[3] Beyhekim Education and Research Hospital, Konya, Türkiye.
[4] Selcuk University, Selcuk University Faculty of Veterinary, Medicine, Department of Biostatistics, Konya, Türkiye.

Correspondence Author: Saadet Kader
E-mail: saadetkader@hotmail.com
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ABSTRACT

Objective: The epidemic which caused by the SARS-CoV-2 virus were defined as COVID-19) and declared as a global pandemic by the World Health Organization (WHO) on March, 2020. Nowadays, many biochemical parameters related to the diagnosis and prognosis of COVID-19 are being investigated. Therefore, we aimed to evaluate D-dimer and neutrophil/lymphocyte ratios (NLR) of COVID-19 patients whom applied to Karapınar State Hospital.

Methods: Patients which consisted of 2523, whom diagnosed with COVID-19 between 11 March 2019 and 29 July 2021 at Karapınar State Hospital were included in the study. Age, gender and social history of the patients were recorded. From the results, the relationships between D-dimer and hemogram were evaluated.

Results: There was a high correlation between the variables HCT and HGB, PLT and PCT, NEUT# and WBC, and MCH and MCV (r=0.981, r=0.944, r=0.923, r=0.925). In addition, there was a high correlation between RBC and HCT and between RBC and HGB variables (r=0.852, r=0.795). There was a moderate correlation between WBC and MO#, MCHC and MCH, PDW and MPV (r=0.562, r=0.639, r=0.64). All the relationships between these variables were positive, and the value of the correlated parameter increases linearly by unit. Also, the highest positive relationships were between HCT and HGB, PLT and PCT, NEUT# and WBC, MCH and MCV. Moreover, D-dimer and NLR were not correlated (r= – 0.015, p=0.49).

Conclusion: In the study, no correlation was observed between D-dimer and neutrophil/lymphocyte ratios of COVID-19 patients. So, more comprehensive and further studies are needed to clarify these results.

Keywords: COVID-19, D-dimer, neutrophil/lymphocyte ratio

1. INTRODUCTION

On March 2020, the epidemic caused by the SARS-CoV-2 virus was declared as a global pandemic by the World Health Organization (WHO) (1). The virus was identified on January, 2020 as a new coronavirus that has not been detected in humans before. It was named as SARS-CoV-2 because of it's showing similarity to the causative SARS-CoV virus. SARS-CoV-2 virus is also thought to be transmitted from animals to humans by a zoonotic infection like SARS-CoV and MERS-CoV (2).

Compared to the non-COVID-19 infections, several studies reported significantly lower WBC or neutrophil counts in the early stages of the disease, although the mean WBC count does not exceed the lower limit to classify them as leukopenia or neutropenia (3). As conversely, leukocyte and neutrophil counts were found to be higher in severe groups with progression of COVID-19 disease, consistent with retrospective studies and other meta-analyses (4). These increase is a result of the susceptibility of secondary bacterial infections in severe COVID-19 (5). Lymphocytopenia is also observed in patients with positive COVID-19. The frequency of lymphopenia suggests that COVID-19 may have an effect on lymphocytes, especially T lymphocytes, including depletion of $CD4^+$ and $CD8^+$ cells, as did SARS-CoV (6, 7).

D-dimer composed of two D fragments of the fibrin, and formed by the activation of the plasmin enzyme. D-dimer display the activation of fibrinolysis and coagulation systems. Elevated D-dimer levels may be observed in physiological and pathological conditions such as surgery, cancer and inflammation (8). The increase in D-dimer value of infected patients is more pronounced in critically ill patients (9). Higher D-dimer levels were observed in non-survivors with COVID-19 compared to the survivors at hospital admission. Therefore, D-dimer monitoring will be helpful for patient triage and management. Several studies suggest that D-dimer

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levels greater than 1 µg/L may be implicated as a marker for severe COVID-19 and mortality (10).

The diagnosis of COVID-19 should be based on clinical and epidemiological history, etiological diagnostic tests that support the diagnosis of infection and/or complications. There are few studies showing the relationship between D-dimer and neutrophil/lymphocyte ratios (NLR) and COVID-19. Hematological and biochemical parameters are very important in the diagnosis, prognosis and clinical assessments of COVID-19. So, further and comprehensive studies are needed to clarify this topic. In this study, we aimed to evaluate the relation between D-dimer and NLR in patients with COVID-19.

2. METHODS

In this single centered retrospective study, a total of 2523 consecutive patients with COVID-19 (between 18-65 year) who were admitted to Karapınar State Hospital between March 2019 and July 2021 were enrolled in the study. The clinical characteristics and laboratory results were obtained. The measurement of D-dimer was performed with Mindray BS-800 (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) clinical chemistry analyzer. D-Dimer concentration was determined by particle-enhanced immunoturbidimetric assay method. Hemogram parameters (neutrophil, lymphocyte, HGB, HCT etc.) were performed with Mindray BC-6800 (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) heamology analyzer which analyzes complete blood count based on laser light scattering (forward and light scatter) and side fluorescent light. The approval of the research was obtained from the ethics committee of Necmettin Erbakan University Medicine Faculty (The date, 03.09.2021; the number, 2021/3375), and all the participants signed their consents before the study.

2.1. Statistical Analysis

To evaluate the data, SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program was used. JASP statistical package program was used to obtain the heatmap graph. In the study, descriptive statistics (mean, standard deviation, median value, minimum, maximum, number and percentile) were given for categorical and continuous variables. The relationship between two continuous variables was evaluated with the Pearson Correlation coefficient, and if the parametric test prerequisites were not linear, the Spearman correlation coefficient was used for evaluation. Less than 0.05 and 0.01 p values were considered as statistically significant.

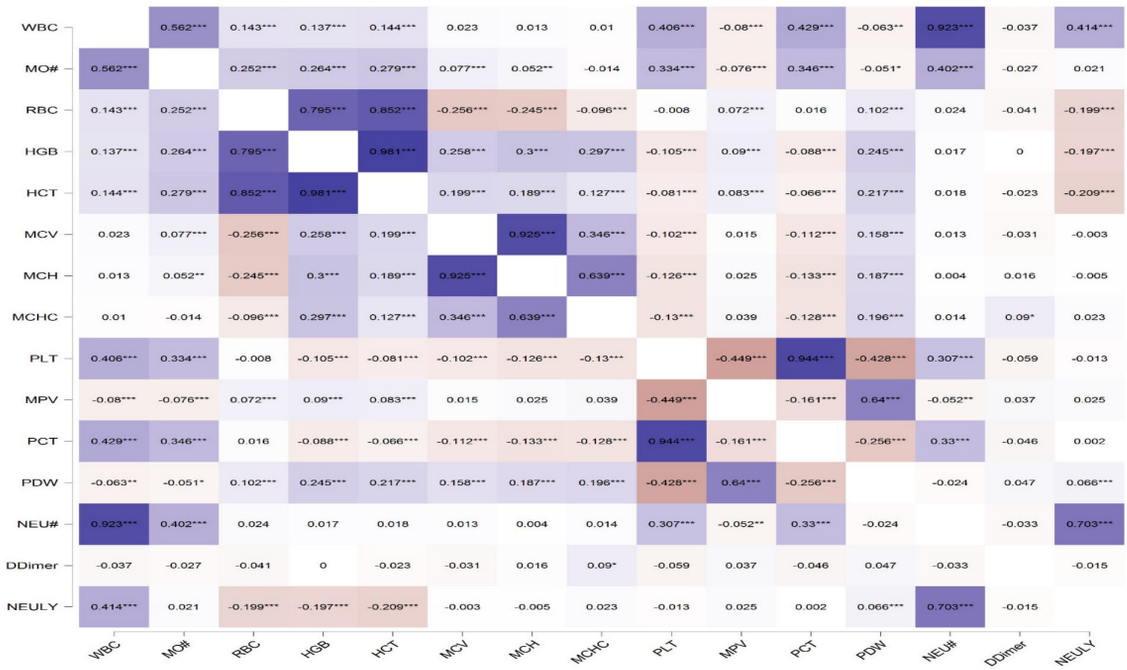

Figure 1. *Heatmap Graph (The heatmap graph shows correlations as symmetrically).*

Table 1. *Descriptive statistics of D-dimer and hemogram parameters in COVID-19 patients.*

Parameter	n	Minimum	Maximum	Mean	Std. Deviation
WBC, ($x10^9$/L)	2523	0.400	32.590	6.8819	2.9966
LYMPH#, ($x10^9$/L)	2523	0.000	7.150	1.5144	0.7543
LYMPH%, (CV%)	2523	0.000	63.500	24.0327	11.1627
MONO#, ($x10^9$/L)	2523	0.000	2.100	0.4523	0.2241
MONO%, (CV%)	2523	0.000	30.000	6.8864	2.8140
RBC, ($x10^{12}$/L)	2523	0.000	8.120	4.7111	0.6460
HGB, (g/dL)	2523	0.000	19.400	13.2141	1.8761
HCT, (%)	2523	0.000	65.800	39.7547	5.3874
MCV, (fL)	2523	0.000	124.900	84.5555	6.3561
MCH, (pg)	2523	0.000	43.900	28.1160	2.5354
MCHC, (g/dL)	2523	0.000	35.800	33.1893	1.3398
PLT, (10^3/µL)	2522	0.000	976.000	243.3727	101.0509
MPV, (fL)	2523	0.000	14.000	9.48962	1.1957
PCT, (%)	2523	0.000	0.830	0.2259	0.0837
PDW, (%)	2523	0.000	19.200	16.1481	0.6130
NEUT#, ($x10^9$/L)	2523	0.000	29.280	4.8242	2.8021
NEUT%, (CV%)	2523	0.000	96.900	67.6218	12.9114
EOS#, ($x10^9$/L)	2523	0.000	0.970	0.0757	0.1031
EOS%, (CV%)	2523	0.000	12.100	1.1440	1.4152
NLR	2521	0.298	69.484	4.2305	4.3313
D-dimer, (µg/mL)	627	100	10000	1236.664	1424.3491

WBC: White blood cell, LY#: Lymphocyte, LY%: Lymphocyte percentage, MONO#: Monocyte, MONO%: Monocyte percentage, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, NEUT#: Neutrophil, NEUT%:Neutrophil percentage,EOS#: Eosinophil, EOS%: Eosinophil percentage, NLR: Neutrophil/lymphocyte ratio

3. RESULTS

All inter-variable relations were evaluated with Spearman correlation coefficient. As shown in Table 1 and 2, there was a high correlation between the variables HCT and HGB, PLT and PCT, NEUT# and WBC, and MCH and MCV (r=0.981, r=0.944, r=0.923, r=0.925). In addition, there was a high correlation between RBC and HCT and between RBC and HGB variables (r=0.852, r=0.795). There was a moderate correlation between WBC and MO#, MCHC and MCH, PDW and MPV (r=0.562, r=0.639, r=0.64). All the relationships between these variables were positive, and the value of the correlated parameter increases linearly by unit as increases. According to Figure 1, the highest positive relationships were observed between HCT and HGB, PLT and PCT, NEUT# and WBC, MCH and MCV. No correlation was observed between D-dimer and NLR in patients with COVID-19 (r= – 0.015, p=0.49).

Table 2. *Relationships between D-dimer and hemogram parameters.*

		WBC	MO#	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	MPV	PCT	PDW	NEUT#	D-dimer
	r	.562**													
MONO#	p	0,001													
	N	2523													
	r	.143**	.252**												
RBC	p	0.001	0.001												
	N	2523	2523												
	r	.137**	.264**	.795**											
HGB	p	0.001	0.001	0.001											
	N	2523	2523	2523											
	r	.144**	.279**	.852**	.981**										
HCT	p	0.001	0.001	0.001	0.001										
	N	2523	2523	2523	2523										
	r	0.023	.077**	-.256**	.258**	.199**									
MCV	p	0.24	0.001	0.001	0.001	0.001									
	N	2523	2523	2523	2523	2523									
	r	0.013	.052**	-.245**	.300**	.189**	.925**								
MCH	p	0.508	0.009	0.001	0.001	0.001	0.001								
	N	2523	2523	2523	2523	2523	2523								
	r	0.01	-0.014	-.096**	.297**	.127**	.346**	.639**							
MCHC	p	0.623	0.489	0.001	0.001	0.001	0.001	0.001							
	N	2523	2523	2523	2523	2523	2523	2523							
	r	.406**	.334**	-0.008	-.105**	-.081**	-.102**	-.126**	-.130**						
PLT	p	0.001	0.001	0.685	0.001	0.001	0.001	0.001	0.001						
	N	2522	2522	2522	2522	2522	2522	2522	2522						
	r	-.080**	-.076**	.072**	.090**	.083**	0.015	0.025	0.039	-.449**					
MPV	p	0.001	0.001	0.001	0.001	0.001	0.446	0.205	0.05	0.001					
	N	2523	2523	2523	2523	2523	2523	2523	2523	2522					
	r	.429**	.346**	0.016	-.088**	-.066**	-.112**	-.133**	-.128**	.944**	-.161**				
PCT	p	0.001	0.001	0.432	0.001	0.001	0.001	0.001	0.001	0.001	0.001				
	N	2523	2523	2523	2523	2523	2523	2523	2523	2522	2523				
	r	-.063**	-.051*	.102**	.245**	.217**	.158**	.187**	.196**	-.428**	.640**	-.256**			
PDW	p	0.002	0.011	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001			
	N	2523	2523	2523	2523	2523	2523	2523	2523	2522	2523	2523			
	r	.923**	.402**	0.024	0.017	0.018	0.013	0.004	0.014	.307**	-.052**	.330**	-0.024		
NEUT#	p	0.001	0.001	0.225	0.381	0.357	0.501	0.826	0.494	0.001	0.009	0.001	0.229		
	N	2523	2523	2523	2523	2523	2523	2523	2523	2522	2523	2523	2523		
	r	-0.037	-0.027	-0.041	0	-0.023	-0.031	0.016	0.09*	-0.059	0.037	-0.046	0.047	-0.033	
D-dimer	p	0.355	0.5	0.312	0.999	0.564	0.436	0.698	0.02	0.144	0.366	0.244	0.248	0.41	
	N	627	627	627	627	627	627	627	627	627	627	627	627	627	
	r	0.041***	0.021	-0.199***	-0.197***	-0.209***	-0.003	-0.005	0.023	-0.013	0.025	0.002	0.066***	0.703***	-0.015
NLR	p	0.001	0.28	0.001	0.001	0.001	0.87	0.812	0.245	0.52	0.03	0.93	0.001	0.001	0.719
	N	2521	2521	2521	2521	2521	2521	2521	2521	2520	2521	2521	2521	627	627

*WBC: White blood cell, LY#: Lymphocyte, LY%: Lymphocyte percentage, MONO#: Monocyte, MONO%: Monocyte percentage, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, NEUT#: Neutrophil, NEUT%: Neutrophil percentage, EOS#: Eosinophil, EOS%: Eosinophil percentage, NLR: Neutrophil/lymphocyte ratio, *p<0.05, ** p<0.01,*** p<0.001*

4. DISCUSSION

From the beginning of the COVID-19 epidemic, the countries surrounding China became the epicenter of the epidemic, and then spread to the whole world that causes the death of millions of people. Scientific studies are very important in terms of struggle with COVID-19 disease (11). Therefore, clinical studies are mainly focus on COVID-19. In these clinical studies, determining the relationship of hemogram

and D-dimer may provide valuable information about the disease, and contribute to the diagnosis and prognosis (12).

The fibrinolytic system breakdown the fibrin system after the clot formation. The D-dimer, which contains the two D fragments of fibrin, is formed by the activation of the plasmin enzyme. This indicates the presence of a broken down fibrin in the bloodstream. Moreover, D-dimer represents activation of coagulation and fibrinolysis systems (13). As common, the D-dimer test is used in clinical practice to exclude the diagnosis of deep vein thrombosis (DVT), pulmonary embolism (PE) and to confirm the diagnosis of disseminated intravascular coagulation (DIC) (14, 15). De Monyé et al. declared that D-dimer levels are elevated in almost all patients with severe DVT. Also, D-dimer elevation may be observed in pathological and physiological conditions (16).

Many studies have been conducted examining the relationship between D-dimer levels and the severity of COVID-19 disease. In a case-control study, Spiezia et al. reported that forty-four COVID-19 patients had increased D-dimer levels with compared the control subjects (17). A recent study declared that D-dimer levels of non-intensive care unit and intensive care unit COVID-19 patients were determined as 1299 and 11.870 µg/L, respectively (18). In our study, D-dimer levels were determined as 1236.664 µg/mL in 627 COVID-19 patients. After hospitalization, elevated D-dimer levels of COVID-19 patients show persistence of multiorgan failure and a precursor to the development of disseminated intravascular coagulation (19). Moreover, a new study demonstrated that total antioxidant status and calculated oxidative stress index levels were correlated with CRP, fibrinogen and D-dimer levels (20). From the studies, it is stated that COVID-19 patients with D-dimer >1000 ng/ml have a 20 times higher risk of mortality which compared to those with lower D-dimer values (21). The D-dimer levels of COVID-19 patients was determined as 1236.664 µg/mL in our study (Table 1). In another study, D-dimer and CRP levels were found to be higher in those with pneumonia than in those without pneumonia (p=0.001 and p=0.001, respectively) (22).

Neutrophils are one of the vital immune cells of the human body. When pathogenic microorganisms invade the body, immune cells tend to rapidly provide chemotactic activation to the site of infection and play a role in host defense and immune regulation (23). Several studies were declared that neutrophils play an significant role in the pathophysiology of COVID-19, especially in the severity of disease. As similar with our study (4.8242x10^9/L), some reports highlight increased neutrophil levels in COVID-19 patients (3.4x10^9/L) (24, 25).

Lymphocytes are the main effector cells of the human immune response. The number of lymphocytes in the body is closely related to the body's immune and defense system against pathogenic microorganisms and negatively correlates with the degree of inflammation (26). As similar with the presented study (1.5144 x10^9/L), a study reported that lymphocyte levels were decreased in COVID-19 patients (1.3 x10^9/L) (27). Also, NLR were determined as 2.4 in patients with COVID-19, as similar with our study 4.2. In the study, we determined that there was no correlation between D-dimer and neutrophil/lymphocyte ratios of COVID-19 patients (r=-0.015, p=0.719 (Table 2). On the basis of our analysis, further studies with multi-centers are necessary to clarify these results.

5. CONCLUSION

Nowadays, D dimer levels and neutrophil/lymphocyte ratios are used for the evaluation of COVID-19 patients. As far as we know, the relation of D-dimer and neutrophil/lymphocyte ratios of COVID-19 patients were not evaluated in studies, yet. From the study, there was no relation between D-dimer and neutrophil/lymphocyte ratios of COVID-19 patients. In conclusion, more sample and prospective studies are needed to elucidate the relationship between D dimer and NLR in COVID-19 disease for clinical evaluations. The presence of comorbidity of the COVID-19 patients was not questioned. Also, patients with COVID-19 were aged between 18-65 years. As a retrospective study, our study has a limitation to obtain the all findings of the COVID-19 patients.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

All authors declare no financial support.

Ethical approval

The study approval was obtained from Necmettin Erbakan University Meram Faculty of Medicine, Non-interventional Clinical Trials Ethics Committee (approval number: 2021/3375)

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Clinical and Experimental Health Sciences

Synthesis of Nanoparticles Loading Indenopyrazole Derivatives and Evaluation of Biological Features

Murat Dogan[1], Umit Muhammet Kocyigit[2], Hayreddin Gezegen[3]

[1] Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Sivas, Türkiye.
[2] Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Türkiye.
[3] Sivas Cumhuriyet University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Sivas, Türkiye.

Correspondence Author: Murat Dogan
E-mail: mdogan@cumhuriyet.edu.tr
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ABSTRACT

Objective: In this study, it was aimed to prepare nanoparticle formulations using chitosan, a cationic natural polymer, and tripoly phosphate, and to perform mechanical characterization and in vitro cell culture studies. In addition, the cytotoxic effects of nanoparticles containing indenopyrazol derivatives against human glioma cells (C6) and human cervical cancer cells (HeLa) were investigated.

Methods: Within the scope of the study, nanoparticles containing indenopyrazole derivative were prepared and characterization of particle size, zeta potential and morphological properties were performed. XTT cytotoxicity test was applied to evaluate the antiproliferative activities of nanoparticles containing these components.

Results: Particle size, zeta potential and morphological properties of nanoparticles were observed to be suitable for application. In vitro cell culture studies showed that nanoparticles containing indenopyrazol derivatives showed better cytotoxic effects in both cell lines.

Conclusion: The results showed that the mechanical properties of nanoparticles containing indenopyrazol derivatives are suitable and can be applied in anticancer activity studies.

Keywords: Chitosan, Indenopyrazole derivatives, Nanoparticle, Antiproliferative activity.

1. INTRODUCTION

Molecules containing indenopyrazole derivatives are compounds that show important bioactive and pharmacological activities in most studies that are widely studied in central nervous system disorders such as Alzheimer's, epilepsy and various cancer types (1). Cancer is a complex disease that can occur due to genetic and environmental factors, and its treatment can be difficult. Methods such as chemotherapy, radiation therapy, antiangiogenic drugs and immunotherapy are widely used in cancer treatment (2,3). In addition to the advantages of these methods, the problems experienced in patient compliance, the emergence of side and toxic effects reduce the treatment effectiveness of the drug (4). The undesirable effects of current treatment methods and drugs trigger the synthesis of new compounds for cancer treatment. Indenopyrazole derivatives also have effects in the treatment of cancer (5, 6). Chemically synthesized molecules can cause toxicity in different tissues or cells with the desired effect. For this reason, drug delivery systems have become a very current and important issue (7, 8). By using biocompatible polymers, targeted nanoparticular systems are synthesized and their bioactivities are investigated. In this way, the bioactivity of the synthesized chemical molecules is increased and any side effects are prevented in the application (9, 10). Chitosan, cellulose, poly vinyl alcohol, sodium alginate and many other polymers are used in the preparation of nanoparticular carrier systems (5, 11). Being biocompatible and biodegradable, showing superior release properties, polycationic structure and easy processing properties, chitosan has made it widely used in the pharmaceutical industry (12, 13). In this study, nanoparticles were prepared by ionic gelation method using chitosan and tri poly phosphate polymers, and their anticancer activities were evaluated by in vitro cell culture studies after mechanical characterization studies.

2. METHODS

2.1. Material

HeLa (ATCC CCL-2) human cervical cancer cells, and C6 (ATCC CCL-107) human glioma cells were obtained from the American Type Culture Collection (ATCC). Dulbecco's

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modified Eagle's medium (DMEM) (ATCC, Manassa, USA), fetal bovine serum (FBS) and phosphate buffer saline (PBS) were obtained from PAA Ltd. (France). L-glutamine-penicillin – streptomycin solution was purchased from Sigma-Aldrich (Steinheim am Albuch, Germany). Chitosan (medium molecular weight, 400 kDa, DD 87) were purchased from Fluka (Germany). XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Roche Diagnostic.

2.2. Determination of Indenopyrazole Derivatives

In this study, indenopyrazole derivatives, whose nanoparticles were prepared and characterization studies were performed, were synthesized by Gezegen et al. within the scope of TUBITAK project. Nanoparticle formulations were prepared by choosing the ones with the best bioactivity results among the synthesized derivatives, mechanical characterization studies and in vitro cell culture studies were carried out. In this study, our main aim is to evaluate the increased anticancer activities of indenopyrazole derivatives embedded in a nanoparticular carrier system.

2.3. Cell Culture Studies

Cell culture studies were performed by modifying the study of Taskin *et al*., 2020 (14). Cytotoxicity of indenopyrazole derivatives and nanoparticles containing these derivatives was measured by the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) cell viability assay, using HeLa and C6 cell lines. Complete mediums of HeLa and C6 cell line are the same properties. Cell lines were cultured in DMEM with 1 % L-glutamine, 10 % FBS, 100 IU/mL penicillin and 10 µg/mL streptomycin in 25 cm^2 polystyrene flasks. The flasks including cells were kept at 37°C within 5 % CO_2 humidified atmosphere and were passaged when they reached around 90 % confluence. Cells were seeded at $10x10^3$ cells/well in 96-well plates with 100 µL DMEM containing 10 % FBS, and incubated overnight. Dimethyl sulfoxide (DMSO) was used as a solvent in indenopyrazole derivatives. The compounds (2a-d) and nanoparticles including compounds were suspended with DMEM with concentration of 500 µg/ml and samples were put in the 96-well plates at determined concentrations (6, 12, 25, 50, and 100 µg/ml). In addition, the same amount of DMSO was inserted in the positive control group. The cells were incubated for 24 h. Then, the medium was removed and wells were washed with 200 µL phosphate-buffered saline (PBS). Following these periods, for determination of living cells, 100 µL of transparent (colorless) DMEM and 50 µL of XTT labelling solution were added to each well and the plates were incubated for 4 h. The absorbance values of XTT-formazan were measured using microplate (ELISA) reader at 450 nm against the control, as untreated cells. According to the results IC_{50} values of derivatives and nanoparticles with derivatives were calculated. All experiments were performed three times and 5-fluorouracil (FU) were used as standard anticancer drug.

2.4. Preparation of Nanoparticles Containing Indenopyrazole Derivatives

The indenopyrazole derivatives loaded chitosan-tripolyphosphate (TPP) nanoparticles were prepared by ionic gelation method as described (15). Briefly, chitosan solution at a concentration (0.40 % w/v) were prepared using glacial acetic acid (% 0.10 V/V) as a solvent. The chitosan dissolution process were performed via a magnetic stirrer. tripolyphosphate solution (0.40 % w/v) including compounds dropped into chitosan solution (0.40 % w/v) under predetermined stirring condition. After two hours of stirring, nanoparticles were separated by centrifugation at 10.000xg for 30 minutes. The supernatant was discarded and particles were washed with bidistilled water. This process was repeated three times. Nanoparticles were stored at +4 ºC after freeze-drying. Since the unique release properties of chitosan-TPP nanoparticles will facilitate the release of indenopyrazol derivatives, it will cause these derivatives with anticancer activity to show activity. Therefore, it was decided to prepare chitosan-TPP nanoparticles

2.5. Characterization of Nanoparticles

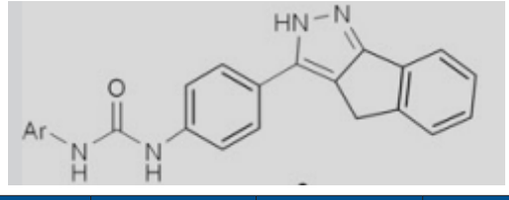
Particle size and zeta potential: Measurements of size and zeta potential of nanoparticles were performed by Zetasizer (NanoZS ZEN3500, Malvern Instrumentals Ltd., UK). The samples were suspended in phosphate buffer saline (PBS, pH 7.4) and each measurement was performed in triplicate.

Scanning electron microscope (SEM): Sample of chitosan nanoparticle was placed on metal grids with double-sided adhesive tape, coated with a gold layer using SCD 005 Sputter coater (Baltec, Liechtenstein) under 0.1 torr at room temperature. The surface morphology of nanoparticles was investigated by scanning electron microscopy (SEM; Carl Zeis-Evo 40, Germany) (16).

3. RESULTS

Indenopyrazole derivative general structure and pre-synthesized indenopyrazole derivatives were shown in Table 1.

Table 1. *Indenopyrazole derivative general structure and synthesized 2a, 2b, 2c, 2d derivatives.*



Product	Ar	Yield [%][a]	MP, °C
2a	Ph	96	355-357
2b	4-MeOPh	98	358-360
2c	4-ClPh	98	355-358
2d	3-CF3- 4-ClPh	85	357-359

Abbreviation: MP, melting point. aYield of isolated product.

3.1. Characterization of nanoparticles loading with compounds

Particle size, zeta potential and polydispersity index of nanoparticles are very important for nanoparticle entry into the cell, escape from the reticuloendothelial system and bioactivity. Therefore, these values should be in the desired ranges. The particle size and zeta potential values of the nanoparticle are in the desired range, which is very important for cell culture studies and therefore for nanoparticles to show antiproliferative activity. Results showed that zeta potential of nanoparticles ranged between 3,26± 0,05 mV and 3,78 ± 0,04 mV. The particle size of the nanoparticles between 452,32 ± 2,20 nm and 486,32 ± 2,90 nm (Table 2). Polydispersity index values of nanoparticles ranged between 0,216 ± 0,03 and 0,287 ± 0,04. The high particle size makes it difficult for the nanoparticle to pass into the cell. For this reason, nanoparticle loaded with 2c indenopyrazole derivative containing 4-chloro phenyl group have the most suitable particle size (452,32 ± 2,20 nm) for use in cell culture studies. Polydispersity index is an indicator of the homogeneity of particle sizes of nanoparticles and the difference between sizes. This value being lower than 0.4 provides an advantage in terms of application. According to the results, it was observed that the 2b indenopyrazole derivative loaded nanoparticle containing the 4-methoxy phenyl aromatic ring had the most appropriate polydispersity index value (0,216 ± 0,03). Results indicated that the nanoparticles were homogeneously dispersed in the phosphate buffer saline (PBS, pH 7.4) without forming aggregates.

Table 2. *Particle size, ζ potential, and PDI index values of nanoparticles.*

Samples	**ζ potential (mV) ± SD	Size (nm) ± SD	PDI ± SD
*NP2a	3,26 ± 0,05	486,32 ± 2,90	0,227 ± 0,05
NP2b	3,78 ± 0,04	467,56 ± 3,60	0,216 ± 0,03
NP2c	3,74 ± 0,02	452,32 ± 2,20	0,232 ± 0,02
NP2d	3,38 ± 0,04	458,44 ± 2,74	0,287 ± 0,04

**NP indicates nanoparticle, **ζ indicates zeta. SD indicates standart deviation.*

3.2. SEM image result of nanoparticles

The morphological properties of the nanoparticles were shown in Figure 1. The morphology of chitosan nanoparticles shows an almost smooth structure.

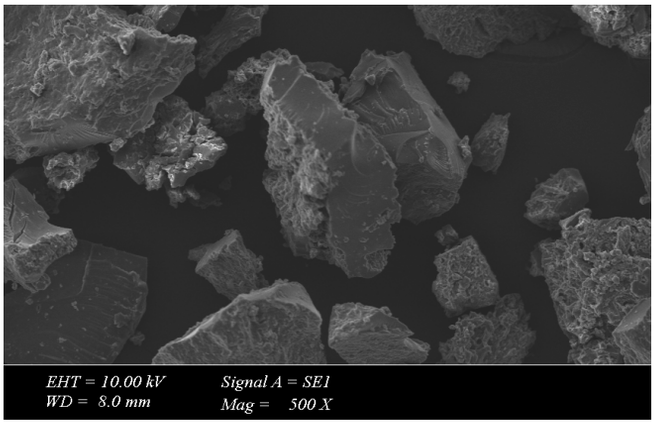


Figure 1. *SEM image of chitosan nanoparticles*

3.3. Evaluation of cytotoxic activity results of nanoparticles and indenopyrazole derivatives

Based on the results of the studies of Gezegen et al. on indenopyrazole derivatives, it was observed that these derivatives have significant efficacy in glioma and cervical cell lines (17). Therefore, in this study, it was decided to study these cells. The cytotoxic activities of 2a-d and 2a-d nanoparticles were evaluated on HeLa and C6 cell lines at 24 hours and 5-Fluorouracil (5-FU) was used as positive control. It was seen clearly from the table that all indenopyrazole compounds 2a-d and nanoparticles containing indenopyrazole compounds 2a-d against the HeLa cell line have better anticancer activity than 5-FU (Table 3). In addition, these derivatives and nanoparticles loaded with these derivatives did not show same effective cytotoxic activity on C6 cell line. The IC_{50} values of 2a-d indenopyrazole derivatives are between 7,06 ± 0,15 µg/mL and 8,09 ± 0,12µg/mL on HeLa cell line. The highest cytotoxic activity was shown by 2d with a 7,06 ± 0,15 µg/mL IC_{50} value on HeLa cells. This derivative contains 3 – trifluorine metil 4-chlorine as a substituent. According to the results, nanoparticles containing indenopyrazole derivatives show more effective cytotoxic activity than samples containing only these derivatives on HeLa cell line. Except for the 2b, both indenopyrazole derivatives and nanoparticles showed significantly better cytotoxic activity than 5-FU on HeLa cell line.

Table 3. *IC50 values of 2a-2d and NPs on HeLa and C6 cell lines (mean ± SD, n = 3).*

Samples	Ar[a]	HeLa IC_{50} (µg/mL)	C6 IC_{50} (µg/mL)
2a	Ph	7,76 ± 0,16	11,96 ± 0,32
NP2a	Ph	7,02 ± 0,21	10,12 ± 0,22
2b	4-MeOPh	8,09 ± 0,12	12,84 ± 0,16
NP2b	4-MeOPh	7,38 ± 0,18	11,05 ± 0,09
2c	4-ClPh	7,32 ± 0,31	11,68 ± 0,17
NP2c	4-ClPh	7,08 ± 0,28	10,98 ± 0,13
2d	3-CF3- 4-ClPh	7,06 ± 0,15	13,65 ± 0,23
NP2d	3-CF3- 4-ClPh	6,64 ± 0,18	11,83 ± 0,16
5-FU	5-FU	8,22 ± 0,24	7,96 ± 0,09

Abbreviations: 5-FU, 5-fluorouracil; NP, nanoparticle, SD, standard deviation. [a]For the formula, see Table 1.

However, indenopyrazole derivatives loaded nanoparticles and only derivatives did not show a more significant cytotoxic effect in the C6 cell line compared to 5-FU. 2c (IC_{50}: 11,68 ± 0,17 µg/mL) and 2d (IC_{50}:13,65 ± 0,23 µg/mL) showed the highest and lowest cytotoxic activity in C6 cell line, respectively. The IC_{50} value of 5-FU was calculated as 7,96 ± 0,09 µg/mL. The IC_{50} values of nanoparticles in the C6 cell line are between 10,12 ± 0,22 µg/mL and 11,83 ± 0,16 µg/mL. The 2a indenopyrazole compound loaded nanoparticle containing the phenyl substituent showed the highest cytotoxic effect. The results we obtained confirm our hypothesis of creating a more effective anticancer activity on cancer cells in in vitro cell culture studies, which is the aim of this study. According to the results, it was observed that nanoparticles showed

greater cytotoxic activity than indenopyrazole derivatives in both HeLa and C6 cell lines.

4. DISCUSSION

Cell culture studies of indenopyrazol derivatives and nanoparticles were carried out in HeLa and C6 cell lines. The fact that the nanoparticles were not in the desired sizes or could be aggregated could have been a limitation of this study. However, according to the results, such a problem was not observed. In order to determine the applicability of nanoparticles, particle size, zeta potential, Polydisperse index value and morphological properties should be at desired values. In order to evaluate these properties, necessary studies were performed and it was determined that the nanoparticles were suitable. Chitosan nanoparticles have enhanced encapsulation and release properties. Thanks to these features, high efficiency is achieved in the application and activity of the active substance they contain. In the light of this information, in our study, chitosan nanoparticles showed a high antiproliferative effect in C6 and HeLa cell lines in in vitro cell culture studies. Indenopyrazol derivatives show different anticancer activity depending on the functional groups in their structures. The indenopyrazole derivatives we evaluated in our study have phenyl, methoxy phenyl, chlorophenyl, trifluorochlorophenyl aromatic functional groups, respectively. According to the IC_{50} values of these derivatives, 2d containing trifluoro chlorophenyl group showed the highest antiproliferative activity, while 2b containing methoxy phenyl group showed the lowest cytotoxic activity in HeLa cell line. According to the results, it can be said that chlorine and fluorine elements attached to the phenyl ring play an important role in increasing anticancer activity in HeLa cell line. When the IC_{50} data of indenopyrazol derivatives in the C6 cell line were evaluated, it was observed that 2c and 2d showed the best and least antiproliferative activity, respectively. It was observed that the efficacy of both indenopyrazole derivatives and nanoparticles on the C6 cell line was lower than that of 5-FU. When the data in the study of Gezegen et al. were evaluated, no significant difference was observed between the IC_{50} values of indenopyrazole derivatives (17). However, it was observed that the IC_{50} values of nanoparticles containing the same amount of indenopyrazole derivatives were significantly lower than the IC_{50} values of indenopyrazole derivatives. It was observed that the preparation and application of nanoparticle formulation increased the antiproliferative activity in C6 and HeLa cell lines, and this result is very meaningful and valuable for the study.

5. CONCLUSION

According to the results of characterization and in vitro cell culture studies of nanoparticles, it was observed that nanoparticles containing indenopyrazole derivative showed antiproliferative effects in HeLa and C6 cells. In addition, it was determined that nanoparticles have higher anticancer activity than only indenopyrazol derivatives. According to the characterization results of the nanoparticles, it was shown that the particle size, zeta potential, polydisperse index and morphological properties were suitable.

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Conflict of interests

The author declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Monocyte to HDL Ratio in Preeclamptic Patients: Can It Be a Predictive Marker?

Sule Yildirim Kopuk[1], Nida Ozer[2], Canan Ozcan[3], Ipek Ulu[4]

[1] Acibadem Maslak Hospital, Department of Obstetrics and Gynecology, Istanbul,Türkiye.
[2] Private Clinic, Istanbul, Türkiye.
[3] University of Health Sciences, Derince Education and Research Hospital, Department of Obstetrics and Gynecology, Kocaeli, Türkiye.
[4] Koru Hospital, Department of Obstetrics and Gynecology, Ankara, Türkiye.

Correspondence Author: Sule Yildirim Kopuk
E-mail: suleyildirim@msn.com
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ABSTRACT

Objective: Preeclampsia (PE) is a severe and high – risk pregnancy complication for both the mother and fetus. Generalized inflammation is a prominent feature of PE. Based on the proinflammatory property of monocytes and the contrary anti-inflammatory mechanism of High-density lipoprotein (HDL), monocyte count to HDL ratio (MHR) could be used as a new marker of systemic inflammation. Our aim is to evaluate the relationship between PE and MHR in terms of diagnosis of PE.

Methods: A retrospective case-control study was recruited preeclamptic and healthy pregnant women in the third trimester of gestation (n=69 and n= 71, respectively).

Results: Maternal age (years), gravity, and body mass index (BMI) were similar in the two groups. The gestational week at delivery was significantly earlier in the PE group than in the control group ($p < 0.001$). Fetal weight in PE was significantly lower than in the control group ($p = 0.001$). Monocyte counts were comparable between the two groups (0.76 ± 0.28 vs. 0.76 ± 0.71; $p = 0.25$). The mean HDL level of PE patients was lower than the control group, but it was not statistically significant (63.87 ± 15.3 vs. 68.23 ± 13.5; $p = 0.77$). The monocyte/HDL ratio was higher in the PE group, but this increment did not reach statistical significance (12.5 ± 5.9 vs. 10.9 ± 4.3, $p = 0.08$).

Conclusion: MHR might be a new marker of inflammation and oxidative stress. The present study did not reach a result indicating a diagnostic marker of PE. Further studies with more cases are needed to evaluate the relationship between MHR and PE.

Keywords: Preeclampsia, monocyte, HDL, MHR, marker

1. INTRODUCTION

Preeclampsia (PE) is a severe and high-risk complication of pregnancy for both the mother and fetus. The prevalence of PE is estimated to be 8% on average (1). The pathogenesis of preeclampsia is still a controversial issue. Many reports concerned with defective placentation, resulting in releasing several antiangiogenic and proinflammatory substances that induce characteristic systemic endothelial dysfunction (2-4). Increased circulating lipid levels cause accumulation within endothelial cells, which causes prostacyclin secretion to decrease, resulting in oxidative stress through endothelial dysfunction (5). The other proposed mechanism is that changes in the pregnant woman's immune system cause an increased inflammatory response, leading to defective placentation, resulting in capillary permeability increment, microvascular thrombosis, and increased vascular tone (6, 7).

Monocytes, one of the main structures in the immune system, secrete proinflammatory cytokines at the site of inflammation (8). During normal pregnancy, monocytes number and activation are increased (9). Although the main cause of monocyte activation during pregnancy is unknown, the placenta may play a leading role in this process. Circulating monocytes in the blood can contact with the syncytiotrophoblast through the placental spaces and activate a proinflammatory phenotype (10, 11).

High-density lipoproteins (HDL) exhibit anti-inflammatory and antioxidant properties (8, 12). HDL protects endothelial cells against low-density lipoprotein cholesterol (LDL-C), preventing monocytes functioning in atherosclerosis and cardiovascular disease (12-14). On the basis of these reports, monocyte count to HDL ratio (MHR) might be a novel marker of oxidative stress and inflammation. MHR is considered as a predictor and prognostic marker for different pathologies (14).

Considering the pathogenesis of PE, we hypothesized that MHR might be regarded as an indicator for disease since PE is an inflammatory process and affects the function of HDL and monocyte count.

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2. METHODS

2.1. Study Population and Design

A total of 140 pregnant were enrolled in the Umraniye Education and Research Hospital's Obstetrics and Gynecology Department between February 2018 and September 2019. According to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, Sixty – nine women were diagnosed with PE (15). As a control group, 71 women were enrolled with a normal pregnancy who have normal blood pressure and experienced a normal course of pregnancy.

All women were in the third trimester, not in active labor, and had intact membranes. Patients with lipid metabolic abnormalities were excluded, as were those who had taken any medication that affected plasma lipid levels in the six months before to enrolling in the trial. Patients were excluded from infectious, inflammatory, or autoimmune diseases, multiple gestations, chromosomal fetal abnormality, chronic and pregnancy-induced hypertension, PE history, diabetes mellitus, molar pregnancy, maternal renal disease, rheumatoid arthritis, and morbid obesity. Alcoholism, smoking, and medical or surgical intervention history were also ruled out. Ethical Committee of Umraniye Education and Research Hospital accepted the study (no: 21.02.2018-18). All participants gave written informed consent.

Demographic characteristics, obstetrics outcomes, and laboratory parameters of patients were saved. The gestational week was calculated by the first date of their last menstrual cycle. The confirmation was done by first-trimester ultrasound scanning of the crown-rump length (CRL).

The peripheral venous blood samples were obtained after a minimum of eight hours of fasting. An automated hematology analyzer (Pentra DX Nexus, Horiba, Japan) was used to measure complete blood counts. DxC700 AU Chemistry Analyzer (Beckman Coulter, USA) was used to measure HDL cholesterol levels in the blood. Monocyte count was divided by serum HDL levels to calculate the MHR.

2.2. Statistical Analysis

All data were analyzed with SPSS, version 20.0. Shapiro–Wilk test was performed to verify normal distribution or not. Mann – Whitney U test or Independent samples t-test were used according to normality test results. Variables were demonstrated as mean, ± standard deviation, or median values. Correlations were calculated by calculating Pearson's correlation coefficient (r). $P < 0.05$ was considered statistically significant.

3. RESULTS

A total of 71 healthy pregnant and 69 preeclamptic pregnant women were included in the study. Age and parity of the women were matched. Table 1 summarizes the pregnant women's demographic characteristics and pregnancy outcomes. Maternal age (years), gravidity, and BMI were comparable in all groups (Table 1). The gestational week at birth was significantly earlier in the PE group than in the healthy group ($p < 0.001$). Preeclamptic patients had a significantly lower fetal weight than healthy pregnant patients (2809.3 ± 778.1 vs. 3188.6 ± 407.4; $p = 0.001$).

Table 1. *Clinical characteristics of study participants.*

	Preeclampsia (n = 69)	Control (n = 71)	p value
Age (years)	29.96±5.3	28.28±5.2	0.08
gravidity (range)	2 (1-7)	2 (1-7)	0.71
Parity (range)	1 (0-6)	1(0-5)	0.35
BMI (kg/m^2)	30.6 ± 4.4	29.9±5.2	0.36
Gestational week at delivery	35(26-40)	37(30-41)	< 0.001
Fetal weight (gr)	2809.3 ± 778.1	3188.6 ± 407.4	0.001

Data are expressed as mean±standard deviation, median (interquartile range)
BMI: body mass index

Monocyte levels were comparable between the two groups (0.76 ± 0.28 and 0.76 ± 0.71; $p = 0.25$). The mean HDL level of PE patients was lower than the control group, but it did not reach statistical significance (63.87 ± 15.3 vs. 68.23 ± 13.5; $p = 0.77$). MHR was higher in PE patients, but this increment did not reach statistical significance (12.5 ± 5.9 vs. 10.9 ± 4.3, p=0.08) (Table 2). WBC count was significantly higher in the PE than in the healthy control group (p=0.01). MHR was positively correlated with WBC count (r= 0.37, p<0.001), but not with age and BMI, respectively (p=0.41, p=0.36).

Table 2. *Laboratory parameters of all groups.*

	Preeclampsia (n = 69)	Control (n = 71)	p value
WBC (x10^9/L) (mean ± SD)	11.4 ± 2.42	9.3 ± 1.63	0.001
Monocyte count (x10^3/mL) (mean ± SD)	0.76 ± 0.28	0.76 ± 0.71	0.25
HDL (mg/dL)	63.87 ± 15.3	68.23 ± 13.5	0.77
MHR	12.5 ± 5.91	10.9 ± 4.3	0.08

WBC — white blood cells; HDL — high-density lioprotein; MHR — monocyte/high density lipoprotein ratio; SD — standard deviation

4. DISCUSSION

In our study, we evaluated MHR in preeclamptic women and compared them with healthy pregnant women. MHR was higher in preeclamptic patients than in the control group, according to our findings. However, this difference was not statistically significant.

During preeclampsia, defective placentation products prompt further activation and maturation of the monocytes (16). Activated monocytes produce cytokines that result in a generalized inflammatory response. Wang et al. analyzed more than three hundred preeclamptic patients and found that absolute monocyte count was significantly higher than the control group (17). In addition, Brien et al. stated that monocyte count was higher in preeclamptic women than in

normal pregnancies (18). Belo et al. reported that monocyte counts were similar in both groups, consistent with the current study. Further, higher WBC counts have been detected in the third trimester preeclamptic pregnancies as our study, but it was not statistically significant (19).

In a healthy pregnancy, lipid metabolic changes occur, with total cholesterol, triglyceride, HDL, and LDL levels rising significantly to fulfill the needs of fetal growth and development (20). In preeclampsia, the oxidatively stressed placenta releases antiangiogenic and proangiogenic factors that induce an exaggerated inflammatory response with generalized endothelial dysfunction (21). HDL positively affects the inflammation and oxidative pathways, such as inhibiting lipid peroxidation and upregulation of cytokine-induced proinflammatory adhesion molecule and chemokine expression by endothelial cells. HDL advocates macrophage transition from pro-inflammatory to anti-inflammatory form and suppresses monocyte progenitor cells to proliferation-differentiation (14, 22). Reyes and Cao et al. stated that HDL levels are lower in preeclamptic patients (23, 24). Konrad et al. reported that HDL levels decreased in the course of worsening from mild to severe preeclampsia (25). Conversely, according to Khaire et al., comparing normotensive pregnant women with preeclamptic women, HDL levels were higher at term PE than the normotensive healthy women (26).

The present study was designed with the idea that PE is an inflammatory process that causes an increase in MHR value. Based on the proinflammatory feature of monocytes, and the contrary anti-inflammatory mechanism of HDL, it was thought that MHR might be used as a new marker of systemic inflammation. MHR was first defined by Kanbay et al. in 2014, and it was reported that a high MHR value could estimate adverse cardiovascular outcomes' risk (27). MHR has been related to different pathologies such as hypertension, abdominal aneurysm, intracerebral hemorrhage, chronic kidney disease, obstructive sleep apnea syndrome (OSA), acute intracranial hemorrhage, gestational diabetes mellitus (GDM), and metabolic syndrome (MS) in Polycystic Ovary Syndrome (PCOS) (28-32).

Selçuk et al. reported that MHR was significantly higher in the non-dipper hypertension (HT) patients than control and dipper HT patients (33). Gembillo et al. stated that MHR was a potential biomarker of inflammation and positively correlated to C-reactive protein. In addition, MHR was significantly higher among resistant hypertension than non-resistant HT patients (34). Sun et al. analyzed the correlation between MHR and OSA in 246 patients with HT. MHR increased with OSA patients' severity, and it was a valuable marker in evaluating OSA risk and severity in hypertensive patients (29).

High blood pressure, obesity, dyslipidemia, and insulin resistance are major components of metabolic syndrome (MS), and the MS is diagnosed in approximately one-third of women with PCOS (35). In this context, researchers have published articles revealing the relationship between MHR, PCOS, and metabolic syndrome (31,36,37). Usta et al. reported that MHR was significantly higher in PCOS than in non-PCOS control groups (11.5 vs. 8.8). In subgroup analysis that PCOS divided into obese and lean PCOS, MHR had higher in obese PCOS than lean PCOS and non-PCOS controls. They summarized that it is a valuable predictor of PCOS (31). Dilşad et al. reported an association between PCOS and obesity. The researcher determined 10.1 cut-off value for prediction for PCOS. In addition, the researcher reported negative correlation between MHR and age in PCOS patients (36). In our study, any correlation was found between MHR with age and BMI.

Çakmak et al. analyzed MHR in PCOS and found MHR to predict metabolic syndrome with a MHR ratio > 9.9, reporting that it may be promising in predicting metabolic syndrome in the early stages of PCOS (37). In our study, MHR for PE and the control group were 12.5 vs. 10.9, respectively. The cut-off value could not be calculated because statistical significance was not found in our study. Based on the knowledge that oxidative stress increases in GDM, Onat et al. compared 60 pregnant with GDM and 52 healthy women; MHR was higher in the GDM patients ($p < 0.05$) (32).

To the best of the present authors' knowledge, there is only one published study investigating MHR in PE by Melekoglu et al. stated that MHR was considerably higher in both preeclampsia and severe preeclampsia groups (p=0.007 and p<0.0001, respectively) (38). Contrary to our study, they reported that the MHR might be valuable for predicting the development of preeclampsia. MHR is a readily available and cheap test that provides relevant information about inflammation and oxidative stress. In our study, MHR could not be used as a diagnostic marker in PE. This situation may change with studies conducted with larger-scale studies.

5. CONCLUSION

In conclusion, this preliminary study demonstrates that MHR cannot be used as a diagnostic marker of PE. An association between MHR and PE has not been proven. However, further studies with more cases are needed to evaluate the relationship between MHR and PE.

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Clinical and Experimental Health Sciences

Are Youtube™ Videos Useful for Biostatistics Education: A Sample of Logistic Regression

Arzu Baygul Eden[1], Neslihan Gokmen Inan[2]

[1] Koc University, Faculty of Medicine, Department of Biostatistics, Istanbul, Türkiye.
[2] Koc University, Engineering College, Department of Computer Engineering, Istanbul, Türkiye.

Correspondence Author: Neslihan Gokmen Inan
E-mail: ninan@ku.edu.tr
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ABSTRACT

Objectives: To examine the quality and educational content of YouTube videos related to logistic regression.

Methods: A comprehensive electronic search was performed for 'Logistic Regression" on YouTube.The first 70 videos were evaluated for each term. Videos were evaluated using Global Quality Score [GQS] checklists and were classified as useful and insufficient by two statisticians.

Results: Of the 70 videos analyzed, 53 were included. The mean GQS value was 3.9+1.1. Fourty videos (75.5%) were classified as useful. Independent users tend to upload videos mostly Lecturer / Ph.D., Lecturer Msc. and Data science course channels. A significant difference was observed in GQS among useful and insufficient videos. The mean GQS scores of useful videos were 4.3 (s.d.:0.8), for insufficient videos were 2.5 (s.d.:0.5).

Conclusion: Specialists or instructors could refer their students to YouTube resources and actively participate in the development of video-sharing platforms for biostatistics.

Keywords: Biostatistics, logistic regression, YouTube, remote learning

1. INTRODUCTION

Logistic regression is a statistical method, which has wide acceptance in various areas, such as biostatistics, machine learning, especially in biostatistics. The roots of Logistic regression come from the early 19th century (1).

Logistic regression is used in biological and medical research in order to determine the effect of independent variables (or just one variable) on a dependent variable. It is a regression model, which uses the logistic function to build a model for the dichotomous dependent variable. There is an important difference between linear regression and logistic regression, which is the characteristic of the dependent variable.

The logistic regression model can be also thought of as a multivariate model when more than one independent factor or covariates were used. Its results don't show only the effect of independent variables individually but also the interaction effect on the dependent variable. Logistic regression has limited assumptions when compared with linear regression, which is the reason of common use of it.

YouTube™ is the most popular video-sharing platform worldwide. YouTube™ is providing informative videos on different topics, as well as videos with entertaining content. Since YouTube™ is not a peer-reviewed platform, in each and every topic there are some useful videos such as misleading videos, which give missing or biased information. There is a number of studies evaluating the quality of information at YouTube™ videos on various topics. It is clear that the quality of content producers increasing day by day (2)).

Remote teaching becomes an important role for education, especially during pandemia. Basilaia and Kvavadze (2020) express that information technologies and communication are used to assist in the acquisition and development of knowledge from particular remote locations. It uses the internet, video/audio and text communication and software to create the learning environment (3)).

Considering the significance of using visual objects and video for teaching, some universities use YouTube as a complementary teaching instrument (4)). Students, researchers and academicians use video resources to learn statistics.

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The aim of this study is to evaluate usefullness of the Logistic Regression videos published in YouTube. One step further purpose is to evaluate the use of Youtube as a source of information for advanced statistical methods, by using the Logistic regression examples. To the best of current knowledge of authors, this study is the first to assess the content of YouTube videos on a specific statistical topic.

2. METHODS

The study was designed to evaluate YouTube videos related to Logistic Regression. Videos were scanned by searching on the YouTube search web site (www.youtube.com) by using the selection filters of Google. The search was performed in English at 18.10.2021 by using the search terms were "Logistic regression" "youtube".

The selection criterias were:

- Only English pages
- Videos with the length of 4-20 minutes.
- Videos uploaded within the past one year.
- Only high-quality videos.

The search produced 70 videos. Seventeen of them were excluded (8 videos non-English, 7 videos were irrelevant, 1 high-speed video, 1 video was difficult to understand). Assessments were performed on 53 eligible videos (Figure 1).

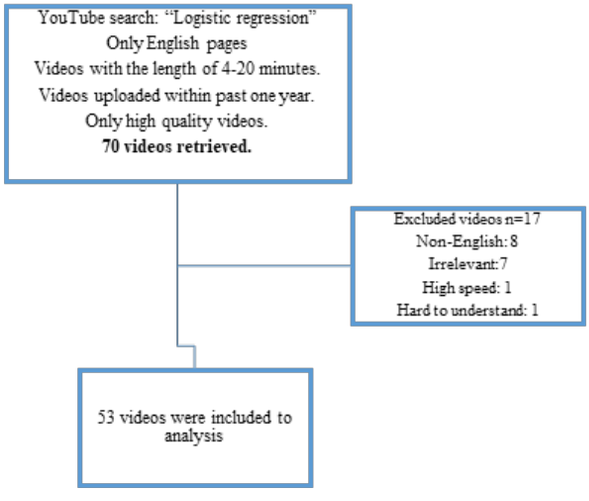

Figure 1. *Flowchart diagram of the selection process*

The web links, watching times, length of video (min), uploading date, video title, number of likes and dislikes, total number of viewes and the source of the videos were recorded in Microsoft Excel database. Days since upload were evaluated by taking the difference between upload date and the date when the search was performed (5)). The overall quality of the videos was examined following GQS criterias (Table 1) Global Quality Scale (GQS), Usefullnes evaluation were calculated by authors.

Table 1. *Global quality scale.*

Score	Global Scale Description
1	Poor quality, poor flow of the site, most information, not at all useful for patients.
2	Generally poor quality and poor flow, sone information listed but many important topics missing, of very limited use to patients.
3	Moderate quality, suboptimal flow, some important information is adequately discussed by others poorly discussed, somewhat useful for patients.
4	Good quality and generally good flow, most of the relevant information is listed, but some topics not covered, useful for patients.
5	Excellent quality and excellent flow, very usef for patients.

Video content was classified as useful and insufficient by the authors. Two statisticians are independently evaluated all the videos which were outside exclusion criteria for usefulness and grouped them into the following categories at the same time. The statisticians were blinded to each other's evaluations, in the event of a discrepancy a final decision was made by a third statistician. The group classifications were: Videos designated as useful information were accurate included scientific and comprehensive information about logistic regression. The videos contained incorrect information or did not contain information on how to contstruct logistic regression model are classified as insufficient videos.

In addition to all quality assessments the interaction index and viewing rate were calculated according to the following formulas (6)).

$$\text{Interaction Index} = \frac{(\text{likes} - \text{dislikes})}{\text{number of views}} x100$$

$$\text{Viewing Rate} = \frac{\text{total no of views}}{\text{number of days since upload}} x100$$

Videos were watched by two authors (ABE, NGİ) blindly, Global Quality Scale and the usefulness score were compared between two authors Any conflicts were corrected by discussion, full consensus has been provided. No ethical approval was needed for this study.

2.1. Statistical Analysis

The normality distribution of continuous variables were evaluated by Shapiro-Wilk's test. Mean, standard deviation, median, minimum and maximum are presented as descriptive statistics. Two normally distributed independent groups were compared by using Student t test. Non-parametric statistical methods were used for values with non-normally distribution. Two non-normally independent distributed groups were compared by using Mann Whitney U test. Statistical significance was accepted when two-sided p value was lower than 0.05. Statistical analysis was performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

3. RESULTS

The videos after searching 70 videos were scanned to evaluate by means of inclusion criteria. The exclusion reasons were: non-English videos (8), irrelevant videos (7), high-speed video (1), the video was difficult to understand (1). 53 videos were eligible and included in the analysis. The videos were classified by the means of source (Table 2). Most videos were uploaded by lecturers at least MSc. Level (58.5%). The length of the videos was 11 minutes, 37 seconds with a standard deviation of 4 minutes, 42 seconds. The mean of the total number of views was 1509.7+1905.8 (min:1, max:9389). The mean interaction index was 2.5+2.6, whereas the viewing rate was 860.8+1285.1 (Table 3).

Table 2. *Source of video.*

Source	n	%
Lecturer / PhD	20	37.7
Lecturer Msc.	11	20.8
Data science course channel	10	18.9
Lecturer	4	7.5
Data engineer	2	3.8
Data scientist	2	3.8
Software engineer	2	3.8
Product developer	1	1.9
Student	1	1.9
Total	53	100

Table 3. *Video characteristics.*

	Mean+SD	Med(min-max)
Length of video (min)	11:37+4:42	11:40(2:02-19:54)
Total views	1509.7+1905.8	808(1-9389)
No of likes	27+25.4	21(0-96)
No of dislikes	0.5+1.3	0(0-5)
Interaction index (%)	2.5+2.6	2(0-15.8)
Viewing rate (%)	860.8+1285.1	397.5(6.7-7250)
Global Quality Scale	3.9+1.1	4(2-5)
	n	%
GQS-1	0	0
GQS-2	7	13.2
GQS-3	13	24.5
GQS-4	14	26.4
GQS-5	19	35.8

Also, videos were classified by the authors as useful and insufficient. Forty (75.5%) of videos were classified as useful whereas thirteen (24.5) of them were assessed as insufficient.

The Global Quality Score assessed eligible videos 7 (13.2%) videos as generally poor, 13 (24.5%) moderate quality, 14 (26.4%) good quality, 19 (35.8) excellent quality.

Interaction index, viewing rate, and the number of dislikes do not differ between useful and insufficient videos (p=0.828, p=0.069). The median global quality scale was 4 in useful videos and 3 in insufficient videos, the difference between them was statistically significant (p<0.001) (Table 4). Also number of likes and number of total views are statistically significantly higher at useful videos (p=0.003, p=0.010).

Videos were classified by sources as lecturers (Msc or PhD) and others (student, data scientists, data engineer, software engineer, lecturer, product developer). Both groups do not have statistically significant difference by means of number of likes, number of dislikes, total views, interaction index and viewing rates. Videos uploaded from lecturers has been scored at higher Global Quality Scale levels relatively (p<0.001) (Table 5).

Table 4. *Comparison between useful, insufficient Youtube videos.*

	Useful (n=40)	Insufficient (n=13)	p
Likes			**0.003**
Mean+SD	31.4+24.8	13.5+23.2	
Med(min-max)	26.5(0-96)	3(0-80)	
Dislikes			0.716
Mean+SD	0.6+1.3	0.4+1	
Med(min-max)	0(0-5)	0(0-3)	
Total views			**0.010**
Mean+SD	1781.1+2076.3	674.6+852.8	
Med(min-max)	935.5(1-9389)	328(3-2361)	
Interaction index (%)			0.828
Mean+SD	2.3+1.7	3.3+4.4	
Med(min-max)	2.2(0-10.7)	1.8(0-15.8)	
Viewing rate (%)			0.069
Mean+SD	895.8+1288.0	753.2+1320.8	
Med(min-max)	465.8(6.7-7250)	268.5(20-4685.7)	
Global Quality Scale			
Mean+SD	4.3+0.8	2.5+0.5	**<0.001**
Med(min-max)	4(2-5)	3(2-3)	

Table 5. *Comparison between source types Youtube videos.*

	Lecturer PhD+Msc (n=31)	Other (n=22)	p
Likes			0.108
Mean+SD	28.4+20	25+31.9	
Med(min-max)	26(2-76)	9(0-96)	
Dislikes			0.383
Mean+SD	0.3+0.7	0.9+1.7	
Med(min-max)	0(0-3)	0(0-5)	
Total views			0.100
Mean+SD	1496.4+1521.6	1528.4+2384.4	
Med(min-max)	879(19-7128)	420.5(1-9389)	
Interaction index (%)			0.396
Mean+SD	2.8+3	2.2+2	
Med(min-max)	2.2(0.3-15.8)	1.9(0-8.7)	
Viewing rate (%)			0.386
Mean+SD	627.9+501.3	1188.9+1880.8	
Med(min-max)	463.7(111.8-2277.3)	334(6.7-7250)	
Global Quality Scale			**<0.001**
Mean+SD	4.4+0.8	3.1+0.9	
Med(min-max)	5(2-5)	3(2-5)	

Other: Student, data scientist, lecturer

4. DISCUSSION

YouTube is a popular video-sharing platform, which is open-access. People mostly use YouTube to access information on any topic. Many people use Youtube videos to get informed about statistical methods. Day by day online content on any topic becomes more popular. YouTube was always thought of as an important source for video content, because of its characteristic of being open access. This situation highlighted the importance of the quality of videos.

The purpose of this study was to evaluate the quality and usefulness of YouTube videos concerning logistic regression. By the way, it was also aimed that, when taking "logistic regression" on the focus, generalize this study by questioning " Are the YouTube videos about advanced statistical methods useful?"

Previous studies mostly evaluated health-related topics (7-15) such as keratoconus, lung cancer, acute myocardial infection, COVID 19 or dentistry. To the best of our knowledge the present study is the first to analyze the content of Youtube videos on a topic of biostatistics.

Logistic regression is a statistical method which includes many basis statistical information in it. On this vision, videos which are thought to be useful should contain all the base information about logistic regression.

In the present study videos were evaluated according to their source. Likes, dislikes, total views, interaction index and viewing rate do not differ between lecturer (MSc. And PhD) sourced videos and videos from other (*Student, data scientist, lecturer)* sources, whereas global quality scale was higher at videos from lecturers than the others. Similarly, Sallı et al.(14) found that, the videos uploaded from independent users have lower quality than videos uploaded from health professionals.

GQS was also higher in useful videos, when comparing insufficient videos, which shows the paralellism between GQS and authors' evaluation. Gaş et al(15)) reported that 59.7% of videos which are uploaded by health professionals were moderate or excellent in usefulness score. This result was similar to the present study.

Correlatively to our study, many of the studies stated that YouTube videos can be used for educational purposes(14, 15) however Fialho et al. (10) stated that there are some misleading videos and contents should be checked by experts.

The present study has some limitations. YouTube is a highly dynamic platform, so gathered information can vary in a very short time. Every day millions of videos are uploading and topic and the content is always changing. Performing these methods of this study can be cause different results, because of the dynamic structure of the platform.

5. CONCLUSION

Most of the videos were classified as useful. Although the quality is varying through videos, people can use YouTube to get information for advanced biostatistics topics by being selective for videos.

Conflict of interests

The authors did not declare any conflict of interest.

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Clinical and Experimental Health Sciences

An Evaluation of Death Depression and Death Anxiety in Patients with Chronic Obstructive Pulmonary Disease

Elif Okur[1], Nesrin Nural[2]

[1] Trabzon University, Tonya Vocational School of Higher Education, Health Care Services, Trabzon, Türkiye.
[2] Karadeniz Technical University, Health Sciences Faculty, Internal Medicine Nursing Department, Trabzon, Türkiye.

Correspondence Author: Elif Okur
E-mail: elifcengiz_ec@hotmail.com

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ABSTRACT

Objectives: The study aimed to determine the level of death depression and death anxiety in patients with COPD.

Method: The sample of this cross-sectional and descriptive study including patients' views consisted of 104 patients diagnosed with COPD and hospitalized in a hospital's pulmonary disease clinic in Turkey. Number, percentage, mean, regression, and correlation analysis were used in the analysis of the data.

Results: The most disturbing symptom of COPD was found to be dyspnea (71.2%), cough (17.3%), and sputum (11.5%). 44.2% of the patients had mild, 40.4% had moderate death anxiety levels, and 51% were in a depressive mood. According to the regression analysis, the death depression total score is predictable based on marital status, disease duration, presence of respiratory disease in patients, and patients' self-care levels (R: 0.556, R2: 0.310, F: 3.401, p: 0.000). The Anxiety about Death Process-Pain and Suffering subscale score is also predictable depending on the marital status, duration of the disease, desire to know the truth in case of deadly disease, and the level of meeting self-care needs (R: 0.563, R2: 0.317, F: 3.520, p: 0.000).

Conclusion: Nurses should monitor the following patients more closely in terms of death depression and death anxiety; those with high dyspnea, those with a history of respiratory disease, single or widowed patients, those with a disease of long duration, those who have difficulties meeting self-care needs and those who do not want to know the diagnosis in case of a deadly disease.

Keywords: COPD, Death, Depression, Dyspnea, Nursing

1. INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is the leading cause of morbidity and mortality in the world (1). Approximately 3 million people die of COPD worldwide each year. It is estimated that the prevalence of COPD will increase in the next 30 years, and deaths due to COPD and related reasons will surpass 4.5 million per year by 2030 due to the gradually increasing smoking rates in developing countries and the aging of the population in developed countries (2). The most apparent symptom of COPD is dyspnea. Patients may also have a long-lasting cough, sputum, and wheezing (1). Dyspnea is the symptom that creates the most fear, panic, and anxiety for COPD patients (3). In addition to physical symptoms, anxiety, and depression are also very typical symptoms in COPD patients. Dyspnea is more common in patients with depression symptoms (4). In a meta-analysis study, COPD is reported to increase the risk of developing depression. It is emphasized that the risk of exacerbation of the disease increases by 43% in COPD patients with depression or anxiety (5). The prevalence of anxiety and depression is higher in COPD than in other chronic diseases. Factors like the feeling of being dependent on others, physical disability, reduced effort capacity, chronic inflammation, the effect of smoking on brain functions, and reduced exercise capacity are listed as the causes of depression and anxiety. The effect of dynamic hyperinflation and hyperventilation is also reported to be significant. The presence of anxiety and depression in COPD has been associated with decreased quality of life and exercise capacity, and an increased frequency of hospitalization, exacerbation, and mortality (2).

According to the study by Strang et al., the anxiety experienced by most patients was associated with "COPD". Patients expressed that they felt as if they were suffocated and strangled, and the feeling of not getting enough

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air caused fear of death (6). In Barnett's study, patients described the moment they experienced respiratory distress as *"unbearable and frightening"* (7). In a phenomenological study, a patient emphasized that when s/he had respiratory distress, s/he "*felt as if s/he was going to die*" and "*felt frightened"* (8).

As dyspnea progresses in patients with the diagnosis of COPD, fear of not being able to breathe intensifies the death anxiety. With the progression of the disease, increasing dependence restricts social activities and can cause patients to have difficulty fulfilling their expected roles in the family and society, resulting in social isolation (9).

Although medical interventions for the treatment of COPD disease are limited, healthcare professionals can still do a lot to improve patients' symptoms and life standards related to the disease. By identifying how symptoms affect patients, they can develop coping strategies.

There are various studies on depression in the literature. Depression is accompanied by many symptoms or diseases. However, there are a limited number of studies describing the relationship between death depression and death anxiety in Turkey (10-12). This study was conducted to evaluate death depression and death anxiety in COPD patients and determine the socio-demographic and disease-related factors influencing them and patients' views on death. In addition to guiding nursing care and practices, the data obtained from the study are thought to shed light on innovative approaches in the future with an improved insight into the issue.

2. METHODS

2.1. Ethical Considerations

Institutional approval for the study was obtained from the General Secretariat of the Trabzon Public Hospitals Association on 22/05/2014 (Number: 96975.576.1704), and ethical permission was received from Karadeniz Technical University Faculty of Medicine Ethics Board (No:2014/58). The participants were informed about the aim of the study, their questions were answered, and their written and verbal consents were obtained.

Study Design: The study was conducted descriptively and cross-sectionally to determine the level of death depression and death anxiety in patients diagnosed with COPD. 104 patients hospitalized with COPD diagnosis in a hospital's pulmonary clinics in northern Turkey were included in the study.

Sample Selection: The study including patients' views was carried out in a descriptive and cross-sectional design at the Pulmonary Clinics of Hospital in Turkey between October 2014 and March 2015. The sample size was determined to be 104 patients after a power analysis was performed in the Open Epi program (9.1% incidence of COPD (13), %95 confidence interval, %80 test power) (14). Inclusion criteria were being 18 and over, being hospitalized with the diagnosis of COPD, being able to communicate, having no vision and hearing loss and psychiatric disease, and being a volunteer.

Data Collection: In the collection of data, the "Patient Information Form", the "Thorson-Powell Death Anxiety Scale (TPDAS)", the "Death Depression Scale (DDS)", and the "Medical Research Council Scale (MRCS)" were used. The data were collected by the researchers through the face-to-face interview technique.

2.2. Data Collection Forms

The Patient Information Form: The Patient Information Form created by the researchers consists of three parts and 19 questions. The first part includes the descriptive characteristics of the patients (age, gender, marital status, education level, family structure, where they spend most of their life, and smoking status). The questions in the second part are about the clinical characteristics of the patients (the most disturbing symptom of COPD, chronic respiratory disease history, duration of the disease, annual hospitalization frequency, knowledge about the disease and prognosis, regular doctor visits, receiving support from the family, the level of meeting self-care needs), and the third part investigates the opinions of patients about death (the importance of religious beliefs in daily life, the desire to know the truth in case of a deadly disease, the expectations of terminal patients).

The Thorson-Powell Death Anxiety Scale (TPDAS): Developed by Thorson and Powell (1992), the Turkish validity and reliability study of the scale was performed by Karaca and Yıldız (2001) (15, 16). The scale consists of 25 items and is scored from 0 to 4, from weak to strong, using a 5-point Likert type. The lowest and the highest points on the scale are 0 and 100. High scores indicate high levels of death anxiety (16). The evaluation of the scores is as follows: 0-25 very low; 26-50 mild; 51-75 moderate; and 76-100 high. Death anxiety is examined in four subscales; anxiety about losing physical and mental functions, anxiety about the life after death, anxiety about decomposition, and anxiety about the death process-pain, and suffering. An increase in scores in all these dimensions is interpreted as an increase in death anxiety. The Cronbach alpha coefficient of the Thorson-Powell death anxiety scale is 0.84 (16). In this study, the Cronbach alpha coefficient of the scale was found as 0.79.

The Death Depression Scale (DDS): Developed by Templer et al. in 1990, the Turkish validity and reliability study of the Death Depression Scale was conducted by Yaparel and Yıldız in 1998 (17, 18). There are 17 items on the scale measuring the emotional states such as death-related depression, sadness, loneliness, terror, and grief. Minimum and maximum scores are 0 and 17 on the scale. 0-8 scores refer to non-depressive mood, and 9-17 refer to depressive mood. The Cronbach alpha coefficient of the Death Depression Scale is 0.74 (18). In this study, the Cronbach alpha coefficient of the scale was found to be 0.74.

The Medical Research Council Scale (MRCS): It is a five-item scale based on a variety of physical activities that produce a sense of dyspnea. The scoring is between 0 and 5, and patients are asked to mark the level of activity that causes dyspnea (19).

2.3. Statistical Analysis

The data were evaluated with the SPSS (Statistical Package for the Social Sciences) 18.0 program. Kolmogorov Smirnov test was used to check the data's conformity to normal distribution. Number, percentage, mean, standard deviation, multiple linear regression analysis, Spearman, and Pearson correlation analysis were used to evaluate the data. Independent t-test, and ANOVA test, which are parametric tests, were used because the variables conformed to the normal distribution in the independent data. In the comparison of the data, the *P*-value of less than .05 was accepted as statistically significant.

3. RESULTS

Patient Characteristics: The results showed that 77.9% of the patients were male, the mean age was 67.65 ± 9.63 years, and 68.3% had nuclear families. The MRCS mean score was found to be 3.58±1.20. The most frequently experienced symptoms of COPD were found to be dyspnea (71.2%), coughing (17.3%), and sputum (11.5%). 56.7% had a history of one or more chronic respiratory diseases (asthma, chronic bronchitis, emphysema, tuberculosis). 37.5% had been followed up with the diagnosis of COPD for 10-29 years, 39.4% were hospitalized 1-3 times a year, 71.2% received family support during their disease, and 83.7% could meet their self-care needs independently (Table 1).

Patients' Views on Death: 71.2% of the patients stated that their religious beliefs were very important in their daily lives. 67.3% wanted to know the truth in case of a deadly disease by reporting the following reasons; "I just want to know" (30.7%), "I want to know to take measures" (19.2%), "I want to know to worship more for the rest of my life" (5.8%), "I believe in death, and I am not afraid of it" (4.8%).

Patients listed their reasons for not wanting to know the truth in case of a deadly disease (25.0%) as follows; "I would feel sad" (15.4%), and "I would feel scared" (3.8%). The expectations of terminal patients from nurses were "to show interest and compassion" (41.3%), "to help and meet the patients' needs" (21.2%), and "to make necessary interventions" (19.2%) (Table 2).

The Death Depression and Death Anxiety: Table 3 shows the distribution of the patients' mean scores of the DDS and TPDAS. On the Death Depression Scale, 51% of patients were found to be in a depressive mood. On the Death Anxiety Scale, 6.7%, 44.2%, 40.4%, and 8.7% of the patients had very low, mild, moderate, and high levels of death anxiety, respectively (Table 3).

Table 1*. Distribution of Descriptive and Clinical Characteristics of Patients (n = 104)*

Descriptive Characteristics		n	%
Age (Mean±SD) 67.65±9.63 years	64 years old and ↓	37	35.5
	65 years old and ↑	67	64.5
Gender	Female	23	22.1
	Male	81	77.9
Marital Status	Married	81	77.9
	Single/Divorced	8	7.7
	Widowed	15	14.4
Education Level	Illiterate /Literate	29	27.9
	Primary school	56	53.8
	Secondary school and over	19	18.3
Family Type	Nuclear	71	68.3
	Extended	33	31.7
The place where the patient spent most of her/ his life	City	35	33.7
	District	21	20.2
	Village	48	46.1
Smoking	Yes	10	9.6
	No	26	25.0
	Quit due to disease	68	65.4
Clinical Characteristics		n	%
MRCS* (Mean±SD) 3.58±1.20	Stage 1	7	6.7
	Stage 2	13	12.5
	Stage 3	24	23.1
	Stage 4	32	30.8
	Stage 5	28	26.9
The most disturbing symptom of COPD**	Dyspnea	74	71.2
	Cough	18	17.3
	Sputum	12	11.5
Having a history of respiratory disease	Yes	59	56.7
	No	45	43.3
The duration of the disease (Mean ±SD) 13.75±13.30 years	Less than 1 year	10	9.6
	1-9 years	37	35.6
	10-29 years	39	37.5
	30 years and ↑	18	17.3
The frequency of hospitalization (Mean ±SD) 3.29±3.28 years	First time	21	20.2
	Once in every 2-3 years	6	5.8
	1-3 times a year	41	39.4
	Four times and ↑ a year	36	34.6
Having information about disease and prognosis	Yes	53	51.0
	No	51	49.0
Having regular doctor visits	Yes	76	73.1
	No	28	26.9
Receiving family support during disease	Yes	74	71.2
	No	30	28.8
Meeting self-care needs	Independent	87	83.7
	Partially dependent***	17	16.3

**MRCS: Medical Research Council Scale*

***COPD: Chronic Obstructive Pulmonary Disease*

**** Those supported by someone and those using assistive vehicles were combined.*

The total score that the patients got from the DDS ranged from 2 to 17, and the total mean score was 8.84±3.56. The total mean TPDAS score was 50.19±15.61, and the highest score was obtained from the subscale of the anxiety about the death process-pain and suffering (15.80±5.17). This is followed by the anxiety about the life after death subscale (15.60 ± 4.96) (Table 4).

Table 2. *Distribution of Patients' Views on Death (n = 104)*

Death-Related Views		n	%
The importance of religious beliefs in daily life.	Very important Important / Not very important	74 30	71.2 28.8
Desire to know the truth in case of a deadly disease	Yes No Depends on conditions	70 26 8	67.3 25.0 7.7
The reasons why the patients want to know the truth in case of a deadly disease (n=70)	I just want to know To take measures To worship more I believe in death, and I am not afraid of it To say goodbye to my loved ones I would be glad to die I would show more interest to my children I would try to heal the hearts I broke	32 20 6 5 3 2 1 1	30.7 19.2 5.8 4.8 2.9 1.9 1.0 1.0
The reasons why the patients would not want to know the truth in case of a deadly disease (n=26)	I would feel sad. I would feel scared. I would not want to leave my loved ones It is difficult to accept In order not to despair Because I'm afraid of being punished in the life after death	16 4 2 2 1 1	15.4 3.8 1.9 1.9 1.0 1.0
The expectations of terminal patients from the nurses (n=93) *	Nurses are expected to show interest and compassion to help and meet the patients' needs to make necessary interventions to give moral support and to have a smiling face to provide religious support to behave normally to relieve pain to be careful/attentive to ask for their last wishes	 43 22 20 14 4 4 3 2 1	 41.3 21.2 19.2 13.5 3.8 3.8 2.9 1.9 1.0

**Since the questions were answered more than once.*

Table 3. *The distribution of the patients' DDS and TPDAS total mean scores.*

Death Depression Scale	n	%
Non depressive mood (0-8 points)	51	49.0
Depressive mood (9-17 points)	53	51.0
The Thorson-Powell Death Anxiety Scale-Anxiety levels	**n**	**%**
Very low	7	6.7
Mild	46	44.2
Moderate	42	40.4
High	9	8.7

The Relationship between Patients' Death Depression Scale-Thorson-Powell Death Anxiety Scale Scores and MRCS scores: According to the correlation analysis, there was a positive correlation between MRCS and DDS mean scores (r=0.231, p<0.05). As the MRCS mean score increased, the DDS mean score increased. The relationship between the MRCS mean score and the subscale of anxiety about decomposition mean score (r=0.222, p=0.023) was statistically significant (p<0.05). Accordingly, as the MRCS score average increased, the anxiety about decomposition sub-score increased. On the other hand, there is a positive significant correlation between the total scores of the DDS and TPDAS in COPD patients (r=0.677, p<0.01), and as the total score of patients increases, the total TPDAS score also increases (Table 5).

Table 4. *Distribution of Patients' DSS and TPDAS Total Scores and TPDAS Sub-Scales Mean Scores (n=104)*

	Min-Max Points to be obtained	Min-Max Points obtained in the study	X±SD
DSS Total Scores	0-17	2-17	8.84±3.56
TPDAS Total Scores	0-100	13-88	50.19±15.61
TPDAS Sub Scales			
Anxiety about losing physical and mental function	0-32	0-31	12.32±6.96
Anxiety about the Life After Death	0-24	0-24	15.60±4.96
Anxiety about Decomposition	0-16	0-16	6.45±4.48
Anxiety about Death Process-Pain and Suffering	0-28	4-28	15.80±5.17

Regression Analyses

Regression analysis revealed that marital status, disease duration, chronic respiratory disease in etiology, and self-care were significantly associated with the death depression scale (R: 0.556, R^2: 0.310, F: 3.401, p: 0.000). (R: 0.556, R^2: 0.310, F: 3.401, p: 0.000). The total score was found higher in widowed patients, those with a disease duration of 30 years or more, those with a history of chronic respiratory disease, and those who could meet their self-care needs semi-dependently.

According to the ANOVA analysis of variance performed to determine the differences between the groups in terms of marital status variable, there was a significant difference in widowed participants compared to married ones (F: 5.653, p: 0.11) (p<0.05). In the etiology of the t-test analysis performed to determine the differences between the groups, there was a significant difference between the groups in patients with chronic respiratory system disease (t: 2.149 p: 0.034) and in patients who could meet their self-care needs semi-dependently (t: 2.007, p: 0.047).

Anxiety about Death Process-Pain and Suffering subscale was significantly associated with marital status, duration of illness, willingness to learn in case of deadly disease, and self-care level (R: 0.563, R^2: 0.317, F: 3.520, p: 0.000).

The total score is higher in singles, those with a disease duration of 10-29 years, those who did not want to learn in case of a deadly disease, and those who could meet their self-care needs semi-dependently. According to the ANOVA analysis of variance performed to determine the differences

between the groups in the marital status variable, a significant difference was found between single and married patients (F: 8.003, p: 0.003), and between widowed and married patients (F:8.003, p: 0.032). In the t-test analysis performed to determine the differences between the groups, a significant difference was found in those who did not want to learn the truth in case of a deadly disease and the others (p<0.001, t: 4.723) (Table 6).

Table 5. *The Relationship between Patients' Death Depression Scale-Thorson-Powell Death Anxiety Scale Scores and MRCS scores (n=104)*

	DDS Total Score	Anxiety About Losing Physical and Mental Function	Anxiety about the Life After Death	Anxiety about Decomposition	Anxiety about Death Process – Pain and Suffering	TPDAS Total score
MRCS	r=0.231 p=0.018**	r=0.116 p=0.241**	r=0.013 p=0.893**	r=0.222 p=0.023**	r=0.010 p=0.919**	r=0.115 p=0.246**
DDS						r=0.677* p=0.000***

**Pearson Correlation Analysis, **Spearman Correlation test p<0.05, ***p<0.01*

Table 6. *Regression Analyses*

Death Depression Scale	B	Standard Error	β	t	P
Age	-0.067	0.039	-0.181	-1.695	0.093
Gender	-0.891	0.865	-0.104	-1.030	0.306
Marital Status	1.719	0.456	0.349	3.770	**0.000**
Family Structure	0.947	0.722	0.124	1.312	0.913
Education level	0.358	0.610	0.068	0.586	0.559
Duration of the disease	0.881	0.416	0.218	2.119	**0.037**
The frequency of hospitalization	-0.224	0.335	-0.069	-0.668	0.506
Having a history of respiratory disease	-1.423	0.679	-0.199	-2.097	**0.039**
Meeting self-care needs	2.428	0.895	0.253	2.713	**0.008**
Receiving family support during disease	-1.386	0.746	-0.177	-1.859	0.066
The most disturbing symptom of COPD	-0.405	0.505	-0.078	-0.802	0.425
MRCS	0.508	0.354	0.172	1.437	0.154
R: 0.556 R^2: 0.310 F: 3.401 p: 0.000					
Anxiety about Death Process-Pain and Suffering Subscale	B	S.E	β	t	P
Age	-0.055	0.055	-0.103	-1.001	0.320
Gender	-1.754	1.240	-0.141	-1.414	0.161
Marital Status	2.124	0.648	0.298	3.277	**0.001**
Education level	1.281	0.932	0.167	1.375	0.173
MRCS	-0.467	0.502	-0.109	-0.931	0.354
Duration of the disease	1.655	0.626	0.283	2.645	**0.010**
The frequency of hospitalization	-0.776	0.515	-0.165	-1.507	0.135
Having information about disease and prognosis	1.180	1.022	0.115	1.154	0.251
The desire to know the truth in case of a deadly disease	2.015	0.744	0.246	2.709	**0.008**
The importance of religious beliefs in daily life	-1.300	1.044	-0.114	-1.244	0.217
Having regular doctor visits	0.827	1.040	0.071	0.795	0.429
Meeting self-care needs	4.084	1.354	0.293	3.016	**0.003**
R: 0.563 R^2: 0.317 F: 3.520 p: 0.000					

4. DISCUSSION

Patients with COPD can experience many symptoms and associated problems. In this study, half of COPD patients were determined to be in a depressive mood (51%), and almost all had mild and moderate (44.2%; 40.4%) levels of death anxiety, respectively.

One of the most significant factors that can cause death anxiety is chronic diseases. However, the presence of any

chronic disease does not always increase death anxiety. Studies show that the presence of physical problems and/or serious health problems, mostly seen in chronic diseases, affects death anxiety (20). In a study conducted in Turkey, moderate and mild death anxiety were seen in patients with Myocardial Infarction (MI) and cancer, respectively (10). Sahan et al. (2018) suggested that the reason why patients with a history of MI had higher levels of death anxiety than cancer patients may be due to facing death unexpectedly. In our study, the total score on the death anxiety scale was found similar to that of MI patients in Sahan's study. This situation may be the result of COPD patients' fear of not being able to breathe due to dyspnea, which makes them feel at the edge of death.

The thought of death has an inevitable impact on human life, and excessive, extreme thought of death negatively affects human psychology (20). Also, a positive correlation was found between death depression and death anxiety total scores ($p<0.01$). As the total death depression score increased, the death anxiety total score increased. Thus, death depression can be considered a reflection of death anxiety.

Dyspnea is the major symptom of COPD disease and the main cause of many other complaints. It can lead to death depression by confronting the individual with death (21). A positive correlation was found between the severity of dyspnea and the death depression and the subscale of anxiety about decomposition mean score in this study. As the MRCS mean score increased, the subscale of anxiety about decomposition mean score increased too. Gokcek et al. (2019) argued that the level of depression due to dyspnea increased, and the risk of depression increased in patients with increasing severity of COPD (21). Marko et al. (2006) observed higher anxiety rates in severe COPD patients (22). Voogda et al. (2011) also revealed a significant positive relationship between exertional dyspnea and anxiety (23). However, Kayhan et al. (2013) determined no statistically significant relationship between dyspnea severity and the scores of depression, state anxiety, and trait anxiety in patients with COPD and asthma (24). Physical limitations associated with dyspnea in COPD patients can cause individuals to feel insufficient, experience a change in role performance, move away from work and social life, and create an inability to fulfill their self-care needs, which may lead individuals to experience depression. Therefore, how patients perceive dyspnea is very important because a high perception of dyspnea indicates high a depressive emotional mood (21).

Regression Analysis Discussion

According to the results of the regression analysis in the study, marital status, duration of illness, chronic respiratory disease in the etiology and self-care for the death depression scale, marital status, duration of illness, willingness to learn in case of a deadly disease, and self-care are predictors for the sub-dimension of death anxiety. On the other hand, no significant relationship was found between age, gender, education level, death depression, death anxiety subscales, and total scores. The reason for the lack of a significant relationship with the age variable may be due to the close distribution (peak value) of the ages of the patients in the sample. On the other hand, female patients' mean scores were found to be higher. In traditional cultures like Turkish culture, men are expected to be braver and stronger than women. Therefore, death anxiety may have been found higher in women depending on this cultural feature. Women are thought to express their feelings more easily because they are emotionally and cognitively different from men. Women express their feelings more easily because they are different from men emotionally and cognitively. For this reason, it is thought that the mean death anxiety scores in women are higher in our study. A human being is a psychosocial entity, and his/her spouse and family have a positive effect on establishing social relations and dealing with difficult life events (25, 26). Therefore, the presence of spouses and children is thought to be effective in reducing death anxiety. In this study, marital status was found to be a significant determinant of death depression and death anxiety. The mean death depression score of widowed patients was significantly higher than that of married patients. The mean death anxiety subscale score was higher in single patients than in married patients, and it was higher in widowed patients than married patients ($p<0.005$). Tutuk and Altun (2014) also reported that the depression mean scores of widowed patients and anxiety scores of those separated from their spouses were higher than the other groups in their study with COPD patients (27). In a study carried out with patients with heart failure, Bayrak et al. (2019) argued that single patients had higher death anxiety than married patients (28). Nal et al. (2016) stated that the death anxiety scores of widowed patients with COPD were higher than married and single patients (29). However, Acehan and Aker (2013) did not find a statistically significant difference between marital status, death depression, and death anxiety (11).

In our study, chronic respiratory system disease in etiology is a predictor of death depression. Prolonged disease and treatment durations in chronic diseases lead to psychosocial problems such as anxiety and depression (30). It is stated in the literature that health problems can increase death anxiety and fears. In other words, as anxiety about the body increases, death anxiety also increases. The individual who monitors changes in his body with anxiety may tend to establish a relationship between disease and death (31).

According to the regression analysis, the duration of diagnosis is a factor that may affect the depression and death anxiety sub-dimensions. The total score is higher in patients with a longer diagnosis period. The total score was found higher in patients with longer disease duration. Consistent with our study, Nal et al. (2016) reported that disease duration affected death anxiety, and death anxiety increased in individuals with COPD as the disease duration increased (29). Tutuk and Altun (2014) emphasized that when the disease duration of COPD patients and their hospital anxiety depression mean scores were compared, the anxiety mean score was found

significantly higher in those who had been ill for 1-5 years, and the depression mean score was higher for those who had been ill for 11 years or more (27). On the contrary, in their study with COPD patients, Kayhan et al. (2013) reported no significant relationship between the severity of depression and the duration of the disease (24). Likewise, Aydemir et al. (2015) found no significant relationship between disease duration and anxiety and depression scores in hospitalized patients with chronic heart disease and chronic respiratory disease (9). In this study, the duration of the disease was determined to affect the death depression, and anxiety. Accordingly, the mean death anxiety score of those with disease duration of 11-20 years was higher than the others. As the duration of the disease increases, the disease progresses, and the frequency and severity of the symptoms increase, so the repeated hospital admissions and hospitalizations increase. In addition, individuals' witnessing some symptoms that evoke closeness to death (e.g., the severity of symptoms and increased frequency of attacks) and continuous exposure to them may increase death anxiety.

Having a deadly disease and not being willing to learn about it are predictors of the death anxiety sub-dimension. This result may be an approach to get rid of existing painful conditions. Similarly, Dougherty et al. (1986) found a positive correlation between death anxiety and denial in their study on cancer patients. It can be suggested that patients who are more anxious about death do not want to face the reality of a deadly disease, and this situation increases their death anxiety (32).

The results showed that death-related depression and death anxiety sub-dimension scores were higher in patients who could meet their self-care needs semi-dependently than those who could meet their self-care needs independently. Nal et al. (2016) found that COPD patients in need of home care had higher death anxiety scores (29). On the contrary, Magrebi Kurt and Akçay (2019) stated that there was no significant relationship between death anxiety and dependency levels in their studies (12). The results of this study are similar to those of Nal et al. (2016) (29). In line with these results, it can be argued that being dependent on the care of others increases death anxiety and death depression.

5. CONCLUSION

This study demonstrated that death depression and death anxiety in COPD patients may have significant behavioral and emotional widespread effects. According to the results, the following patients should be evaluated in terms of death anxiety and death depression and necessary support should be provided; patients who are single, who are without spouse support, who has a disease of long duration, who has a history of chronic respiratory disease, who do not want to know the truth in case of a deadly disease and who are dependent on others in meeting self-care needs. It is concluded that dyspnea increases death depression and death anxiety. Therefore, it is recommended that nurses should monitor COPD patients with increasing dyspnea levels more closely in terms of death anxiety and death depression. Health professionals working with COPD patients need to pay attention to determining how patients can cope with death anxiety, develop effective strategies, increase the support resources, and ensure continuity of education and treatment after discharge. In the treatment of COPD, not only medical treatment but also a psychosocial approach should be adopted. It is recommended to conduct further qualitative research on nurses working with COPD patients and patients receiving COPD treatment to determine their views on death.

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Clinical and Experimental Health Sciences

A Prospective Study Concerning the Effect of Pharmaceutical Care Services on Patients with Heart Failure

Neda Taner, Barkın Berk

Istanbul Medipol University, Faculty of Pharmacy, Department of Clinical Pharmacy, Istanbul, Türkiye.

Correspondence Author: Neda Taner
E-mail: ntaner@medipol.edu.tr
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ABSTRACT

Objective: Heart failure (HF), caused by an abnormality in cardiac function, is the inability of heart tissue to pump blood or deliver sufficient oxygen, resulting in abnormal diastolic volume. Drug-Related Problems (DRPs) can cause significant but preventable morbidity and mortality once specific medication errors and their contributing factors are identified. The aim of this prospective study is to determine the effect of pharmaceutical care in patients with heart failure in a Turkish hospital.

Methods: A total of 160 patients with heart failure (80 patients in the control group, 80 patients in the intervention group) were examined at a university hospital. The results of the Pharmaceutical Care Survey were evaluated in accordance with the objective of the study. In addition, using the Pharmaceutical Care Network Europe (PCNE) classification system V8.01, the role and importance of the clinical pharmacist in identifying, preventing and resolving drug-related problems encountered during the treatment of two groups was assessed. The number and causes of potential DRPs were taken into scrutiny.

Results: Comparing the results of the Pharmaceutical Care Survey in both groups at the end of the 6th month, the study group shows a significant improvement in the rates of "forgetting to take medication" (2.9%) and "experiencing any side effects from your drug" (4.5%). Compared to other problems, ineffectiveness of the drugs used in treatment was reported as the most common drug-related problem (n=23; 28.7%) in the study group (p<.05). 72.5% of the proposed interventions were accepted and the problem was found to be resolved in 31% of the patients.

Conclusion: In this study, it is discussed that clinical pharmacists can play an active role in resolving DRPs in heart failure patients. It is therefore can be predicted that the training of information and warnings conveyed by the clinical pharmacist to the intervention group will make a significant contribution to the health of the patient within the framework of pharmaceutical care.

Keywords: Clinical Pharmacy, Drug-related problem, Heart Failure, Pharmaceutical Care

1. INTRODUCTION

Heart failure (HF) is a serious, life-threatening chronic condition associated with certain filling and drainage abnormalities in heart structure, function, rhythm, or conduction that rupture (1, 2). Chronic diseases are the main cause of morbidity and mortality worldwide. The prevalence of heart failure varies mainly between adult and elderly populations with a treatment approach that slows disease progression and relieves patients' symptoms (3).

Preclinical heart failure is four times more common than symptomatic heart failure. The criteria for diagnosing chronic heart failure (CHF) were established in accordance with the guidelines for the diagnosis and treatment of heart failure published by the European Society of Cardiology in 2016, emphasizing that self-care is an essential part of HF management and the drugs used in the pharmacotherapy of heart failure (4, 5, 6).

For HF pharmacotherapy, the drug related problem (DRP) is one of the main problems that need to be defined, identified and used to solve the situation. The DRP is defined as the effect of a disease that is intended to be achieved with medication treatment, with an existing or potential negative situation stemming from the drug itself. Because DRPs deal with current or potential problems and can be identified as side effects or drug failures, the grading of the urgency of the situation in terms of resolution depends on potential injuries, harms, and the risk rate of that harm to the patient,

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which correlates primarily with the Rational drug use (RUD) concept. This is also a key point where clinical pharmacists have an important role to play when it comes to involving patient care pharmacists in DRP therapy with other healthcare professionals (7, 8). Many studies have shown that pharmaceutical care reduces PRM status by 50-80% by reducing the number of adverse drug reactions (ADRs), length of hospital stay and maintenance costs (9, 10) .

The aim of the present study is to identify the clinical pharmacist's role in the prevention and elimination of drug-related problems, particularly in patients with heart failure, in a full-capacity university hospital setting, which was attempted to determine with a Pharmaceutical Care Survey (ordered by the classification of the European Association of Pharmaceutical Care Network (PCNE)) (11).

2. METHODS

This was a prospective study that randomly enrolled 160 adult patients of both genders. Patients in the study group were admitted to the hospital with a diagnosis of heart failure, who were hospitalized, and discharged. At discharge, the patients in the study group were randomly divided into two subgroups of 80 and designated as the control and intervention groups

The patients' registration sequence numbers during their hospital stay were processed in the random number generator program and the random numbers generated by the program were organized and the first 80 patient groups were assigned as a control group and the second 80 patient groups as an intervention group.

Simultaneously, the PCNE classification (version 8.01) was performed for both groups to monitor DRPs, and the validated "Pharmaceutical Care Survey" was used to assess the role and contribution of the clinical pharmacist in improving its measuring criteria (12).

The roles assumed in the association of physicians and clinical pharmacists for both groups are as follows: Physicians performed the clinical follow-up of the control group after discharge. Apart from their binding recommendations, they gave no further information. To the study design, there was no further information attempt by clinical pharmacists on this group.

In the intervention group, in addition to the doctors' usual clinical follow-ups, the clinical pharmacists repeated their information on the drug related problems in the PCNE classification and applied the "Pharmaceutical Care Survey", which would measure the improvement in patient monitoring.

The first step in the study was the discharge step of the patients. Three further steps were created to enable outpatient follow-up after discharge. These steps are referred to as:

1. Month Interval Outpatient Polyclinics: This is the step of following the routine control and recommendations of all patients summoned by doctors one month after discharge. The doctors performed routine examinations for all patients in the control and study groups. In addition to medical interventions, clinical pharmacists informed only 80 patients verbally in the intervention group about their prescription medications, gave them dietary advice, and answered patient questions. Standardization to ensure the readability of patient-pharmacist verbal interactions was accomplished by adapting the Pawson review protocol questions as communication tools, e.g. *"what works, for whom, in what circumstances, in what respects, to what extent and why"* (13,14). They also applied the "Pharmaceutical Care Survey" to patients.

3. Month Interval Clinical Pharmacy Department Communication: Clinical pharmacists attempted to answer questions and **inform** patients **over the phone** about the disease and their medications by calling patients in the intervention group.

6. Month Interval Routine Outpatient Clinics: In the fourth step of the study, the procedure carried out in the second step (month 1) was repeated again by the same team.

While the cardiologist performed a classic standard practice clinical assessment in both groups, the clinical pharmacist applied the PCNE classification system and a Pharmaceutical Care Survey.

3.1. Ethical Aspects

The study was approved by the Ethics Committee of Non-Interventional Clinical Investigations of Istanbul Medipol University (approval number 186 and date 16.05.2017).

3.2. Statistical Analysis

The descriptive statistics included the average, standard deviation, lowest and highest values. The number of DRPs was presented as an (%). The independent samples t-test was employed for the analysis of quantitative independent data. Categorized data were analyzed using the Chi square or Fisher exact test when required. A confidence interval of 95% and p value of <.05 were considered significant. The SPSS 22.0 statistical software package was used for all statistical analyses.

3. RESULTS

The aim of the present study is to identify drug-related problems, especially in patients with heart failure, to plan interventions for these problems, and to evaluate the effect of the clinical pharmacist in the cardiology room considering the acceptability/rejection of the interventions.

The gender distribution was 45% females and 55% males and 36% females and 64% males in the accompanying

intervention group. Gender distribution was not statistically significant at the p=.261 levels.

According to the Pharmaceutical Care Survey, patient responses to questions were converted to percentages of frequency and statistically evaluated using the chi-square test. Table 1 summarizes the differences in patient responses to Questions (Qs) 2, 4, 5, 7, 8, 9 and non-significance between the two groups is indicated.

Statistically significant differences were calculated for the remaining questions (p<.05). More specifically, compared to the control group, the intervention group was more likely not to forget to take their medication and was more aware of the importance of taking it. (Q1); experienced side effects of their medications (Q3); paid attention to changes in their body weight (Q6); and they were better informed and more aware of developing a productive cough (Q10).

As for the control and intervention groups, the detailed DRP data mentioned below, collected from the Pharmaceutical Care Survey, were reassessed at the end of the 6-month interval.

According to this assessment, 2 patients (2.9%) in the intervention group answered yes and 67 patients (97.1%) answered no due to the condition "forgot to take medication". On the other hand, 29 patients (36.2%) in the control group answered yes and 51 patients (63.7%) answered no.

The responses of the patients in the intervention group to the parameter "experience of side effects of the recommended drug" were "yes" in 3 patients (4.5%) and "no" in 64 (95.5%) patients, while these values were at 39 patients (48.8%) of the control group answered yes and 41 (51.2%) patients answered no.

The answers to the question "diet-related body weight gain" were as follows: 19 (29.2%) of the patients in the intervention group yes, 46 (70.8%) no; 43 (53.8%) of the patients in the control group answered yes and 37 (46.2%) no.

The responses to the question "development of a productive cough" are as follows: 16 (23.2%) of the intervention group answered yes, 53 (76.8%) no; 35 (43.8%) of the control group answered yes, 45 (56.2%) no. A statistically significant difference in the development of the "no" and "yes" answers to these question parameters was found between the groups and is shown in Table 1.

As shown in Table 2, when examining patient-reported reasons for DRPs, the most common in the control group were inappropriate medications or combinations of medications, while in the intervention group no medication was prescribed despite the current indication.

Table 3 shows the types of DRP. According to the results, all patients required intervention for a problem related to the medication they were taking that was relevant to their disease. The adverse drug event that occurred was the most common DRP (50%) with a statistically significant difference (p<.05) compared to all other problem types in the control group. Inadequate drug response was the most common DRP (28.7%) with a statistically significant (p<.05) compared to the remaining problem types in the study group. Cost-related problems were not identified in any of the patients.

Of the interventions of this study, 72.5% were accepted, 6.2% were not accepted, and 21.2% were included in the other category.

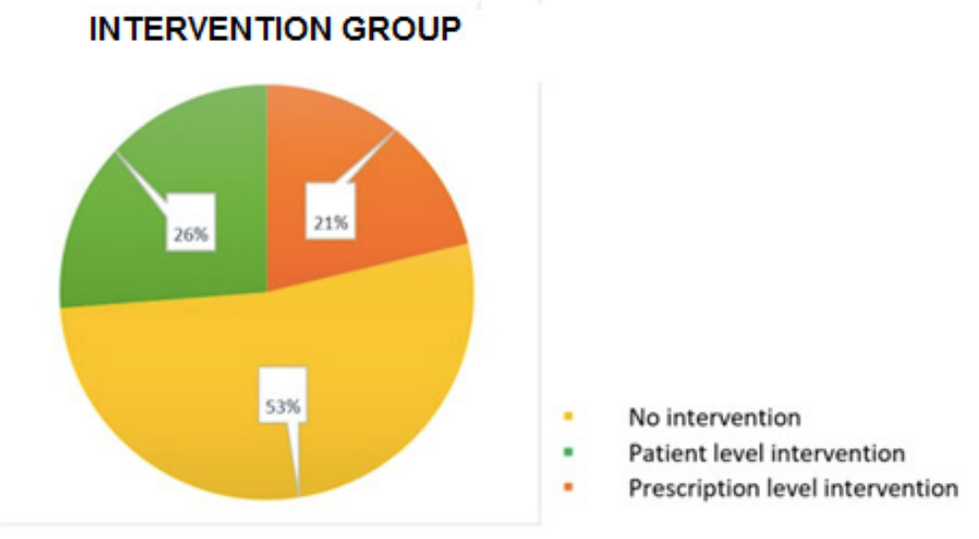

Figure 1. *Types of clinical pharmacist intervention*

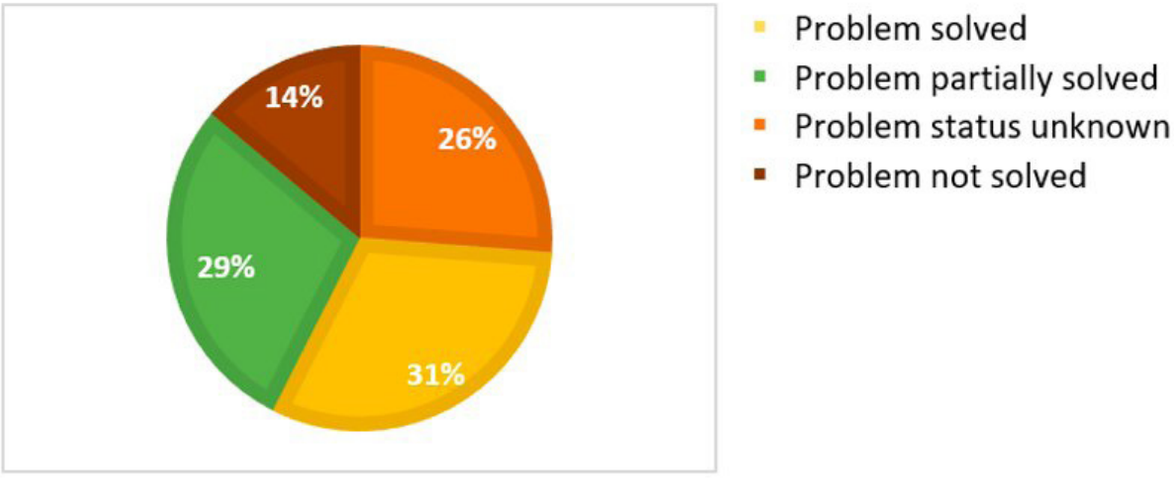

Figure 2. *Clinical Pharmacist Intervention Results*

Table 1. *Comparison of the Sixth-Month Pharmaceutical Care Survey*

		Intervention		Control		p
		n	%	n	%	
Have you ever forgotten to take your medicine or preferred not to take it?	Yes	2	2.9%	29	36.2%	p<.05
	No	67	97.1%	51	63.7%	
Do you know what you need to do when you miss a dose of your medicine?	Yes	51	83.6%	61	76.2%	p>.05
	No	10	16.4%	19	23.8%	
Have you experienced any side effects from your medication?	Yes	3	4.5%	39	48.8%	p<.05
	No	64	95.5%	41	51.2%	
Do you have a blood test regularly?	Yes	54	94.7%	73	91.2%	p>.05
	No	3	5.3%	7	8.8%	
Do you smoke?	Yes	6	8.8%	13	16.2%	p>.05
	No	62	91.2%	67	83.8%	
Have you observed any changes in your weight several times?	Yes	19	29.2%	43	53.8%	p<.05
	No	46	70.8%	37	46.2%	
Have you observed any swelling or increased swelling in your ankles?	Yes	40	63.5%	46	57.5%	p>.05
	No	23	36.5%	34	42.5%	
Have you noticed any shortness of breath or an increase in your breathing problems during exercise?	Yes	56	82.4%	64	80.0%	p>.05
	No	12	17.6%	16	20.0%	
Have you noticed any shortness of breath or an increase in your breathing problems during sleep at night?	Yes	34	52.3%	46	57.5%	p>.05
	No	31	47.7%	34	42.5%	
Do you have a productive cough?	Yes	16	23.2%	35	43.8%	p<.05
	No	53	76.8%	45	56.2%	

Table 2. *Causes of DRPs in Control and Study Groups*

		Intervention		Control	
		n	%	n	%
C	Inappropriate drug	0	0.0%	2	2.5%
	Inappropriate combination of drugs or drugs and herbal medication	6	7.5%	21	26.2%
	No drug treatment in spite of existing indication	20	25.0%	13	16.2%
	Numerous drugs are prescribed for indication	0	0.0%	1	1.2%
	Drug dose too low	2	2.5%	1	1.2%
	Drug dose too high	6	7.5%	14	17.5%
	Dosage regimen not frequent enough	2	2.5%	1	1.2%
	Dosage regimen too frequent	3	3.8%	2	2.5%
	Necessary information not provided	1	1.2%	0	0.0%
	Wrong drug, strength or dosage advised	3	3.8%	1	1.2%
	Wrong drug or strength dispensed	1	1.2%	0	0.0%
	Inappropriate timing of administration and / or dosing intervals	4	5.0%	0	0.0%
	Drug under–administered	1	1.2%	0	0.0%
	Drug over-administered	2	2.5%	0	0.0%
	Drug not administered at all	3	3.8%	0	0.0%
	Wrong drug administered	1	1.2%	2	2.5%
	Patient uses less drug than prescribed or does not take the drug at all	3	3.8%	0	0.0%
	Inappropriate timing or dosing intervals	7	8.8%	8	10.0%
	Patient uses the drug in a wrong way	5	6.2%	1	1.2%
	Patient unable to use the drug as directed	1	1.2%	0	0.0%
	Medication follow-up is not done properly	9	11.2%	13	16.2%

Table 3. *Types of problems in control and intervention groups*

		Intervention		Control	
		n	%	n	%
P	No effect of drug treatment	15	18.8%	5	6.2%
	Effect of drug treatment not optimal	23	28.7%	24	30%
	Untreated symtomps or indications	19	23.8%	9	11.2%
	Advers drug event occuring	13	16.2%	40	50%
	Unnecessary drug treatment	4	5%	0	0%
	Unclear problem or complaint	6	7.5%	2	2.5%

4. DISCUSSION

In the study by Roblek et al. (2016), in which DRPs were evaluated in 213 patients with heart failure, 66 clinically significant DRPs were found in 51 patients. As a result of the interventions performed, it was found that the number of patients with DRP at discharge was significantly lower (10 versus 31; p=.0049). The results show the importance of clinical pharmacist intervention to reduce the number of patients with drug-related problems (15).

In another study, Sadik et al. (2005) evaluated the effect of a pharmacist-led pharmaceutical care follow-up program on optimizing pharmacological therapy in 160 patients with heart failure, and at the end of 12 months, enlightened patients were unaffected by the prescribed medications (85 vs. 35) and lifestyle changes (75 vs. 29) showed higher compliance than control patients (16).

In the study by Varma et al. (1999) 83 patients with heart failure were educated about lifestyle changes, monitoring of

their symptoms, and pharmacologic treatments to evaluate the outcome of a pharmaceutical care program. As a result of the 12-month study, it was documented that patients who received training showed better adherence to therapy and increased physical performance (17).

In this study, as in the studies by Sadik and Varma et al., the results obtained showed that patient awareness increased, particularly in the intervention group. Patients in the intervention group, who were informed and trained in the field of pharmaceutical care, forgot to take their medication less often than the control group and became more aware of the importance of taking medication. They were found to be more aware of the side effects they may experience associated with drug therapy. They also paid more attention to maintaining their body weight.

In the studies by Chambela et al. (2020) to emphasize the importance of pharmaceutical care in 81 heart failure patients, found that DRPs were reduced in all patients in the intervention group compared to baseline DRPs. The ADR, which was 17.5% at the start of the study, fell to 8.8% at the end of the 12th month. In the control group, the ADRs remained at the same level from the start to the end of the study (18).

In the study by Adriano et al. (2017) found that pharmaceutical care services prevent the development of DRP by 50-80% and reduce the number of ADRs (9). In the study by Winterstein et al. (2002) reported that in a study of patients hospitalized for DRP, about 60% of DRP could be prevented (19). Shastry et al. (2019) evaluated drug interactions and adverse drug reactions in 120 patients with ischemic heart disease and reported that 40% of the adverse drug reactions identified were preventable (20).

In this study, DRPs were shown to completely resolve as a result of the interventions in 31% of patients. For 26% of the patients who underwent the intervention, the results of the interventions are unknown for various reasons.

In the study conducted in the clinical pharmacy unit of Lycksele Hospital in Sweden, interventions for DRPs were performed in 88% of cases. The rate of patients with medication-related problems was 66% (68/103), and the most common DRPs were inappropriate medication use (39/133), drug interaction (21/133), incomplete medication (12/133, and overdose (12/133) (21).

In the study by Dempsey et al. (2017) to identify the important role of pharmacists in 60 patients diagnosed with heart failure and to document the prevalence of problems related to drug therapy, 304 drug-related problems were identified. In 22% of drug-related problems, pharmacists intervened to change the medication regimen. According to the results obtained, all patients in this study required intervention due to some problems with their pharmacotherapies. It was found that 53% of patients in the study group required intervention at the prescribed level. 72.5% of the interventions were successful, while 6.2% did not accept them. Patient-level interventions were performed in 26% of patients. The most common drug-related problems in patients have been reported as an untreated indication or inadequate treatment and drug interactions (22).

In our study, the most common drug-related problem in the group of patients in whom we conducted the drug survey was non-optimal drug response (28.7%). In the uninformed and educated control group, adverse drug reactions (50%) were the most common problem. When examining the underlying causes of drug-related problems, inappropriate drug use or combination of drugs was the most common cause in the control group; non-prescribing of medication despite current indication is the most common reason identified in the intervention group. Our findings are like these studies in relation to the types and causes of drug problems.

The results of these two studies, supported by the Lycksele Hospital Study and Dempsey et al. (2017) point out the importance of advice and information, training and the role of the clinical pharmacist in relation to pharmaceutical care services and show that this makes a positive contribution to the health of the patient. Like the other studies mentioned here, these studies also agree with the results of our present study.

As indicated in Table 1, the collected data from the "Pharmaceutical Care Survey" were reassessed at the end of the 6th month with regard to the control and intervention groups. After that, patients in the intervention group were informed about the parameters "forgetting to take medication", "experience of side effects of the recommended medication, diet-related increase in body weight and occurrence of productive cough". If this information process is counted as training, the positive results in the intervention group are statistically significant compared to the control group.

Drug-related problems are common in most people with various illnesses. Patient education or training is an important criterion in solving drug-related problems. Although patient education does not cover all pharmaceutical care services, it occupies an important place within the clinical pharmacist's services. When evaluating the results, the intervention group treated with medication differed significantly from the control group. This result can be considered a good measure of the importance of pharmaceutical care and the functional benefit of clinical-pharmaceutical services.

In this study, it is argued that clinical pharmacists can play an effective role in improving the DRPs of heart failure patients, and it can be predicted that the information and warning training provided by the clinical pharmacist to the intervention group will make a significant positive contribution for the patient's health as a follow-up of pharmaceutical care.

This study has some limitations. First, it was conducted in a single center with a limited number of patients. Additionally, the limitations here are to only assess the prevalence of DRP-related PCNE criteria's in a single center. Despite the limitations of this study, it also validates the importance

of clinical pharmacy services in identifying, resolving, and preventing DRPs in heart failure consultations. Our DRP findings from this study become more tangible when the extent to which patient education can contribute to treatment is replicated at the multicenter and country levels.

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Clinical and Experimental Health Sciences

Evaluation of Nursing Care in the Early Postpartum Period

Seda Karacay Yikar[1], Ebru Gozuyesil[2], Evsen Nazik[1], Ebru Var[3]

[1] Cukurova University, Faculty of Health Sciences, Department of Obstetrics and Gynecologic Nursing Adana, Türkiye.
[2] Cukurova University, Faculty of Health Sciences, Department of Midwifery, Adana, Türkiye.
[3] Adana Sehir Egitim ve Arastirma Hastanesi, Adana, Türkiye.

Correspondence Author: Seda Karacay Yikar
E-mail: sedakrcyyikar@gmail.com
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ABSTRACT

Objective: Evaluation of the care given in the postpartum period is vital in terms of the quality of care. The aim of this study was to evaluate nursing care in the early postpartum period.

Methods: This descriptive and cross-sectional study was conducted in a postpartum clinic between December 2016 and January 2017 with 130 mothers in postpartum period, and 18 nurses and midwives working in the same postpartum clinic. Data were collected using a personal information form, and the Postpartum Nursing Care Evaluation Tool (PPNCET). The data analysis was conducted using percentages, arithmetic mean, independent samples t-test, Mann-Whitney U test, and Kruskal-Wallis test.

Results: The mean age of the mothers was 26.80 ± 4.90, and of the nurses and midwives was 35.83 ± 12.14. The mean PPNCET score of the mothers was 141.58 ± 32.03. The mean care subscale score of the mothers was 69.88 ± 12.06 and of the education subscale was 71.70 ± 19.97. The mean PPNCET score of the nurses and midwives was 153.50 ± 23.18. The mean care subscale score of the nurses and midwives was 73.38 ± 8.84, and the mean education subscale score was 80.11 ± 14.33.

Conclusions: The mean PPNCET score of mothers and nurses and midwives were higher than the average. Higher PPNCET scores of the nurses and midwives compared to the mothers suggests that either the postpartum care given was not perceived as adequate by the mothers, or the care was not served in adequate quality. To increase postpartum care satisfaction of the mothers, involving nurses and midwives in the care process more is suggested. In line with these results, planning in-service training programs to develop the knowledge and skills of the nurses and midwives would be beneficial in increasing the quality of service.

Keywords: postpartum care, nursing care, women, nurse

1. INTRODUCTION

The postpartum period is an important transition period in which physical and psychological changes are encountered by the woman, the new-born, and the family. It is also characterized by regressive and progressive changes occurring in the mother. This period is a challenging time in which a transit to parenting occurs, in addition to rapid physiological changes, while undertaking new roles and responsibilities (1, 2). The aim of care during this period, in which the mother and the new-born both need care more than during any other time, is to facilitate the accommodation of the woman to the physiological and psychological changes, prevent high risk conditions, start the process of bonding between the mother and the child, and to have the mother gain the abilities to take care of herself and the baby (2, 3, 4).

The duration of postpartum hospitalization is 24 hours for vaginal delivery and 48 hours for caesarean section delivery in Turkey. Women who give birth in Turkey are followed three times after the birth at the hospital (first at 1 hour, then between 1-6 hours, and 6-24 hours), and three times at home (between the days of 2-5, 13-17, and 30-42 after birth) (5). Maternal mortality and morbidity rates are high during the postpartum period, and maternal mortality is unacceptably high in the world. According to the WHO (2017) maternal mortality report, 810 women die every day due to pregnancy or birth related problems (6). 99% of all maternal deaths occur in the developing countries. The risk of maternal mortality is highest within the first 24 hours postpartum. Maternal health problems, such as bleeding, occur at this period, and can cause maternal deaths (5). According to the Annual Turkish Health Statistic (2019) data, maternal mortality rate is 13.1 in every 100 000 in Turkey (7).

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Most maternal deaths are avoidable since the healthcare solutions to prevent or manage complications are well known. All women need access to antenatal care during pregnancy, skilled care during childbirth, and care and support after childbirth (5). A healthy and successful adaptation to the postpartum period depends on the physical care, education, and counselling services given by the healthcare personnel to the mother, the baby, and the family (8). Feeling comfortable physically affects the mother's active contribution to the care of self and the baby positively, as well as her success in the continuation of the care and their physical and mental health. Therefore, nurses and midwives should evaluate the healthcare requirements of the mothers in the postpartum period, and provide necessary care and support, facilitating their accommodation to the role of mothering and decreasing the problems that might be encountered in the postpartum period (1, 2). The system and management of care is as important as the evaluation of the efficacy and the quality of the care given in the postpartum period (4).

The World Health Organization reports that only 35% of the women around the world benefit from postpartum services (9). According to the Turkey Demographic and Health Survey (TDHS-2018) data, the rate of receiving postpartum care varies between 83% and 97% in Turkey and depends on the level of education of the mother, age of the mother at the time of the birth, the location, and the region (10).

Early discharge is a routine procedure in the postpartum period. However, most of the signs and symptoms of the postpartum complications occur following discharge. Therefore, it is vital to define early signs of complications and identify mothers under risk, informing the mothers about the alarming signs by performing an extensive evaluation before the discharge (education to women, providing brochures, etc.) (11). In a study by Dag and associates, 66.4% of mothers did not receive information about the discharge and 31.8% of the individuals giving the information were nurses or nursing students (8). On the contrary, some studies in the literature indicate a high rate of mother satisfaction in the postpartum period. Among the mothers, 56%, 88%, and 90% reported that they were satisfied with the care they received in the early postpartum period in a study by Valbo and associates (12).

Patient satisfaction is a multi-dimensional concept including implementation of care, interaction between the patient and the caregiver, presence and continuation of care, adequacy of the caregivers, and communication skills, and has been accepted as an indicator of quality of care (13). Postpartum period is a stage where additional care is needed for the mother and the baby. Care during the postpartum period should be planned to meet the needs of the mother, the new-born, and the family. Care implications practiced in the postpartum period are prevented risks, guided, and helped to orientation family's new position in terms of physical and psychosocial, develop positive health behaviours. Midwives and nurses should critically scrutinize themselves and should re-evaluate themselves in terms of the care and service they provide to increase satisfaction and quality of the healthcare. An evaluation of satisfaction of the women provides an opportunity to fulfill the needs and contributes to professional development (14). Therefore, it is important to evaluate the satisfaction of the group in terms of the implementation and the given education, and to assess the quality of the implementation. The aim of this study was to evaluate nursing care in the early postpartum period**.**

2.METHODS

2.1. Study Design

The descriptive and cross-sectional study was performed at a postpartum clinic in a government hospital between December 2016 and January 2017. Only mothers who delivered a baby through vaginal birth were hospitalized at this postpartum clinic.

2.2. Setting and Sample

The study included two groups. First group included 130 mothers who were hospitalized at the postpartum clinic. Mothers who gave birth by vaginal delivery, who were willing to participate in the study, who had no communication problems, who were at least primary school graduates, who spoke Turkish, and which is next to baby were included in the study. The mothers evaluated postpartum nursing care they received during their hospital stay. The duration of postpartum hospitalization is 24 hours for vaginal delivery in Turkey.

Power analysis was performed for the sample size. In the power analysis performed using GPower 3.1 (http://www.gpower.hhu.de/), sample size calculated using 80% power and alpha = 0.05, and one-sample t-test, was 118 women at postpartum period. Considering the probability of women leaving the study, the study was conducted with 130 mothers.

The second group of the study included all the nurses and midwives working in the postpartum clinic without selecting a sample, which included 18 nurses and midwives working in the same postpartum clinic. Women receive care from nurses and midwives in obstetric clinics in Turkey, and nursing tasks and roles are defined by law, as well as by other regulations and directives. In Turkey, nurses are authorized to provide prenatal and postnatal care to women, with the exception to deliver patients' babies.

2.3. Materials

The data were collected using the "Personal Information Form" for mothers and nurses and midwives, and the "Postpartum Nursing Care Evaluation Tool (PPNCET)" for the evaluation of the nursing and midwifery care.

Personal Information Form for Mothers: The form was prepared by researchers and included mothers' socio-demographic characteristics (age, level of education, working status, economic

status, family type, etc.) and obstetric characteristics (number of deliveries, number of living children).

Personal Information Form for Nurses: This form was prepared by researchers and included socio-demographic characteristics of the nurses and midwives (age, level of education, and marital status), obstetric characteristics (number of living children), and professional characteristics (duration of employment as a nurse or midwife, duration of employment at the postpartum clinic, working hours at the postpartum clinic).

Postpartum Nursing Care Evaluation Scale (PPNCET***):*** This tool was developed by Yıldız Eryılmaz in 1999 to evaluate the care and the education received by the mothers who gave birth through the normal vaginal route (15). The scale is bidirectional and used both with mothers who are receiving care and with the nurses and midwives who are giving care. Using both scales is possible, while it can be used as a single scale directed to the mothers alone or to the nurses alone. The scale consists of two subgroups with a total of 45 items. The subgroups include care and education (items 1-21 related to care, and items 22-45 related to education). The answers are given within a four-point Likert-type scale. Each item is evaluated with the responses of "completely agree," "agree," "partially agree," and "do not agree." The highest score for each item is four and the lowest score is one. The total scores of the complete scale, care subscale, and education subscale are 180, 84, and 96, respectively. These scores are valid for both the mothers and the nurses. High scores for the mean scores of evaluation of postpartum nursing care reflect the efficacy of the care given. Cronbach's alpha internal consistency coefficient was 0.96 for the nurses and midwives, and 0.88 for the mothers. In this study, the Cronbach's alpha internal consistency reliability of the scale was 0.97 for the mothers, and 0.98 for the nurses and midwives.

2.4. Data Collection

A face-to-face interview method to administer the questionnaires by the researcher was used. Data were collected before the mothers were discharged, and at suitable times for nurses and midwives. The interviews lasted approximately 20 minutes.

2.5. Data Analysis

The statistical analyses were performed using the SPSS for Windows version 16.0. Percentage, arithmetic mean, independent samples t-test, One-Way ANOVA Mann-Whitney U test, and Kruskal-Wallis test were used to analyze the data. The level of significance was accepted as $p < .05$.

2.6. Ethical Considerations

The School of Medicine Institutional Review Board reviewed and approved the study in 2016 (50-10/05.02.2016). The women included in the study were informed about the purpose of the study. They were also informed that the information collected would not be read by anyone apart from the researchers, and that the information would be used for scientific purposes, and in this way, verbal consent was obtained. Verbal permission was obtained to use the scale.

3. RESULTS

Descriptive and obstetric characteristics of the mothers, and the nurses and midwives are presented in Table 1.

Table 1. *Descriptive and obstetric characteristics of mothers and Nurses/Midwives*

Descriptive and obstetric characteristics	Mothers (N=130) n	%	Descriptive characteristics	Nurses/ Midwives (N=18) n	%
Age			**Age**		
19 years and younger	7	5.4	20-29	7	38.9
20-24	45	34.6	30-39	3	16.7
25-29	36	27.7	40 years and older	8	44.4
30-34	32	24.6			
35 years and older	10	7.7			
Level of Education			**Level of Education**		
Primary School	66	50.8	High School	3	16.7
Secondary School	36	27.7	Associate degree	6	33.3
High School	28	21.5	Bachelor's	9	50.0
Family Structure			**Marital Status**		
Nuclear Family	93	71.5	Married	10	55.6
Large Family	37	28.5	Single	8	44.4
State of Employment			**Profession**		
Employed	20	15.4	Nurse	7	38.6
Unemployed	110	84.5	Midwife	11	61.1
Level of Income			**Experience year**		
income < expenditure	54	41.5	5 years and less	6	33.3
income = expenditure	65	50.0	6-10 years	3	16.7
income > expenditure	11	8.5	11 years and more	9	50.0
Parity			**Experience year**		
1	30	23.1	**at the Postpartum**	4	17.6
2	40	30.8	**Clinic**	6	35.3
3 and above	60	46.2	1 year and less	8	47.1
			2-4 years		
			5 years and more		
Presence of Episiotomy			**Parity**		
Present	77	59.2	None	8	55.6
None	53	40.8	1	3	16.7
			2	4	22.2
			3	3	16.7
The Room Stayed in at the Hospital			–	-	
A private room	18	13.8			
A regular room	112	86.2			

The comparison of Postpartum Nursing Care Evaluation Scale (PPNCET) and subscale mean scores of mothers, nurses and midwives are presented in Table 2. The mean total PPNCET scores of mothers in this study was 141.58 ± 32.03, while the mean PPNCET scores of the nurses and midwives was 153.50 ± 23.18. Mean scores of mothers for PPNCET care and education subscales were 69.88 ± 12.06 and 71.70 ±

19.97, respectively, and the mean scores of the nurses and midwives of the care and education subscales were 73.38 ± 8.84 and 80.11 ± 14.33, respectively.

Table 2. *Comparison of PPNCET and Subscale mean scores of Mothers and Nurses/Midwives*

PPNCET and Subscales	PPNCET Min-Max	PPNCET Mothers	PPNCET Nurses/ Midwives	
Care Subscale	21-84	69.88+12.06	73.38+8.84	t=-1.187 p=0.237
Education Subscale	22-96	71.70+19.97	80.11+14.33	t=-1.722 p=0.087
Total PPNCET	43-180	141.58+32.03	153.50+23.18	t=-1.581 p=0.116

[a]*Independent Samples t*

An examination of the relationship between descriptive and obstetric characteristics of the mothers and the total PPNCET scores demonstrated that mothers who had graduated from primary school, lived in nuclear families, whose income met their expenditure, who had parity 2, who were not given episiotomy, and stayed in a normal room in the hospital, had the highest PPNCET scores. The total PPNCET mean scores were not statistically significantly associated with age (KW = 6.086, *p* = 0.107), level of education (*KW* = 0.07, *p* = 0.964), family structure (t = 1.215, *p* = 0.227), state of employment (MWU = 881.500, *p* = 0.158), parity (F = 0.263, *p* = 0.7709), or staying in normal room in the hospital (MWU = 937.500, *p* = 0.634) of the mothers. There was a statistically significant relationship between the total mean scores of the PPNCET and the mothers' levels of income (KW = 9.820, *p* = 0.007) (Table 3).

The mean scores on the PPNCET care subscale were statistically significantly associated with the mothers' level of income (KW = 12.713, *p* = 0.002). There was also a statistically significant association between the mean scores on the PPNCET education subscale, and the mothers' level of income (KW = 7.685, *p* = 0.021), and between the mean scores on the PPNCET education subscale and receiving episiotomy (t = – 1.927, *p* = 0.05) (Table 3).

An examination of the relationship between descriptive and obstetric characteristics of the nurses and midwives and the total PPNCET scores demonstrated that nurses and midwives who were aged between 20-29, who were high school graduates and nurses, who were single, who had worked five years or less, who worked in the postpartum clinic for more than a year, and who had not given birth, had the highest PPNCET scores (Table 4).

There was a statistically significant relationship between the mean scores of the PPNCET care subscale and the nurses and midwives' age (KW = 7.239, *p* = 0.027), marital status (MW-U = 9.000, *p* = 0.006), and experience in years at the postpartum clinic (KW = 5.758, *p* = 0.05). The mean scores on the PPNCET education subscale were statistically significantly associated with the nurses/midwives' age (KW = 10.396, *p* = 0.006), and experience in years at the postpartum clinic (KW = 6,277, *p* = 0,04). There was also a statistically significant relationship between the mean scores on the total PPNCET, and the nurses and midwives' age (KW = 9.535, *p* = 0.009), marital status (MW-U = 14.000, *p* = 0.021), and experience in years at the postpartum clinic (KW = 6.577, *p* = 0.037) (Table 4).

Table 3. *Comparison of descriptive and obstetric characteristics of mothers with PPNCET*

Descriptive and obstetric characteristics	PPNCET Care Subscale X±SS	PPNCET Education Subscale X±SS	PPNCET Total X±SS
Age			
19 years and younger	66,28±11,62	56,42±21,58	122.71±32.75
20-24	66,15±14,05	69,08±21,38	135.24±34.12
25-29	74,00±10,59	74,80±18,57	148.81±27.70
30-34	71,50±10,91	74,71±19,47	146.22±29.59
35 years and older	69,20±5,24	73,40±15,32	142.60±19.55
	KW= 8.776[b]	**KW= 5.300**[b]	**KW= 6.086**[b]
	P= 0.067	**P= 0.258**	**P= 0.107**
Level of Education			
Primary School	70,33±12,29	71,89±20,45	142.23±31.64
Secondary School	69,69±11,99	70,58±20,79	140.28±31.36
High School	69,07±12,02	72,71±18,33	141.79±28.80
	KW=0.452[b]	**KW=0.105**[b]	**KW=0.07**[b]
	P=0.798	**P=0.949**	**P=0.964**
Family Structure			
Nuclear Family	70,81±11,82	72,83±19,93	143.66±30.75
Large Family	67,54±12,52	68,86±20,06	136.41±30.54
	t=1.402[a]	**t=1.021**[a]	**t=1.215**[a]
	P=0.163	**P=0.311**	**p=0.227**
State of Employment			
Employed	73,75±9,54	77,35±15,56	151.10±23.51
Unemployed	69,18±12,37	70,68±20,57	139.86±31.67
	KW=885.500[b]	**KW=906.500**[b]	**MWU=881.500**[b]
	P=0.165	**P=0.210**	**p=0.158**
Level of Income			
income < expenditure	66,00±12,10	66,51±19,87	132.52±30.50
income = expenditure	73,35±11,40	76,06±19,73	149.42±29.91
income > expenditure	68,45±10,36	71,45±17,13	139.91±26.37
	KW=12.713[b]	**KW=7.685**[b]	**KW=9.820**[b]
	P=0.002	**P=0.021**	**p=0.007**
Parity			
1	70,70±13,03	68,80±21,88	139.50±3395
2	69,45±12,98	75,00±18,96	144.45±31.01
3 and above	69,76±11,09	70,96±19,67	140.73±29.27
	F=0.096[c]	**F=0.901**[c]	**F=0.263**[c]
	P=0.909	**P=0.409**	**P=0.770**
Presence of Episiotomy	68,63±12,22	68,93±20,59	137.57±31.36
Present	71,69±11,71	75,73±18,49	147.43±29.15
None	**t=-1.427**[a]	**t=-1.927**[a]	**t=-1.813**[a]
	P=0.156	**P=0.05**	**p=0.07**
The Room Stayed in at the Hospital	69,94±10,94	69,61±18,06	139.56±27.03
A private room	69,87±12,28	72,04±20,32	141.92±31.40
A regular room	**KW=995.000**[b]	**KW=919.500**[b]	**MWU=937.500**[b]
	P=0.990	**P=0.549**	**p=0.634**

[a]*Independent Samples t,* [b]*Test Kruskal-Wallis* [c]*One-Way ANOVA*

Table 4. *Comparison of descriptive characteristics of Nurses/ Midwives with PPNCET*

Descriptive Specifications	PPNCET Care Subscale X±SS	PPNCET Education Subscale X±SS	PPNCET Total X±SS
Age			
20-29	80.00+4.76	89.28+9.82	169.29+14.33
30-39	75.66+6.42	87.00+4.35	162.67+10.69
40 years and older	66.75+7.90	69.50+13.34	136.25+20.90
	KW=10.396[b]	**KW=7.239**[b]	**KW=9.535**[b]
	p=0.006	**p=0.027**	**p=0.009**
Level of Education			
High School	77.33+8.14	88.00+10.44	165.33+18.58
Associate degree	73.33+7.11	78.83+15.09	152.17+22.03
Bachelor's	72.11+10.50	78.33+15.46	150.44+25.65
	KW= 0.429[b]	**KW=0.805**[b]	**KW=0.615**[b]
	p=0.807	**p=0.669**	**p=0.735**
Marital Status			
Married	68.30+7.36	74.10+12.63	142.40+19.75
Single	79.75+6.08	87.62+13.34	167.38+19.32
	MW-U= 9.000[d]	**MW-U=19.000**[d]	**MW-U=14.000**[d]
	p=0.006	**p=0.061**	**p=0.021**
Profession			
Nurse	76.14+8.21	84.57+12.06	160.71+20.21
Midwife	71.63+9.15	77.27+15.47	148.91+24.18
	MW-U= 28.500[d]	**MW-U=31.000**[d]	**MW-U=30.000**[d]
	p=0.363	**p=0.495**	**p=0.440**
Experience year			
5 years and less	80.66+4.84	91.83+7.83	172.50+12.64
6-10 years	76.66+6.02	83.66+9.07	160.33+13.05
11 years and more	67.44+7.68	71.11+13.38	138.56+20.74
	KW=0.852[b]	**KW=3.322**[b]	**KW=1.724**[b]
	p=0.356	**p=0.068**	**p=0.189**
Experience year at the Postpartum Clinic	82.50+1.29	95.00+1.41	177.50+2.51
	70.83+3.54	76.83+6.24	147.67+8.38
1 year and less	70.75+10.9	7512+17.50	145.88+28.17
2-4 years	**KW= 5.758**[b]	**KW=6,277**[b]	**KW=6.577**[b]
5 years and more	**p=0.05**	**p=0,04**	**p=0.037**
Parity			
None	78.50+6.34	86.25+13.03	164.75+19.09
1	75.00+6.92	85.33+9.50	160.33+15.94
2	70.50+7.32	74.75+14.38	145.25+21.57
3	62.00+8.71	65.66+13.79	127.67+22.50
	KW= 5.022[b]	**KW=2.722**[b]	**KW=3.973**[b]
	p=0.081	**p=0.256**	**p=0.137**

[b]*Kruskal-Wallis,* [d] *Mann-Whitney U test*

4. DISCUSSION

Some of the primary issues affecting satisfaction are presentation and perception of care. Currently in health care services, meeting the necessities and requirements of the women and increasing their satisfaction constitutes an important dimension of quality in service care. Past experiences of the individual, as well as age, gender, level of education, social characteristics, health status, diagnosis, mental health, and such characteristics are among the factors that affect satisfaction. However, in addition to these characteristics, meeting the expectations of the patient about care, and providing physical and mental comfort, are among the important factors to increase satisfaction (16, 17, 18).

In a study by Larkin and associates, the mothers expected behavioral and communication skills from midwives (19). The quality of the desired obstetric care in the postpartum period was evaluated from the point of view of mothers in a qualitative study by Chan and associates. In the study, all the participants reported that the most important characteristic of the nurses as an indicator of quality was a positive attitude, and nurses were defined as compassionate, emphatic, polite, friendly, and valuable (20). The mothers' expectation of postpartum care was high in the study by Mirzaei and associates. In the study, the rate of matching the expectations was low, and the women reported to be unsatisfied with the care they received (2). It was reported also in the study by McCarter,Law, Cabullo and Pinto that the expectations of the mothers in the postpartum period were unmet (21). This suggests that mothers have high expectations of nurses and midwives during the postpartum period, as was reported in the related studies. During the postpartum period, women have the right to receive quality health care from the health institutions in line with their expectations.

When the distribution of the mean PPNCET scores of the mothers according to their sociodemographic properties were evaluated, age, level of education, marital status, status of employment, number of deliveries, and presence of a companion had no effect on the mean total scores of the scale ($p > 0.05$). Parallel to the present study, similar sociodemographic and obstetric specifications were found not to affect the satisfaction from the care in different studies (22, 23).

In the present study, a statistically significant relationship was found between the level of income of the mothers, and mean PPNCET scores ($p < 0.05$). In addition, mean PPNCET scores were higher in individuals whose income were equal to expenses, namely, a normal level of income. This result suggests that the expectations of women with normal level of income were met.

A statistically significant relationship was found between the age and marital status of the nurses and midwives, and mean PPNCET care subscale scores ($p < 0.05$). The mean scores of individuals with an age range of 20-29 years and single were higher. Productivity in caregiving may be decreased with married individuals due to stress, family, and professional load. Also, the fact that the young individuals were newly graduated, and therefore their knowledge is more current, might have a role in the high mean scores of the care subscale of the nurses and midwives. A statistically significant relationship was found between the age of the nurses and midwives and duration of work at the obstetrics clinic, and the mean education subscale scores ($p < 0.05$). The mean scores of individuals in the age range of 20-29 years, and who

had worked less than one year at the obstetrics clinic, was higher. This result suggests that the younger individuals, and nurses and midwives, who had recently started working at the clinic, presented more quality care.

It is important to develop provided care to increase patient satisfaction in the health sector. Care satisfaction of women during hospitalization is an important factor affecting the satisfaction of all the hospital services (4). In the present study, care was evaluated from the perspective of mothers receiving care and nurses giving care, and in general, the mean total PPNCET scores were higher than average. This result demonstrates that mothers were satisfied with the care given during the postpartum period. In the studies by Valbo and associates, and Kesuma and Chongsuvivatwong, mothers reported being satisfied with care received during the early postpartum period (12, 24). In a study by Ertem and Sevil, the effect of care provided by nurses on women's satisfaction, as well as quality of care given during the postpartum period, was evaluated. In the study, 94.3% of the women in the experimental group and 31.4% of the women in the control group were satisfied with the care they received (25). In a study by Chan and associates, women reported being satisfied with the care received. In another study, in which the level of satisfaction of women hospitalized at the gynecology and obstetrics clinics associated with the care was evaluated, women were satisfied with the care. Also, in general, the mean total scores of PPNCET of the nurses and midwives were higher than the average. This result demonstrates that the care provided during the postpartum period was good (20).

In the present study, the total scores and the education and care subscale scores of the nurses and midwives were higher than the mothers' scores. This result can be interpreted in two ways. First, care in the early postpartum period may not be adequately perceived by the mothers. Early discharge of the mothers in the early postpartum period precludes them to benefit adequately from the health care. In general, the duration of hospitalization in Turkey in cases without any complications is accepted to be at least 24 hours after vaginal birth, and at least 48 hours after cesarean birth (26).

Early postpartum discharge means hospital discharge of mothers within 24 hours after vaginal birth or 48 hours after caesarean section. In recent years, early discharge of mothers and newborns after an uncomplicated birth has been more accepted. Among the mothers, 90% wanted to be discharged after resolving problems related to breast feeding, and therefore, desired to stay in the hospital for more than 72 hours. In a study by Lindberg and associates, 61.3% of mothers wanted to be discharged no earlier than 72 hours, and 94.9% wanted to stay in the hospital as long as they wanted. These results suggest that early discharge may affect the postpartum care process perceived by the mother. Also, the care requirements defined by the mothers and health care personnel are different during the postpartum period. This condition precludes the requirements of the mothers to be met and decreases the efficacy of the postpartum care (4). These factors might have affected the perception of the care by the mothers.

Secondly, since nurses apply postpartum care and education services in line with the education they have received, their level of awareness is likely high, and thus the mean PPNCET, and education and care subscales of the nurses and midwives, are higher compared to the mothers. Postpartum care was evaluated from the perspective of care givers and care receivers in a study by Valbo and associates, and whether the education on baby care provided to the mothers was adequate was investigated, where 40.7% of the mothers and 51% of the workers reported that the education given was adequate. The findings of the present study are parallel to the findings of Valbo and associates (12).

5. CONCLUSIONS

It was determined that women and nurses/midwives had high scale total mean scores of PPNCET. Total mean scores of PPNCET nurses/midwives are higher than mothers. This result shows that mothers are less satisfied with the care they receive. The highest score obtainable from the PPNCET is 180, and mothers and nurses in this study received 141.76 ± 32.03 and 153.50 ± 23.18 scores, respectively. PPNCET scores of the mothers receiving postpartum care, and nurses giving care, were higher than the mean scores. This result suggests that the care and education given during the postpartum period was effective. Income level and receiving episiotomy affected the total mean scores of PPNCET of the women. In line with these results, planning the in-service training programs directed to develop knowledge and skills of the nurses and midwives would be beneficial to increase the that the nurses/midwives should evaluate the care and the quality of care they give during the quality of service presentation. Also, it is recommended postpartum period from the perspective of care receivers. In order to assess the postpartum care more effectively, and to increase the satisfaction of the postpartum care, involving the mothers more, and allowing them to make decisions regarding the care, is recommended.

This study has several limitations. First, this study included 130 postpartum women who gave birth at a single hospital in southern Turkey, limiting the generalizability of the results. Third, some factors such as the physical environment, the number of nurses/midwives and the time they spend with their patients may affect the nursing care received by mothers that were not evaluated in this study. Despite its limitations, this study may be helpful in guiding further research.

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Clinical and Experimental Health Sciences

The Effect of Duration of Active Labor Phase on Postpartum Fatigue and Comfort

Nuray Goker, Zumrut Bilgin

Marmara University, Health Science Faculty, Midwifery Department, Istanbul, Türkiye.

Correspondence Author: Zumrut Bilgin
E-mail: zumrutbilgin45@gmail.com

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ABSTRACT

Objective: This study aims to examine the effect of the duration of the active labor phase on postpartum fatigue and comfort.

Methods: This descriptive study was conducted in a maternity hospital in Istanbul, Turkey date 2018. Nulliparous pregnant women without any risk (n: 120) participated in the study. Data were collected using the "Information Form", the "Visual Analogue Scale", the "Postpartum Comfort Scale" and the "Visual Analogue Scale for Fatigue", and statistically analyzed.

Results: The mean age of the women was 23.66±4.24 years. 22.5% felt very tired in the postpartum period. Their mean duration of active labor phase was 5.00±1.88 hours. Their Postpartum Comfort Scale and Visual Analogue Scale for Fatigue mean scores were 131.30±13.39 and 96.67±47.82, respectively.

Conclusion: Duration of active labor phase did not affect postpartum fatigue and comfort of women.

Keywords: Active phase, comfort, fatigue, labor

1. INTRODUCTION

Childbirth and postpartum period is a normal physiological process for women. Normal birth consists of four stages (1). The first stage of labor consists of the latent and active phases. This phase begins with cervical dilation of 4 cm accompanied by regular uterine contractions and ends with full cervical dilation at 10 cm. The duration of the active phase varies depending on whether the woman is multiparous or primiparous. The mean cervical dilatation rate in the active phase has been reported as 1.2 cm/hour in nulliparous and 1.5 cm/hour in multiparous. Although the average duration of the active phase in primiparous women is accepted as 4.9 hours, it is considered normal to last up to 11.9 hours (2, 3). The duration of the first stage of labor affects women's comfort. The long duration of the active phase at birth causes fatigue in the mother in the early postpartum period. Fatigue negatively affects both the mother's self-care and the baby's care and breastfeeding (4, 5).

The duration of normal labor varies depending on various factors. One of the most important factors affecting the duration of labor is the parity of the mother. Labor takes longer in primiparas. In particular, the prolongation of the first stage causes problems during and after birth. The most common problems are expressed as pain and fatigue. While fatigue causes the prolongation of the second stage, it affects the comfort of the mother in the postpartum period. Postpartum fatigue usually occurs within the first 36 hours after birth. Fatigue affects the mother's comfort by affecting the mother's functions (5, 6).

A prolonged labor and postpartum fatigue negatively affect both women's life comfort and their childbirth and postpartum care satisfaction (7). It is very important to identify and prevent the factors that cause fatigue in pregnant women, in order to increase their comfort and satisfaction during and after labor. Alleviating labor and postpartum fatigue positively affects postpartum comfort and satisfaction of women. In addition, qualified midwifery and nursing care/support services increase their physical/social comfort and childbirth satisfaction (8).

Prolongation of any phase of labor, especially of the first and active phases, may cause fatigue in mothers, negatively affecting their pushing performance in the second stage of labor and life comfort in the postpartum period. Henderson et al. (2018) reported that 13% of women feel "exhausted" in the first few days postpartum and 5% in the next 3 months. However, only two-thirds or less of these women reported experiencing fatigue or severe fatigue (5). Therefore, it is very important to identify and prevent the factors that cause

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fatigue in mothers during labor. There are no studies about the effect of active phase duration on postpartum fatigue. This study was conducted to determine the effect of duration of active labor phase on postpartum fatigue and comfort of women.

2. METHODS

2.1. Type of Research

This study is a descriptive type.

2.2. Population of the Research and Selection of Sample

The study was conducted between June 04 and November 30, 2018 in the maternity unit of a training and research hospital in Istanbul, Turkey. At the hospital where the research was conducted, care services are provided to both low-risk and high-risk pregnant women. The population of the study consisted of nulliparous women who were referred to the delivery room of this maternity hospital and had no labor risk (pregnant women without complications).

The sample size was calculated before the study data were collected. G-power analysis was performed to calculate the sample size of the study. When the number of groups was determined as one (1) in the sampling calculation, the number of women to be included in the sample was calculated as 120, considering the effect size (f): 0.30, type I error rate (α) 0.05, type II error rate (β): 0.05, and power of the study (1 – β): 0.95. A total of 120 women were included in the study with a 95% confidence interval (9). However, considering the data loss, a total of 142 women were interviewed. It was determined that cesarean section was performed in 11 women due to fetal distress during labor, 6 women due to non-progressive delivery indication, and postpartum complications developed in 5 women. Therefore, a total of 22 women were excluded from the study.

Study inclusion criteria: Nulliparous women who were between the ages of 18-35 years and 37-41 pregnancy weeks and had no complications were included in the study.

Study exclusion criteria: Pregnant women who had those who did not want to participate in the study, and those who underwent cesarean section were excluded from the study.

2.3. Data Collection Tools

Data were collected using the "Participant Information Form", the "Visual Analogue Scale (VAS)", the "Postpartum Comfort Scale (PCS)" and the "Visual Analogue Scale for Fatigue (VAS-F)".

One of the researchers was present for research in the hospital's maternity unit during or out of working hours. The subject of the research was explained to the pregnant women in the maternity unit and their consent was obtained. The forms were filled in by the researcher with face-to-face method within 10-15 minutes.

Participant information form: The form was prepared by the researchers in line with the literature (4,5), and includes questions about the participants' sociodemographic and obstetric characteristics, duration of labor, physical and emotional status, fetus characteristics, postpartum fatigue and comfort.

The visual analogue scale (VAS): The visual analogue scale was developed by Hayes and Patterson in 1921, and the reliability and validity of the scale for assessing pain was performed by Price et al. in 1983. Cronbach's alpha value of the scale was reported to be between 0.71 and 0.90.17,18 VAS is a scale that is used to evaluate pain. The patient indicates the pain s/he feels on a 10cm scale ranging from no pain on one side and severe pain on the other side. This line is used to measure the level of pain. In this study, it was used to assess pregnant women's level of pain based on their self-report. This scale is also used for the evaluation of labor pain in addition to the evaluation of general pain (10, 11).

Visual Analog Scale for Fatigue (VAS-F): The scale was developed by Lee et al. (1990), and adapted to Turkish by Yurtsever (1999). It consists of 18 items and two subscales, including fatigue and energy. It includes 10 cm long horizontal line with positive statement on one end and negative statement on the other. The person is asked to mark the appropriate part for him/her. Then the value is determined by measuring the part marked with a ruler Yurtsever (2003) found the Cronbach's alpha value of the scale as 0.90 (12). In this study, the Cronbach's alpha value was calculated as 0.71.

Postpartum Comfort Scale (PCS): The General Comfort Scale was developed by Kolcaba (1992) and adapted to Turkish by Kuğuoğlu and Karabacak (2007). The PCS was developed from the General Comfort Scale by Karakaplan and Yıldız Eryılmaz to evaluate the physical, psychosocial and sociocultural comforts of women who undergo cesarean section or have normal childbirth. This five-point Likert type scale consists of 34 items in total. The lowest and highest scores on the scale are 34 and 170 (13). The Cronbach's alpha value of the scale was found as 0.78. In this study, the Cronbach's alpha value calculated as 0.78.

2.4.Ethical Considerations

Ethical approval from Marmara University Faculty of Medicine Clinical Research Ethics Committee (Ethics Committee Approval Date: 2018/09.2018.417) and permission from the institutions where the study was conducted, and approval from the participants were obtained.

2.5.Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) 21 demo software program. Chi-square test was used to compare quantitative categorical data, and Mann Whitney-U test to compare continuous data with normal distribution. Spearman's correlation coefficient was calculated. In addition, mean, standard deviation, number,

and percentages were calculated. The results were evaluated at 95% confidence interval and 0.05 significance level.

3.RESULTS

3.1.Participants' Characteristics

In this study, 8.3% of the women had bachelor's degree, 9.2% were employed, 37.5% had extended family, and 15.8% reported to receive insufficient support from their spouses during pregnancy. The mean age of the women was 23.66±4.24 years, the mean height was 160.38±5.68 cm, the mean pre-pregnancy weight was 59.48±8.96 kg, and the total mean weight gain during pregnancy was 12.13±4.18 kg.

It was determined that 13.3% of the women had two or more pregnancies, 18.3% did not plan their pregnancy, 6.7% used oral iron medication during pregnancy, and 20% felt tired during the prepartum period. A statistically significant relationship was found between the women's VAS mean scores according to status of having a problem requiring psychological help (p<0.05)(Table 1).

A statistically significant relationship was found between the VAS-F mean scores of women who used iron/vitamins during pregnancy (91.62±20.96) and those who did not (104.75±15.43)(p<0.05)(Table 1).

The mean VAS-F score was 88.08±21.21 for women who did not feel constantly tired during pregnancy, 88.42±20.86 for those who felt occasionally tired, and 99.23±19.24 for those who felt constantly tired, and a statistically significant relationship was found between them (p<0.05)(Table 1).

The mean VAS-F score was 86.11±23.87 for women with regular sleep and 95.12±19.00 for those with irregular sleep, and a statistically significant relationship was found between them (p<0.05)(Table 1).

No statistically significant difference was found between the women's VAS-F mean scores according to planning status of pregnancy, perception of spouse and social support, and situation of having an emergency for consulting doctor during pregnancy (p>0.05)(Table 1).

A statistically significant relationship was found between the women's PCS mean scores, planning status of pregnancy and state of feeling tired (p<0.05).

The PCS mean score was 132.81±13.47 for women with planned/wanted pregnancy, 125.09±10.87 for those with unplanned but wanted pregnancy, and 130.40±16.57 for those who got pregnant via treatment/medical assistance (p<0.05)(Table 1). A statistically significant difference was found between the women's PCS mean scores according to state of constantly feeling tired during pregnancy (p<0.05) (Table 1).

The PCS mean score was 136.25±13.13 for women who did not feel tired during pregnancy, 133.36±11.32 for those who sometimes felt tired, and 126.47±14.32 for those who felt tired all the time, and a statistically significant relationship was found between them (p<0.05) (Table 1).

No statistically significant difference was found between the women's PCS mean scores according to status of having a problem requiring psychological help, perception of partner support, use of medication during pregnancy, and situation of having an emergency for consulting doctor during pregnancy (p>0.05)(Table 1).

Table 1. *Comparison of the women's individual characteristics and scale scores (n:120).*

Obstetric data	Visual Analogue Scale for Fatigue Mean ± SD	Postpartum Comfort Scale Mean ± SD
Problem requiring psychological help		
No	92.22±20.92	131.39±13.40
Yes	108.50±0.70	125.50±16.26
t; p	*8.177; 0.001*	*0.616; 0.539*
Perception of partner and social support (spouse, family support)		
Inadequate	*94.89±17.91*	*127.26±13.95*
Adequate	*92.04±21.40*	*132.05±13.22*
t; p	*0.544; 0.587*	*1.438; 0.153*
Planned pregnancy		
Planned/Wanted	91.22±21.58	132.81±13.47
Unplanned/Wanted	96.90±18.72	125.09±10.87
Via Treatment/Medical Assistance	96.80±14.34	130.40±16.57
F, χ^2 ;p	*0.769; 0.466*	*3.074; 0.074*
Use of medication during pregnancy		
No	104.75±15.43	126.50±8.99
Iron/Vitamin	91.62±20.96	131.64±13.62
t, Z ;p	*2.261; 0.050*	*1.049; 0.296*
Having an emergency for consulting doctor during pregnancy		
No	93.06±20.74	131.47±13.23
Yes	91.58±21.22	131.02±13.78
t, Z ;p	*0.377; 0.707*	*0.179; 0.859*
Feeling tired all the time during pregnancy		
No	88.08±21.21	136.25±13.13
Sometimes	88.42±20.86	133.36±11.32
Yes	99.23±19.24	126.47±14.32
F, χ^2 ;p	*4.102; 0.019*	*5.613; 0.005*
Sleep pattern		
Regular	86.11±23.87	131.91±12.63
Irregular	95.12±19.00	131.04±13.76
t, Z ;p	*2.187; 0.031*	*0.321; 0.749*

t: Independent Sample t test. F: One way ANOVA. Z: Mann Whitney U test. χ2: Kruskal Wallis test. p<0.05

3.2.Participants' Labor-Related Variables

There was no statistically significant relationship between the women's VAS-F mean scores, hunger and satiety status, status of having hydration during labor, status of having induction during labor, status of having analgesics during labor, status of having amniotomy during labor, status of having bladder

drainage on obstetric table, and status of having episiotomy during birthdelivery (p>0.05)(Table 2).

Table 2. *Comparison of labor-related variables and scale scores (n:120).*

Variables	Visual Analogue Scale for Fatigue Mean ± SD	Postpartum Comfort Scale Mean ± SD
Status hunger and satiety		
Hungry	93.78±21.09	130.73±13.28
Full-solid	78.60±17.71	137.60±8.90
Full-liquid	94.50±19.91	129.50±12.79
Full – both solid and liquid	82.37±19.12	137.75±17.33
F; p	*1.559; 0.203*	*1.153; 0.331*
Application of hydration during labor		
No	91.14±22.08	129.83±13.75
Yes	93.40±20.09	132.27±13.15
t; Z; p	*0.724; 0.470*	*0.979; 0.330*
Application of induction during labor		
No	93.26±21.10	131.82±13.73
Yes	91.31±20.61	130.48±12.95
t; Z; p	*0.496; 0.621*	*0.530; 0.597*
Application of analgesics (pethidine 50 mg) during labor		
No	92.74±20.95	131.03±13.87
Yes	91.72±20.87	132.13±11.95
t; Z; p	*0.229; 0.819*	*0.385; 0.701*
Application of amniotomy during labor		
No	99.70±17.50	130.04±12.87
Yes	91.43±19.05	130.13±12.82
Naturally ruptured	90.07±23.20	132.86±14.17
F; χ^2 ;p	*1.870; 0.159*	*0.623; 0.538*
Bladder drainage on obstetric table		
No	93.04±21.66	131.49±13.82
Yes	91.44±19.36	130.69±12.68
t; p	*0.383; 0.703*	*0.297; 0.767*
Application of episiotomy		
No	87.75±19.06	134.00±18.65
Yes	92.66±20.96	131.20±13.27
F; p	*0.462; 0.645*	*0.409; 0.684*

t: Independent Sample t test. F: One way ANOVA. Z: Mann Whitney U test. χ2: Kruskal Wallis test. p<0.05

In addition, there was no statistically significant relationship between the women's PCS mean scores, hunger and satiety status, status of having hydration during labor, status of having induction during labor, status of having analgesics during labor, status of having amniotomy during labor, status of having bladder drainage on obstetric table, and status of having episiotomy during birthdelivery (p>0.05)(Table 2).

3.3. The Interventions Applied During Labor and Duration of Birth

There was no statistically significant relationship between the women's gestational week, status of hunger and satiety, status of having hydration, amniotomy and analgesics during labor, woman's age, pre-pregnancy body weight, fetuse's body weight and duration of the second stage of labor (p>0.05)(Table 3).

Table 3. *Intervention at birth, information about mother and fetus, comparison of the duration of the second stage of labor and the active labor stage (n:120).*

Duration of active labor phase					Test statistics	
Variables	≤5 Hours		>5 Hours		*t; χ^2*	*p*
	n	%	n	%		
Pregnancy week according to the last menstrual period						
37-40 weeks	65	66.3	33	33.7	1.312	0.859
41 weeks and over	15	68.2	7	31.8		
Status hunger and satiety						
Hungry	55	61.8	34	38.2	4.862	0.182
Full-solid	3	60.0	2	40.0		
Full-liquid	15	83.3	3	16.7		
Full – both solid and liquid	7	87.5	1	12.5		
Application of induction during labor						
No	54	74.0	19	26.0	4.477	0.034
Yes	26	55.3	21	44.7		
Application of hydration during labor						
No	32	66.7	16	33.3	0.00	1.000
Yes	48	66.7	24	33.3		
Application of amniotomy during labor						
No	15	62.5	9	37.5	0.243	0.886
Yes	30	68.2	14	31.8		
Naturally ruptured	35	67.3	17	32.7		
Application of analgesic during labor						
No	62	68.1	29	31.9	0.364	0.546
Yes	18	62.1	11	37.9		
The woman's pushing performance in the second stage						
Sufficient	44	81.5	10	18.5	13.627	0.001
Insufficient	5	33.3	10	66.7		
Crystals were used	31	60.8	20	39.2		
***Continuous variables**	**Mean ± SD**		**Mean ± SD**		**t**	**p**
Age of the woman	23.55±4.12		23.88±4.51		0.394	0.694
Pre-pregnancy body weight of women	58.45± 8.62		61.55±9.38		1.802	0.074
Body weight of fetuses	3219.38±387.69		3289.75±348.67		0.968	0.335
Duration of the second stage of labor	29.00±14.37		39.00±23.67		2.871	0.005

χ2= Chi Square Test, Kruskal Wallis test, t=Independent Sample t test, p<0,05

However, a statistically significant relationship was found between the induction during labor, woman's pushing performance in the second stage, duration of the second stage of labor, and duration of active labor phase (p<0.05) (Table 3).

The mean duration of active labor phase of the women was 5.00±1.88 hours, where the shortest and longest active phases lasted 2 and 14 hours, respectively. The mean duration of the second phase of labor was 29.00±14.37 minutes for women with active labor phase ≤5 and 39.00±23.67 minutes for those with active labor phase >5 (p<0.05)(Table 3).

3.4. Women's Postpartum Physical and Emotional States

The relationship between the women's VAS-F mean scores, emotional self-perception, perception of the environment, need for support, and state of feeling safe after labor was statistically significant (p<0.05). But the relationship between their VAS-F mean scores, emotional self-perception, perception of the environment, need for support, and state of feeling safe after labor was statistically insignificant (p>0.05)(Table 4).

Table 4. *Comparison of the women's postpartum physical and emotional states and scale scores (n:120).*

Perceptions	Visual Analogue Scale for Fatigue Mean ± SD	Postpartum Comfort Scale Mean ± SD
The mother's perceive the environment		
Very uncomfortable	95.33±20.40	111.66±9.09
Very comfortable	92.35±20.95	132.33±12.79
t; Z; p	*0.340; 0.734*	*3.897; 0.001*
The mother's perceive herself physically		
Energetic	90.69±25.99	138.15 ± 13.75
A little bit tired	88.83±18.55	130.58 ± 13.49
So tired	103.33±17.49	126.48 ± 10.22
F; χ^2 ;p	*5.107; 0.007*	*5.656; 0.005*
The mother's perceive herself emotionally		
Happy / joyful	93.09±21.58	132.11±13.15
Sad / Anxious	93.33±24.11	134.00±29.51
Surprised / panicked	92.66 ±17.59	127.41±15.76
Restless / nervous	96.00±28.58	128.00±6.24
Excited / enthusiastic	83.62 ±14.60	127.75±6.67
F; χ^2 ;p	*0.396; 0.811*	*0.547; 0.701*
The mother's state of feeling safe after birth		
Doesn't feel safe	113.00±39.23	136.00±24.97
Feels safe	91.97±20.20	131.17±13.14
t; Z; p	*1.739; 0.085*	*0.614; 0.541*
The mother needs any support		
Needs no support	89.83±21.70	133.32±14.06
Needs her mother's support	96.34±19.99	123.47±9.65
Needs her husband's support	99.00±16.65	133.29±10.80
F; χ^2 ;p	*1.355; 0.260*	*4.005; 0.009*

	VAS-F		PCS	
	r**	p	r*	p
Postpartum pain	0.193	0.034	-0.312	0.001
Duration of active phase	0.081	0.377	0.004	0.969

**r=Pearson Correlation Coefficient. **r= Spearman Rho Correlation Coefficient. p<0.05*

The women's PCS scores, physical self-perception, perception of the environment, and need for support had a statistically significant relationship (p<0.05). But the relationship between their PCS mean scores, emotional self-perception, and state of feeling safe after labor was statistically insignificant (p>0.05)(Table 4).

A statistically significant relationship was also found between the women's pain values in the postpartum period and their PCS and VAS-F mean scores (p<0.05)(Table 4).

4. DISCUSSION

In this study, the findings of the research, in which we examined the effect of the duration of the active birth phase on postpartum fatigue and comfort, were discussed in line with the relevant literature.

In this study, the mean age of the women was 23.66±4.24 years, and the mean weight gained during pregnancy was 12.13±4.18 kg, which are consistent with the 2018 Hacettepe University Institute of Population Studies data (14). A statistically significant linear correlation was found between the VAS-F and PCS scores of women who felt constantly tired during pregnancy. Those who felt tired during pregnancy had higher VAS-F mean scores (Table 1). Those who felt tired during pregnancy had lower PCS mean score than those who did not (Table 1). Studies on the relationship between sleep patterns and fatigue have shown that irregular sleep has a negative effect on fatigue (15, 16).

In the study, those with full satiety had higher PCS mean score and lower VAS-F mean score than those with hunger (Table 2). Regarding oral food intake restriction in labor, the American College of Obstetricians and Gynecologists (ACOG) suggest that pregnant women without complications can consume oral fluid without grains. Other studies support oral food intake during labor (17, 18). However, an oral liquid-solid food intake restriction policy is applied to women in the hospital where the study was conducted. There was no statistically significant relationship between the women's hunger levels at the time of birth and the length of the active phase of labor (Table 3). Other studies reported different results suggesting that oral liquid/food intake shortened or did not affect the duration of active labor phase (19-22). The most important cause of women's fatigue may be associated with routine restriction or interruption of food intake during labor.

In this study, the active phase duration of 66.7% of women who were given hydration (fluid support) during delivery was ≤5 hours, and the active phase duration was >5 hours in 33.3% of those who were not given hydration. The hydration given to the mother during labor did not affect the duration of the active labor phase (Table 3). The Institute for Clinical Systems Improvement (ICSI) reported that an administration of intravenous (IV) fluids during labor shortened delivery time (23, 24). The different result in the present study may be due to other factors that affect the duration of active labor phase. Labor induction affected the duration of active labor

phase (Table 3). Similar to this study, other studies have also reported that labor induction affects the duration of active labor phase (23,25).

The present study also found no statistically significant relationship between amniotomy, VAS-F and PCS scores and duration of active labor phase (p>0.05)(Table 2, Table 3). Another study supports this study result (26). The present study determined that episiotomy, a routine procedure administered to nulliparous women in the hospital where the study was conducted, did not affect the women's VAS-F and PCS scores (Table 2). Akgün and Duran Aksoy (2020) did not find a significant difference between the mean postpartum comfort questionnaire (PPCQ) scores of women who underwent episiotomy and those who did not. According to Yilmaz et al. (2021) stated that the comfort level of all women who underwent or did not undergo episiotomy was moderate, and there was no difference in terms of the mean postpartum comfort (PPC) score. This result of the study showed similarities with the studies (27, 28).

The duration of active labor phase statistically significantly differed according to the women's pushing performances. As the pushing performance increased the duration of active labor phase shortened. The self-pushing rate of women who had an active phase duration of ≤5 hours was 81.5%, indicating that those who exhibited less effort during labor could push the baby out more effectively (Table 3). The woman's pushing efforts facilitate the descent of the fetus into the pelvis. The woman's forceful pushing of the fetus shortens the second stage of labor. However, prolongation of labor and efforts to push the fetus for a long time cause fatigue in women (29).

There was a statistically significant relationship between the women's physical self-perception, perception of the environment, need for support and PCS scores (Table 3). Women who perceived the environment very uncomfortable had higher PCS scores. Akgün and Duran Aksoy (2020) found a positive and significant relationship between the postpartum comfort scores of mothers who expressed their general health status very well/well in their study (27). Capık et al. (2014) found that mothers who gave birth vaginally had higher postpartum physical and sociocultural comfort (4). Postpartum comfort is very important in terms of mothers' adaptation to the postpartum period and their roles.

The VAS-F mean scores of women who felt energetic or slightly tired or very tired were compared, the mean score of those who felt very tired was found to be significantly higher as expected (Table 4). The mean score in this study was higher than the mean score reported by Çapık et al. (2014) (4). Aktaş and Karaçam (2017) stated that many factors affect fatigue and that the postpartum fatigue level of mothers is high (8). Akgün and Duran Aksoy (2020) found a positive and significant relationship between the postpartum comfort scores of mothers who expressed their general health status as very good or good. The results of the study support the results of other studies (27). It is very important to support mothers with fatigue problems and to increase their comfort by easing their fatigue.

No statistically significant difference was found between the women's PCS mean scores according to duration of the second stage of labor (Table 4). A negative weak linear statistically significant relationship was found between the women's postpartum pain values and PCS scores (Table 4). According to this result, PCS scores decrease as women's postpartum pain values increase (30). Fatigue and pain are the most common problems faced by women in the early postpartum period (31). Factors such as pain and fatigue during and after birth affect the comfort of mothers negatively (4). According to Wilson and Kolcaba (2004), one of the most important factors influencing the decrease in physical comfort is pain (32). In the study conducted by Taylor and Johnson (2013), it was stated that postpartum fatigue in the first week of postpartum is associated with pain experienced in the last 24 hours. (33). In order to increase the postpartum comfort of the mother, interventions to reduce pain should be planned and implemented.

A positive, weak, linear, statistically significant correlation was found between postpartum pain scores of women and VAS-F scores. According to this finding, VAS-F scores increased as women's postpartum pain increased (Table 4). Troy reported that primiparous women are more likely to experience postpartum fatigue (34). Other studies show that postpartum fatigue is associated with postpartum pain (8,35,36). In parallel with the women's pain score, energy use increases, which causes the woman to feel more tired.

Friedman was the first to describe the progress of primiparous women in the active phase as 1.2 cm/hour (2). In the literature, different results have been given regarding the duration of the active birth phase (37, 38, 39). In this study, the shortest and longest periods of active labor were 2 and 14 hours, respectively. The mean duration of the active phase was 5.00±1.88 hours, which was very close to the time reported by Coşkun and Ünal (5.11 hours) (40).The relationship between pain and fatigue in mothers in the early period is thought to be related to the birth process.

5. CONCLUSION

As a result of the study, from the women's obstetric and demographic characteristics; feeling tired all the time, feeling safe in the environment and need for support during labor affected postpartum comfort. Medical interventions applied during labor did not affect postpartum comfort. The duration of the active labor phase did not affect postpartum comfort.

As a result: Relevant interventions should be made to relieve pain and fatigue of pregnant women in order to increase their comfort during childbirth and postpartum period. Ineffective applications should be excluded from routine labor interventions. The effect of hunger and satiety status of women should be examined in detail and, unless necessary, oral food intake should not be restricted during labor. The

reliability of the results should be increased by repeating the study with a larger sample.

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Clinical and Experimental Health Sciences

Examination of Predictive Factors Healthy Lifestyle Behaviours and Compare Coping Styles with Stress of Adolescents with and without Hearing Loss: A Comparative Study

Kamer Gur[1], Eda Kilinc Isleyen[2], Elif Yayikci[3], Kemal Deger[4], Ezgi Tekin[5]

[1] Marmara University, Faculty of Health Sciences, Public Health Nursing Department, Istanbul, Türkiye.
[2] Pamukkale University,Faculty of Health Sciences, Public Health Nursing Department, Denizli, Türkiye.
[3] Kirklareli State Hospital, Kirklareli, Türkiye.
[4] Mardin State Hospital, Mardin, Türkiye.
[5] Malazgirt District Health Directorate, Mus, Türkiye.

Correspondence Author: Eda Kilinc Isleyen
E-mail: kilinc_edaa@hotmail.com
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ABSTRACT

Objective: Examination of predictive factors healthy lifestyle behaviours and compare coping styles with stress of adolescents with and without Hearing Loss (HL).

Methods: A comparative cross-sectional design was employed. The participants comprise high school students with (n= 272) and without (n= 272) HL. Participants completed questionnaires on coping styles with stress and Adolescent Lifestyle Profile Scale 2 (ALP-R2). Descriptive statistics, chi-square, t-test, and multiple linear regression analysis were used to analyze the responses given to the above instruments.

Results: Adolescents without HL (116.46±16.89) had significantly higher scores on the lifestyle profile scale than another group (113.68±16.12) (p<.000). The difference in coping styles (pray, hobbies and exercise etc.) with stress of two group was determined (p<.000). According to multiple linear regression analysis, independent variables explained 27% of the change in adolescent lifestyle profile in adolescents with HL (R2=.273). BMI (β=-.258), economic status (β=-.439), and mother education level (β=.146) were significant predictors affecting lifestyle profile in adolescents with HL. Also, independent variables explained 17% of the change in adolescent lifestyle profile in adolescents without HL (R2=.170). BMI (β=-.327) and economic status (β=-.233) were significant predictors in adolescents without HL.

Conclusion: Lifestyle profiles of adolescents with HL are lower than those without HL. Adolescents with HL are more vulnerable in terms of a lifestyle behaviours and coping style with stress. By using these findings, nurses can make school-based interventions on positive life perspective, stress management to increase lifestyle profiles.

Keywords: Adolescent, hearing loss, healthy lifestyle, stress and coping, stress management

1. INTRODUCTION

Healthy lifestyles are defined as behavioral patterns that help prevention and promotion people's health and well-being (1). Especially, the adolescent period is considered an important period in which positive behaviors are acquired for health promotion (2). For a healthy life, it is important to implement lifestyle behaviors that health prevention and promotion during adolescence.[1,3,4]. Because adolescents comprise most of the demographical distribution of the population and that these individuals are the adults of the future, significant priority must be given to the healthy growth and development of this group (2,4). It is easier to promote health responsibility in childhood and adolescence. Healthy lifestyle behaviors should be made daily life habits in the adolescent years (5).

Although adolescence is generally healthy periods in one's life, some important health and social problems may begin to evolve or reach a peak at this time. Examples include nutrition and weight conditions, obesity, inadequate physical activity, depression etc. (6). These problems affecting the lifestyle profiles of adolescents are more common in adolescents with HL (7). Adolescents with HL may face many difficulties when using healthcare services. For example, they may need support with communication (such as lip-reading or sign language) while receiving healthcare (7). In addition, adolescents with HL are at risk of reduced access to healthcare, social withdrawal, and lowered self-esteem due to difficulties in communicating with others. For this reason, negatively affects the nutrition, physical activity, stress management behaviors and quality of life of adolescents

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with HL (7,8). Studies have found that adolescents with HL have more sedentary behavior and less physical activity than adolescents without HL (9,10).

In addition, adolescents with HL face many stresses in their daily life (7). The reason for this stress was determined as social interaction (with peers), classroom environment, and academic difficulties. Among adolescents, various coping styles have been found to be effective at perceived stress levels (11). For this reason, it is also important to determine stress management, interpersonal relationships, and positive life expectancy, which determine the healthy lifestyle behaviours in adolescents with hearing loss.

Even though there many studies describing the healthy lifestyle behaviors of adolescents (1,3,12,13) the healthy lifestyle behavior profile of adolescents with HL is not clear. The aim of the study is examination of predictive factors healthy lifestyle behaviours and compare coping styles with stress of adolescents with and without HL.

2. METHODS

2.1. Study Design and Setting

This study is of comparative cross-sectional research design. The study was carried out at four high schools for students with and without HL between 2018-2019 academic year in Istanbul, Turkey (N= 644). Two of the schools had students with HL enrolled but the other two schools were made up of adolescents without HL. The schools for the HL and the other schools were situated in similar areas and had similar socio-economic backgrounds.

2.2. Participants

The "convenience sampling" method was used in the sample selection of the study. The research aimed to reach the whole of the population. Adolescents with and without HL who met the sample criteria were included. The inclusion criteria of adolescents with HL were no intellectual disability, parental consent, and knowing sign language. The inclusion criteria of adolescents without HL were no intellectual disability and parental consent. Exclusion criteria were the presence of an intellectual or other disability (vision etc.). During the academic year, 170 adolescents were recruited from the first, 102 from the second school (schools that had adolescents with HL) for the study. A total of 272 adolescents were recruited from the third and fourth schools (schools with adolescents who could hear). The response rate of the study was 84.47%.

2.3. Measures

Sociodemographic questionnaire

The authors created this questionnaire based on their survey of the literature. This form contains basic descriptive questions such as age, gender, parental education, and health insurance.

Hearing loss form

The form that was applied to adolescents with HL contained two questions on the degree and classification of the HL. School nurses evaluate students' degree of hearing and speech level during their examinations before enrollment at school. In doing this, they make use of the Omaha Classification System to identify the hearing and speech difficulties defined in the physiological domain and the specific symptoms and findings related to each issue. An appropriate nursing diagnosis is made based on this system. The degrees of HL, speech levels were taken from these records.

Coping styles with stress form

The authors created this questionnaire based on their survey of the literature (14,15). This form contains basic descriptive questions such as pray, hobbies, exercise, breathe deeply and have a shower.

Adolescent Lifestyle Profile Scale 2 (ALP-R2)

This scale was developed in 2006 by Hendricks, Murdaugh and Pender to measure adolescent health-promoting behavior (16). The scale was revised in 2009 (ALP-R2). The scale was adapted into Turkish by Ilhan (17). ALP-R2 is a 4-point (1=never to 4=always) Likert-type of scale composed of 44 items and 7 sub-scales. The subscales are health responsibility, physical activity, nutrition, positive life perspective, interpersonal relations, stress management, spiritual health. The minimum possible score on the scale is 44; the maximum is 176. The total Cronbach's α coefficient was .88. The CFA results showed that the structure of the model/scale has an acceptable goodness of fit. The study, this scale the total Cronbach's α coefficient was .87. Positive health promotion behaviors increase as the score on the scale rises.

2.4. Procedures

The questionnaires for hearing adolescents were distributed to them during class time. The purpose of the study was explained to the adolescents and their consent was obtained. The students filled out the forms under the supervision of the researchers. The instruments for the adolescents with HL were adapted into sign language after consultation with a sign language specialist. The following processes were used; a) First, in order to adapt the ALP-R2 into sign language (translation and back translation), we enlisted the help of an expert in sign language; b) then, individual interviews were held in the form of a pilot study with a team of experts, teachers from the school, the researchers, and 6 students in the effort to make an evaluation of the translated questions; c) afterwards, modifications were made based on the feedback from the discussions; d) we then assessed the pilot study initiated with 6 students. There were words in the pilot study that the students could not understand. Again, a consultation was held with specialist

in sign language to determine whether the items on the questionnaire were comprehensible and appropriate for the level of the adolescents with HL. The data of this study were collected face to face by the researchers in the classroom environment. The necessary explanations were given to adolescents with HL in sign language by researchers who have a sign language certificate. Then, consent was obtained from those who wanted to participate in the study. Questionnaire were presented to the participants. During the completion of the questionnaires, a researcher was assigned for each adolescent with HL. The researchers made explanations in sign language for questions. As a result, both a questionnaire and sign language were used to collect data.

2.5. Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences, Version 22.0. Descriptive statistics were used to determine the scores of the adolescents on the ALP-R2 and sociodemographic characteristics etc. The normal distribution of the data in this study was examined with the Kolmogorov Smirnov test and the data showed a normal distribution (p>0.05). The socio-demographic difference between the adolescents with and without HL were examined using the chi-square test. T-test was used to explore the differences between the adolescents with and without HL ALP-R2 scale and subscale scores. The chi-square test was used to determine the difference between coping styles with stress of adolescents with and without HL. Multiple linear regression analysis was performed to determine the predictors affecting the lifestyle profile of adolescents with and without HL. P<0.05 was considered statistically significant.

2.6. Ethical Considerations

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Marmara University Health Sciences Institute (19.11.2018/No: 219). Permission was obtained from the Provincial Directorate of National Education for the work to be carried out in the high schools. The informed consent of the volunteer participating adolescents and their parents was obtained.

3. RESULTS

Of the adolescents in the study with HL, 55.9% were male while the female of 53.3% of those with hearing. Among the adolescents with HL, 84.9% had a BMI of 18.5-24.9 kg/m^2, while the BMI of 88.9% of those with hearing was 18.5-24.9 kg/m^2 (Table 1).

The classification of HL and speech levels of adolescents with HL is shown in Table 2. 36.8% of adolescent's difficulty hearing normal sounds of talking, even with a device. 49.6% of adolescents had inability to talk due to not understanding (Table 2).

The ALP-R2 scale mean score for adolescents with HL was 113.68±16.12 and 116.46±16.89 for the adolescents who could hear (p<.000). The mean scores of the adolescents with HL on the subscales were, from highest to lowest, nutrition (20.28±3.28), positive life perception (16.0±3.52), interpersonal relations (15.53±3.22), stress management (15.53±2.81), spiritual health (16.20±3.37), physical activity (15.25±3.61), and health responsibility (14.89±3.69). The mean scores of the adolescents without HL on the subscales were, from highest to lowest, nutrition (19.54±3.53), stress management (17.84±2.70), interpersonal relations (17.77±4.02), positive life perception (17.0±3.4), spiritual health (15.32±3.07), physical activity (14.54±3.7), and health responsibility (14.45±3.45). The adolescents with HL had lower scores on these subscales except for spiritual health (Table 3).

There was a statistically significant difference between the two groups in terms of the methods they used to cope with stress (p<.000) (Table 4).

The difference between the participants' coping or not, and their lifestyle profiles and sub-dimensions were examined. Accordingly, the ALP-R2 score of those who prayed as a coping method in adolescents with HL was significantly higher than those who did not (p<.05). Among adolescents without HL, ALP-R2 scores of those who exercised as a coping style were significantly higher than those who did not (p<.05) (Table 5).

According to the multiple linear regression analysis results, when the significance level corresponding to the F value is examined, it is seen that the established models are statistically significant (F=12.326; p<.001, F=8.903; p<.001).

According to multiple linear regression analysis, independent variables explained 27% of the change in adolescent lifestyle profile in adolescents with HL (R^2=.273). Multiple linear regression analysis indicated that BMI, economic status, and mother education level were significant predictors affecting lifestyle profile in adolescents with HL (t=-4.865, p<.001; t= 2.561, p<.05; t=-7.793, p<.001). One unit increase in the BMI variable causes a decrease of %0.258 on the lifestyle profile (β=-.258). One unit increase in the mother education level variable provides an increase of %0.146 on the lifestyle profile (β=.146). In addition, one unit decrease in the economic status variable causes a decrease of %0.439 on the lifestyle profile (β=-.439). Also, independent variables explained 17% of the change in adolescent lifestyle profile in adolescents without HL (R^2=.170). BMI and economic status were significant predictors affecting lifestyle profile in adolescents without HL (t=-5.683, p<.001; t=-3.840, p<.001). One unit increase in the BMI variable causes a decrease of %0.327 on the lifestyle profile (β=-.327). In addition, one unit decrease in the economic status causes a decrease of %0.233 on the lifestyle profile (β=-.233) (Table 6).

Table 1. *Sociodemographic characteristics of adolescents (n= 544)*

Demographic Characteristics		Adolescents with hearing loss (n= 272)		Adolescents without hearing loss (n= 272)		Statistic
		Mean	SD	Mean	SD	
Age		17.11	1.33	16.18	0.98	
		n	%	n	%	x^2; p
Gender	Female	120	44.1	145	53.3	**4.59; 0.03**
	Male	152	55.9	127	46.7	
BMI	18.5-24.9 kg/m^2: Nonoverweight	231	84.9	242	88.9	1.96; 0.16
	25 and above: Overweight	41	15.1	30	11.1	
Number of siblings	Single child	14	5.1	18	6.6	**38.50; 0.00**
	1 sibling	46	16.9	100	36.8	
	2 siblings	78	28.7	81	29.8	
	More than 2 siblings	134	49.3	73	26.8	
Father education level	Illiterate	18	6.6	23	8.5	**115.27;0.00**
	Primary school	141	51.8	41	15.1	
	Middle School	61	22.4	50	18.4	
	High school	39	14.3	86	31.6	
	University	13	4.8	72	26.5	
Mother education level	Illiterate	43	15.8	23	8.5	**95.23;0.00**
	Primary school	142	52.2	72	26.5	
	Middle School	57	21	46	16.9	
	High school	22	8.1	74	27.2	
	University	8	2.9	57	21.0	
Social insurance	Yes	253	93	262	96.3	**2.95; 0.02**
	No	19	7	10	3.7	
Level of economic	Bad	52	19.1	32	11.8	6.73; 0.81
	Middle	89	32.7	89	32.7	
	Good	102	37.5	123	45.2	
	Pretty good	29	10.7	28	10.3	

SD: Standard Deviation

Table 2. *Characteristics of hearing and hearing levels of adolescents with hearing loss (n= 272)*

Characteristics		n	%
Hearing loss classifications	Difficulty hearing normal sounds of talking, even with a device.	100	36.8
	Difficulty hearing with a device only in crowded environments	17	6.3
	Difficulty hearing loud sounds even with a device	8	2.9
	No hearing/No response	81	29.8
	I have no problem with hearing with a device	66	24.3
Speech level classification	Inability to talk	69	25.4
	Inability to talk due to not understanding	135	49.6
	Talking with inappropriate sentence structures	68	25.0

Table 3. *Distribution of adolescents' adolescent lifestyle profile scale mean scores (n= 544)*

	Adolescents with hearing loss (n = 272)	Adolescents without hearing loss (n = 272)	Statistic
	Mean (SD)	**Mean (SD)**	**t; p**
Health responsibility **(min=7; max=26)**	14.89±3.69	14.45±3.45	1.41; 0.158
Physical activity (min=6; max=24)	15.25±3.61	14.54±3.72	2.26; **0.024***
Nutrition (min=11; max=28)	20.28±3.28	19.54±3.53	2.52; **0.012***
Positive life perspective (min=8; max=24)	16.00±3.52	17.00±3.43	3.49; **0.001****
Interpersonal relations (min=7; max=24)	15.53±3.22	17.77±4.02	8.42; **0.000*****
Stress management (min=9; max=24)	15.53±2.81	17.84±2.70	9.78; **0.000*****
Spiritual health **(min=7; max=24)**	16.20±3.37	15.32±3.07	3.17; **0.002****
ALP-R2 total (min=68; max=167)	**113.68±16.12**	**116.46±16.89**	3.52**; 0.000*****

SD: Standard Deviation
**p<.05 **p<.01 ***p<.001*

Table 4. *Comparison of coping styles with stress between two group of adolescents*

		Adolescents with hearing loss (n = 272)		Adolescents without hearing loss (n= 272)		Statistic
Coping Styles		**n**	**%**	**n**	**%**	**x^2; p**
Pray	Yes	136	50.0	74	27.2	29.81;**.000***
	No	136	50.0	198	72.4	
Hobbies	Yes	50	18.4	136	50.0	60.42;**.000***
	No	222	81.6	136	50.0	
Exercise	Yes	29	10.7	68	25.0	19.08;**.000***
	No	243	89.3	204	75.0	
Breathe Deeply	Yes	33	12.1	66	24.3	13.44;**.000***
	No	239	87.9	206	75.7	
Have a shower	Yes	37	13.6	82	30.1	21.78;**.000***
	No	235	86.4	190	69.9	

**p<.001*

Table 5. *Difference in lifestyle profile between adolescents who do and do not do coping strategies (n= 544)*

		Health responsibility		Physical activity		Nutrition		Positive life perspective		Interpersonal relations		Stress management		Spiritual health		ALP-R2 total	
Coping style		**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**	**Mean (SD)**	**t;p**
Pray (1)	**Yes**	15.74± 3.79	**3.88 ;.00****	15.49± 3.44	1.07 ;.284	20.65± 3.35	1.87;.06	16.11± 3.60	.73;.46	15.37± 3.06	1.86 ;.23	15.30± 2.80	1.36 ;.17	16.04± 3.07	**3.96 ;.00****	114.72± 15.81	**2.40;.01***
	No	14.04± 3.40		15.02± 3.77		19.91± 3.18		15.80± 3.45		14.91± 3.37		15.76± 2.80		14.60± 2.91		110.05± 16.15	
Pray (2)	**Yes**	14.59± 3.35	.39 ;.69	14.32± 3.81	.59 ;.55	18.94± 3.37	1.71;.08	16.94± 3.47	.16;.86	17.70± 3.34	.18 ;.85	17.56± 2.68	1.04 ;.29	16.57± 3.35	.97 ;.33	116.60 ±	.45;.64
	No	14.40± 3.49		14.62± 3.69		19.76± 3.57		17.02± 3.42		17.80± 4.25		17.95± 2.71		16.08± 3.38		117.66± 16.85	
Hobbies (1)	**Yes**	14.68± 3.29	.45 ;.65	15.88± 3.57	.98 ;.17	20.28± 3.30	.00;.99	15.68± 3.76	.61;.53	14.98± 3.16	.39 ;.69	15.80± 2.79	.74 ;.45	14.82± 2.86	1.28 ;.20	112.12± 17.17	.13;.89
	No	14.94± 3.78		15.11± 3.61		20.28± 3.28		16.02± 3.48		15.18± 3.24		15.47± 2.81		15.43± 3.12		112.45± 15.91	
Hobbies (2)	**Yes**	14.71± 3.45	1.21 ;.22	14.78± 3.84	1.07 ;.28	19.56± 3.54	.10;.91	17.30± 3.51	1.46;.14	18.13± 4.41	1.49 ;.13	18.18± 2.78	2.05 ;.05	16.23± 3.48	.16 ;.87	118.93± 17.11	1.52;.12
	No	14.20± 3.45		14.30± 3.60		19.52± 3.53		16.69± 3.33		17.41± 4.77		17.51± 2.59		16.16±3 .27		115.82± 16.58	
Exercise (1)	**Yes**	15.24± 4.20	.53 ;.59	16.55± 3.71	**2.05 ;.04***	20.62± 2.92	.58;.55	16.82± 3.88	1.40;.16	15.41± 3.20	.47 ;.63	17.00± 2.37	**3.01 ;.00****	14.93± 2.56	.72 ;.46	116.58± 17.10	1.48;.13
	No	14.85± 3.63		15.10± 3.58		20.24± 3.32		15.85± 3.47		15.11± 3.19		15.35± 2.81		15.37± 3.13		111.89± 15.96	
Exercise (2)	**Yes**	15.51± 3.35	**2.94 ;.03***	15.92± 3.72	**3.60 ;.00****	20.89± 3.65	**3.73 ;.00****	17.36± 3.48	1.01;.31	17.60± 2.92	.40 ;.68	18.23± 2.75	1.36 ;.17	16.97± 3.44	**2.18 ;.03***	122.51± 17.11	**2.93;.00****
	No	14.10± 3.42		14.08± 3.62		19.09± 3.38		16.88± 3.41		17.83± 4.33		17.72± 2.68		15.94± 3.31		115.66± 16.29	
Breathe Deeply(1)	**Yes**	13.87± 3.14	1.68 ;.92	14.69± 3.19	.94 ;.34	19.24± 3.42	1.95;.05	16.81± 2.90	1.49;.13	14.63± 3.46	.96 ;.33	15.12± 3.12	.89 ;.37	15.15± 3.35	.34 ;.73	109.54± 14.28	1.08;.28
	No	15.03± 3.74		15.33± 3.66		20.42± 3.24		15.84± 3.59		15.21± 3.19		15.59± 2.76		15.34± 3.04		112.78± 16.35	
Breathe Deeply(2)	**Yes**	14.25± 3.23	.54 ;.58	14.24± 3.69	.75 ;.45	19.33± 3.18	.55;.57	16.65± 3.51	.95;.33	17.83± 3.05	.13 ;.89	17.98± 2.93	.46 ;.64	15.62± 3.13	1.61 ;.10	115.92± 16.08	.80;.42
	No	14.52± 3.53		14.64± 3.74		19.61± 3.64		17.11± 3.40		17.75± 4.29		17.80± 2.63		16.38± 3.43		117.84± 17.15	
Have a shower(1)	**Yes**	14.02± 2.55	1.53 ;.12	14.97± 3.11	.51 ;.60	20.13± 3.29	29;.76	16.21± 3.57	.47;.63	15.75± 3.62	1.24 ;.21	15.56± 3.17	.80 ;.93	14.94± 3.08	.80 ;.42	111.62± 13.29	.31;.75
	No	15.02± 3.83		15.30± 3.69		20.30± 3.28		15.91± 3.52		15.04± 3.15		15.52± 2.75		15.38± 3.08		112.51± 16.54	
Have a shower(2)	**Yes**	14.65± 3.70	.60 ;.54	14.39± 4.01	.42 ;.66	19.75± 3.57	.63;.52	17.09± 3.49	.29;.76	18.09± 3.05	.86 ;.38	18.13± 2.61	1.13 ;.25	16.49± 3.44	.92 ;.35	118.62± 17.77	.79;.42
	No	14.37± 3.35		14.60± 3.60		19.45± 3.51		16.96± 3.41		17.63± 4.36		17.72± 2.74		16.07± 3.34		116.84± 16.52	

1= Adolescents with hearing loss 2= Adolescents without hearing loss
**p<.05 **p<.001*

Table 6. *Predictors affecting the lifestyle profile of adolescents with and without hearing loss (n= 544)*

Adolescents with hearing loss	β	t	p	F	Model (p)	R2	Durbin Watson
Constant	-	18.896	**.000****	12.326	**.000****	.273	1.573
Gender	-.073	-1.395	.164				
BMI	-.258	-4.865	**.000****				
Number of siblings	-.083	-1.509	.132				
Father education level	-.060	-1.041	.299				
Mother education level	.146	2.561	**.011***				
Social insurance	.056	1.079	.282				
Economic status	-.439	-7.793	**.000****				
Hearing loss classifications	.069	1.302	.194				
Speech level classification	-.001	-.020	.984				
Adolescents without hearing loss	**β**	**t**	**p**	**F**	**Model (p)**	**R2**	**Durbin Watson**
Constant	-	19.994	.000*	8.903	**.000****	.170	1.487
Gender	-.010	-.183	.855				
BMI	-.327	-5.683	**.000****				
Number of siblings	.013	.231	.818				
Father education level	-.001	-.012	.991				
Mother education level	.012	.199	.843				
Social insurance	-.110	-1.939	.054				
Economic status	-.233	-3.840	**.000****				

Note: Multiple linear regression analysis is regression coefficient, β is standardized regression coefficient, R2 is proportion of variation in dependent variable explained by regression model, p is level of statistical significance.
**p<.05 **p<.001*

4. DISCUSSION

The need to enhance healthy lifestyle behaviors in adolescents to prevent health behavior–related chronic conditions and damaging health outcomes in the long term has become a national imperative.

It was seen in the comparison of the two groups that there were no differences in physical activity and nutrition scores. It may be said therefore that HL did not have an impact on these dimensions. In both groups, the adolescents exhibited the highest score in the nutrition, the lowest in the health responsibility subscales. In studies, where the ALP-R2 were applied to high school students, it was reported that the participants recorded the highest score in the interpersonal relations subscale, and the lowest score in the health responsibility dimension (3,18). The inadequacy adolescents showed in taking on health responsibility in both studies was in keeping with the behavior of the adolescents in our study. In other words, in both groups, the participants showed a weakness in assuming responsibility for their own health, and their health education behavior and tendency to seek help when needed was inadequate. This might be explained by the over-confidence of youth, a tendency that is in essence risky behavior.

Although the physical activity and nutrition subscale scores were slightly higher among the adolescents with HL, the difference was not statistically significant (p>.005). These findings suggest that adolescent with HL engage in the same level of activity as their peers. Similarly, it was also promising to observe that there was no difference between the groups in the participants' selection and planning of meals. The similarity in the BMI values of the two groups supports this finding.

The adolescents with HL in our study exhibited lower mean scores than their peers in the subscales of positive life perspective, interpersonal relations, stress management, but a higher mean score in the spiritual health dimension. A difference could be seen between the two groups. The positive life perspective subscale assesses the degree of an individual's positive outlook on life, encompassing aspects of hope or hopelessness. The interpersonal relations subscale determines an individual's communication with family and friends and assesses the level of continuity achieved. Stress management assesses the level at which an individual recognizes sources of stress, stress control mechanisms and coping levels. Spiritual health assesses an individual's beliefs, morals, and values (3,18).

The finding that the adolescents with HL had a more negative approach to life, were weaker in interpersonal relations and

less skilled in stress management indicates that this is a matter that must be underlined. One of the main techniques adolescents with HL use to cope with stress is prayer. Using active coping methods such as working with a hobby, spending time to rest and relax, doing exercises, performing breathing exercises, or showering was less common. The HL may be responsible for this. It might also be that the adolescents' hesitation in working with a hobby, doing exercises and assuming other lifestyle habits may be affecting their ability to take on a positive life perspective, engage in fruitful interpersonal relations and practice stress management. HL may be irreversible but methods and behaviors of coping with stress may be changed and improved at any time.

On top of the stress, they experience by being in the period of adolescence just like their peers, adolescents with HL may also feel the effects of stress factors specific to their disability (19,20). Study findings suggest that adolescents with HL not only feel the effects of stress in terms of daily stressors but are also prone to issues brought about by HL, their experiences with social interactions, the classroom setting and academic difficulties (20). More importantly, these individuals are usually introverted. They tend to cope through withdrawal, it is believed, because of their perceived high level of stress (20,21). Studies concur that the method of turning inward is a nonfunctional coping technique that contributes nothing to solving problems in the long run.[20] Our results have confirmed this. Adolescents with HL tend to use the technique of prayer more than turning to active coping behaviors such as working with a hobby, doing exercises, taking a shower, or doing breathing exercises to cope with stress. The results of studies demonstrate that schools, teachers, and health professionals need to effectively support adolescents with HL in their struggle to cope with the stress of daily living (20).

Adolescents with HL usually have a limited capability for speech (22). Of the adolescents with HL participating in this research, 25.4% exhibited an inability to talk, 25% talked using inappropriate sentence structures, and 12.9% used unintelligible sentences or sounds in their speech. Adolescents with HL who use speech as a means of communication are more likely to miss or misunderstand what is being taught in the learning setting and may benefit less from the knowledge imparted compared to their peers who can hear (23,24). It is for this reason also that interaction and relations between peers may be difficult for young people who are adolescents with HL. The barriers to communication may have a negative effect on the interaction of adolescents with HL (24,25). Adolescents with HL generally have a higher rate of experiencing issues in their social contacts and relations with peers compared to their hearing counterparts. They are also confronted with difficulties in participating in social activities, they have trouble with self-acceptance, and are challenged by having to defend themselves and cope with issues of perceived stigmatization (26). It has also been reported that these individuals find it difficult to find leisure time activities for themselves and suffer from problems they experience in their participation in social activities. Adolescents with HL cannot participate like their peers in activities such as listening to music or events that depend on vocal commands. HL can diminish the capacity to participate in leisure time activities. Data have shown that the degree of HL and coping style are associated with the level of stress perceived by adolescents with HL (27). All this knowledge reported in the literature is consistent with the findings of our own study.

Social withdrawal and low self-esteem are the primary sources of stress in adolescents with HL (7). The health responsibility and spiritual health subscale scores of adolescents with HL who prefer to pray as a state of coping with stress were significantly higher. In a research conducted with children with HL, it was determined that praying has a positive contribution to spiritual health in children who grow up culturally and religiously based on faith (28). In our study, it is thought that the adolescents chose to pray as a coping style because they live in a region with Muslim beliefs.

Stress management is positively affected if adolescents with HL prefer exercise as their coping style. When the literature is examined, it has been determined that exercising increases stress management in adolescents with HL (29,30). The reason for this is that because of physical activity, adolescents experience the pleasure of accomplishing something and the feeling of relaxation and feeling happy (7,21,30). Also, while the life profile level of exercisers is significantly higher in disabled adolescents without HL, there is no difference in disabled adolescents with HL. When the reason is discussed, it is thought that exercise has a positive effect on health responsibility, physical activity, nutrition, and spiritual health.

When the predictors affecting the lifestyle profile in adolescents are examined, BMI, mother education level and economic status are effective in adolescents with HL, while BMI and economic status are effective in the other group. Increase in BMI of adolescents negatively affects their lifestyle profile (1,10). The reason for this can be attributed to the high consumption of fast food and sedentary behavior of adolescents in Turkish society (31,32).

The decline in economic level of adolescents negatively affects the lifestyle profile. This finding is supported by the literature (1,3,33). However, the decline in economic status affects the lifestyle profile of those without HL %0.233 times, while it affects %0.439 times for those with HL. When the reason of this situation is discussed, the opportunities of poor adolescents with HL, such as access to healthy nutrition and health services, are affected more negatively (15,20). For this reason, adolescents with HL have more disadvantages and their lifestyle profiles are lower.

In our study, while the increase of mother education level in adolescents with HL affects the lifestyle profile positively, it does not have any effect in those without HL. When the reason for this is discussed, the intense stress experienced by adolescents with HL pushes them to seek social support (30). Adolescents with HL frequently seek social support from the family, especially the mother (14,20,29). To provide adequate and correct support to adolescents with HL, it is important

that the mother education level is high (29). When previous studies were examined, it was found that high mother education level of adolescents with HL influences positive health behaviors and stress management in adolescents (30,34).

Strengths and Limitations

The strength of this study is that it was conducted with a difficult-to-study group with HL. Also, it is the first study in the literature to measure the lifestyle behaviors of this group. A limitation is that the research was carried out in only four schools.

5. CONCLUSION

Significant differences were found in this study comparing adolescents with and without hearing loss. The lifestyle profile of adolescents with HL is lower to that of hearing adolescents. Thus, when considered from a general perspective, adolescents with HL have much more of a need to improve their healthy lifestyle behaviors. There are differences between the two groups in the subscales of positive life perspective, interpersonal relations, stress management and spiritual health. Outside of spiritual health, the mean scores of the adolescents with HL were lower. In both groups, the adolescents exhibited the lowest score in the health responsibility subscale. There is a difference in coping styles with stress (pray, hobbies and exercise etc.) of two group. In adolescents with HL, those who prayed as a coping style had higher lifestyle profiles than those who did not. While BMI, mother education level and economic status affected the lifestyle profiles of adolescents with HL, only BMI and economic status affected those without HL. Adolescents with HL are less capable of effective stress management and suffer therefore from a relatively higher level of stress.

Adolescents with HL are more vulnerable in terms of healthy lifestyle behaviours and coping style with stress. Nurses can make school-based interventions on positive life perspective, interpersonal relations, stress management, spiritual health, nutrition, physical activity, and health responsibility to increase lifestyle profiles in adolescents. Accessible psychological support resources, guidance and psychological counseling services should be provided to all schools with groups with special needs such as hearing loss. In addition, it is thought that increasing mother support and strengthening the communication with the mother on this special group will positively affect the lifestyle profiles. In accordance with the philosophy of mental health and psychiatric nursing, it can be suggested to examine the healthy lifestyle behaviours, stress management, coping styles and family support in more detail on vulnerable groups (adolescents with hearing, vision, speech, and physical disabilities etc.)

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Clinical and Experimental Health Sciences

Perceptions of Turkish Nursing Students on Nursing Diagnoses

Munevver Sonmez[1], Oznur Gurlek Kisacik[2]

[1] Atilim University, Faculty of Health Science, Nursing Department, Fundamentals of Nursing Department, Ankara, Türkiye.
[2] Afyonkarahisar Health Science University, Fundamentals of Nursing Department, Faculty of Health Science, Afyonkarahisar, Türkiye.

Correspondence Author: Munevver Sonmez
E-mail: munevverunlu@gmail.com

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ABSTRACT

Objective: This study aimed to determine how Turkish nursing students' perceived nursing diagnosis.

Methods: This descriptive and cross-sectional study was carried out with 655 nursing students in the Departments of Nursing in the Health Sciences Faculties of two universities, in the Aegean and western Black Sea Region, between 15 February and 5 April 2020. The Students Information Form and Perceptions of Nursing Diagnosis Scale were used for data collection. The independent samples t-test, one-way ANOVA test, and the Mann-Whitney U-test with Bonferroni-correction were used to determine the differences between the groups. Pearson correlation analysis was used to determine the effects of independent variables

Results: The mean age of the nursing students was 21.12±1.39; 76.6% of them were female and 36.8% were in the second-year. 53.7% of the nursing students reported that they had difficulty in making nursing diagnoses. The overall Perceptions of Nursing Diagnosis Survey score of the nursing students was found to be 2.46±051. Statistically significant difference was found between Perceptions of Nursing Diagnosis Survey scores in terms of gender (p=0.012), the necessity of nursing diagnosis (p<0.001), and having sufficient knowledge about nursing diagnosis (p=0.019).

Conclusions: The findings of this study have revealed that Turkish nursing students' perceptions of nursing diagnoses are positive. It is important that use effective teaching methods in teaching nursing diagnoses in fundamental nursing education, to give more importance to nursing diagnoses. It is recommended to plan qualitative studies to in-depth examine students' perceptions with randomized controlled studies involving innovative educational interventions in the future.

Keywords: Nursing diagnosis, nursing students, Perceptions of Nursing Diagnosis Scale

1. INTRODUCTION

Defining specific responses to health problems and biological processes in order to achieve positive health care outcomes and make the necessary interventions is an indispensable part of nursing (1). Nurses around the world use nursing diagnoses to correctly define the patients' health care problems, risk status, and willingness to engage in health promotion and improvement (2). A nursing diagnosis defines the basic concepts of nursing and the function of nurses in the provision of health services, as well as constituting the basis for planning, implementing, and assessing nursing care (1,3).

Using a standard terminology to make nursing diagnoses contributes to the continuity quality of nursing care and the visibility of nursing practices. It also enables the recognition of the scientific aspect of nursing by strengthening the communication between nurses, the other members of the health care team and patients (3,4). Therefore, it is important to determine and use the correct nursing diagnosis, identification of nursing diagnosis is the principal component of the diagnosis step of the nursing process, in order to obtain positive patient care outcomes. (7,8). There is some evidence that professional patient care based on the use of nursing diagnoses with a standardized terminology positively affects patient care outcomes (4,9-12).

Nursing students are considered to be prospective health care professionals. Nursing education aims to teach nursing students how to provide individualized patient care based on nursing process (13). Giving nursing students the necessary knowledge and skills to use nursing diagnoses contributes to their understanding of their professional roles and to achieve better quality patient care outcomes by using nursing diagnoses when they start their professional life (14). However,

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making nursing diagnoses requires education, proficiency and experience. National and international studies (15,16) have shown that determining the correct nursing diagnosis is still a difficult process for nursing students (17-19). Nursing students' perceptions of what nursing diagnoses are, their attitudes towards nursing diagnoses, and the importance they attach to nursing diagnoses may affect patient care by affecting the use of such diagnoses (13). A number of studies have reported that negative perceptions regard to nursing diagnoses can negatively affect the use of nursing diagnoses (2,20,21).

In Turkey, the requirement to care for patients in accordance with the nursing process was limited to the education in nursing undergraduate programs until 2007. However, revisions made in the legal regulations between 2007-2010 related to nursing have made the delivery of patient care services based on the nursing process in the clinical environment into a legal obligation made. The regulations for improving healthcare quality standards in Turkey have required using the nursing process as a standard for patient care since 2015. Given that students will continue to use nursing diagnoses when they begin their professional life, having a positive attitude towards the diagnostic process, and being able to correctly make and apply diagnoses are fundamental to a successful nursing education. Determining the perceptions of nursing students regarding nursing diagnoses can guide us to identify areas in teaching nursing diagnoses that need to be improved. This study thus aimed to determine how Turkish nursing students' perceived nursing diagnoses and the factors affecting this variable.

2. METHODS

2.1. Study Design and Setting

This descriptive and cross-sectional two-centered study was conducted between 15 February and 5 April 2020 in the Departments of Nursing in the Health Sciences Faculties of two universities in Afyonkarahisar and Zonguldak. The Ethical committee approval for the study was obtained from the Clinical Research Ethics Committee of the one of the universities (Date/No:2020/99), and the written permission was obtained from the administrators of the Committee of the faculties where the study was conducted. The nursing students participating in the study were also informed about the objective of the study and their written and verbal informed consent were obtained. The data were also analyzed and reported in such a way that the participants cannot be identified.

The sample of this study consisted of all second-, third – and fourth-year nursing students of these two universities. 655 participants who were second, third, and fourth-year nursing students during the 2019-2020 academic year, who had received theoretical education on the Nursing Process, and Nursing Terminologies and Classifications within the scope of a Fundamentals of Nursing course who had experience in clinical practice, and who gave their informed consent were included in the study. First-year nursing students in these universities were not included in the study, because they did not take Fundamentals of Nursing course, their experience of using the nursing process was not adequate and they had no experience of clinical practice. 655 of the 790 students answered the questionnaires. The overall participation rate in the study was 82.9%.

2.2. Data Instrument

The Student Information Form and Perceptions of Nursing Diagnosis (PND) Scale were used as data collection tools.

The Student Information Form

This form was prepared by the researchers after reviewing the literature and obtaining expert opinions. It contained 13 questions about the characteristics of the nursing students and their views about nursing diagnoses. The content validity of the information form was evaluated by five academic experts in the Nursing Process, and Nursing Terminologies and Classifications. No changes were recommended by the experts. After the evaluation process, a preliminary study was conducted with 10 nursing students to determine the comprehensibility of the questions.

Perceptions of Nursing Diagnosis (PND) Scale

This instrument was developed in 1991 by Olsen et al. (22). The Turkish validity and reliability study of the scale was conducted in 2013 by Korhan et al., and the final version of the scale consisted of 26 items (23). The measuring tool is a 5-point Likert-type scale in which 1 means ''strongly agree'' while 5 means ''strongly disagree''. The 26 items reflect nurses' perceptions about the use, practicality, aims, results, goals and limitations of nursing diagnoses within four thematic areas. The score obtained from the scale ranges between 1 and 5; lower scores indicate a more positive PND (22). In the Turkish validity and reliability study, the Cronbach's alpha value was 0.84. In this study, the Cronbach's alpha value was found to be 0.89.

2.3. Data Collection

An appropriate time period out of the course and practice hours was selected for the nursing students to answer the questions in the data collection tools. After the students were informed about the aim of the study, the data collection forms were distributed, and the completed questionnaires were collected as face to face by researchers. In order to the confidentiality of the data, the students were asked to fill in the data collection forms anonymously and to put the completed forms in sealed envelopes. The data collection forms required an average of 15-20 minutes to complete. The educational process in Turkey, including universities, has been interrupted since March 16, 2020, because of the outbreak of COVID-19. Therefore, some of the data were collected using

an online survey via Google Forms.165 students, who were previously planned to be reached by face-to-face interview method, answered the items on the same data collection forms online.

2.4. Data Analysis

The data were analyzed using the SPSS version 22.0 (Armonk, NY: IBM Corp). Skewness-Kurtosis values and the Kolmogorov-Smirnov test were used to evaluate whether the data were distributed normally. The descriptive statistics of continuous variables in the study were shown with mean, standard deviation, and minimum and maximum values, while descriptive statistics of categorical variables were shown by frequency and percentage. To determine the differences between the groups, the independent samples t-test, one-way ANOVA test, and the Mann-Whitney U-test with Bonferroni-correction were used. Pearson correlation analysis was used to determine the effects of independent variables. $p<0.05$ was accepted as statistically significant.

3. RESULTS

3.1. Characteristics of the Nursing Students

The mean age of the nursing students was 21.12±1.39; 76.6% of them were female and 36.8% were in the second-year. 82.9% of nursing students reported that they had not participated in extracurricular activities about nursing diagnosis. 95.3% of them stated that they used nursing diagnosis in clinical practice and 86.4% stated that nursing diagnosis was necessary. 65% of the nursing students reported that they found their current knowledge about nursing diagnosis to be partially adequate and 82.3% of them stated that they need more education on the topic. 53.7% of the nursing students reported that they had difficulty in making nursing diagnoses, while 89.3% of them used a guide to make them (Table 1).

3.2. Nursing Students' PND Scale Scores

The overall PND score of the nursing students was found to be 2.46±051. The mean scores on each of the sub-dimensions were as follows: for ''delineation and promotion of nursing profession'' 2.13±0.71; for ''clear representation of patient status'' 2.92±0.64; for ''ease of use'' 2.62±0.64; and for ''conceptual orientation'' 2.79±0.66 (Table 2). It was found that there were statistically significant differences in the overall PND scores in terms of gender ($p=0.012$), the necessity of nursing diagnosis ($p<0.001$), and having sufficient knowledge about nursing diagnosis ($p=0.019$) (Table 3).The significant differences between the mean scores on the sub-dimensions according to the characteristics of nursing students are presented in Table 3.

Table 1. *Nursing students' characteristics (n= 655)*

Characteristics	Mean ± SD		
Age (Years)	21.12±1.39		
Number of days in clinical practice (weekly)	2.19±1.31		
	Categories	**n**	**%**
Gender	Female	502	76.6
	Male	153	23.4
Year of education	Second	241	36.8
	Third	206	31.5
	Fourth	208	31.8
Previous extracurricular activities about Nursing Diagnosis	Yes	112	17.1
	No	543	82.9
Type of extracurricular activities (n= 112)	Conference	75	66.9
	Congress	18	16.2
	Clinical practice	19	16.9
Use of nursing diagnosis in clinical practice	Yes	624	95.3
	No	31	4.7
Necessity of nursing diagnosis	Yes	566	86.4
	No	89	13.6
Having sufficient knowledge level about nursing diagnosis	Yes	152	23.2
	Partially	426	65.0
	No	77	11.8
Wanting to receive education about the nursing diagnosis	Yes	539	82.3
	No	116	17.7
Having difficulty to determine the nursing diagnosis	Yes	352	53.7
	No	303	46.3
Using guides to determine the nursing diagnosis	Yes	585	89.3
	No	70	10.7
Type of guides (n= 585)	NANDA Diagnosis list	526	89.9
	Clinical nurses	33	5.6
	Lecturer	26	4.5

Table 2. *Nursing students' perception of nursing diagnosisby total scale and subscales scores (N= 655)*

Subscales	Min	Max	Mean±SD
Delineation and promotion of nursing profession	1.00	5.00	2.13±0.71
Clear representation of patient situation	1.00	5.00	2.92±0.64
Ease to use	1.00	5.00	2.62±0.64
Conceptual orientation	1.00	5.00	2.79±0.66
Total score	**1.00**	**5.00**	**2.46±051**

SD: standart deviation

Table 3. *Comparison of nursing students' perceptions of nursing diagnosis scale scores according to their characteristics*

Characteristics	Delineation and promotion of nursing profession	Clear representation of patient situation	Ease to use	Conceptual orientation	Total score
Gender	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Female	2.07±0.65	2.92±0.58	2.61±0.61	2.78±0.61	2.43±0.45
Male	2.35±0.84	2.91±0.78	2.66±0.73	2.79±0.80	2.58±0.68
Test value and significance *	t= – 3.857 **p<0.001**	t= 0.250 p=0.85	t= – 0.727 p=0.46	t= – 0.130 p=0.89	t= – 2.522 **p= 0.012**
Year of education					
Second	2.03±0.60[a]	2.95±0.56	2.66±0.59	2.78±0.60	2.42±0.44
Third	2.20±0.70[b]	2.85±0.59	2.64±0.67	2.69±0.62[a]	2.47±0.49
Fourth	2.19±0.82[b]	2.96±0.75	2.55±0.67	2.89±0.74[b]	2.50±0.61
Test value and significance**	F= 4.080 **p= 0.017**	F= 1.768 p= 0.17	F= 1.942 p= 0.14	F= 4.811 **p= 0.008**	F= 1.332 p= 0.26
Previous extra curricular activities about nursing diagnosis					
Yes	2.13±0.69	2.89±0.67	2.62±0.62	2.70±0.65	2.47±0.61
No	2.17±0.82	2.93±0.63	2.64±0.74	2.80±0.60	2.48±0.49
Test value and significance*	t= 0.519 p= 0.60	t= – 0.582 p=0.56	t= 0.270 p= 0.78	t= – 1.547 p= 0.12	t= 0.068 p= 0.94
Necessity of nursing diagnosis					
Yes	2.07±0.68	2.92±0.62	2.58±0.60	2.74±0.69	2.43±0.49
No	2.59±0.75	2.92±0.71	2.87±0.82	2.79±0.65	2.73±0.61
Test value and significance*	t= – 6.347 **p<0.001**	t= 0.033 p= 0.97	t= – 3.040 **p= 0.003**	t= 0.620 p= 0.53	t= – 4.237 **p<0.001**
Having sufficient knowledge level about nursing diagnosis					
Yes	2.09±0.66	2.87±0.73	2.53±0.73[a]	2.78±0.75	2.44±0.46[a]
Partially	2.18±0.82	2.91±0.61	2.60±0.57[a]	2.78±0.62	2.46±0.63
No	2.27±0.74	3.06±0.54	2.91±0.74[b]	2.84±0.64	2.62±0.50[b]
Test value and significance**	= 2.529 p= 0.08	F= 2.459 p= 0.08	F= 9.488 **p<0.001**	F= 0.348 p= 0.70	F= 3.075 **p= 0.019**
Use of nursing diagnosis in clinical practice					
Yes	2.13±0.70	2.80±0.73	2.61±0.63	2.78±0.65	2.45±0.50
No	2.27±0.84	2.93±0.63	2.69±0.76	2.82±0.78	2.54±0.69
Test value and significance*	t= – 1.116 p= 0.26	t= 1.104 p= 0.27	t= – 1.543 p= 0.12	t= – 0.324 p= 0.74	t= – 0.798 p= 0.42
Having difficulty to determine the nursing diagnosis					
Yes	2.16±0.69	2.98±0.66	2.72±0.62	2.87±0.68	2.47±0.46
No	2.11±0.73	2.87±0.61	2.50±0.65	2.71±0.62	2.46±0.54
Test value and significance*	t= 0.855 p= 0.39	t= – 2.344 **p= 0.019**	t= 4.262 **p<0.001**	t= – 3.164 **p= 0.002**	t= 0.269 p= 0.78
Wanting to receive education about the nursing diagnosis					
Yes	2.09±0.69	2.89±0.67	2.56±0.76	2.78±0.65	2.45±0.49
No	2.32±0.80	2.93±0.63	2.63±0..61	2.81±0.71	2.54±0.61
Test value and significance*	t= – 2.809 **p= 0.006**	t= 0.500 p= 0.61	t= 0.943 p= 0.34	t= – 0.410 p= 0.68	t= – 1.831 p= 0.06
Number of days in clinical practice (weekly)	r***= 0.059 p= 0.13	r= 0.054 p= 0.16	r= – 0.057 p= 0.14	r= 0.104 **p= 0.008**	r= 0.060 p= 0.12

**Independent Samples-t testwas used*
***One-Way Anova test was used*
****Pearson correlation coefficient*
[a, b]Different superscripts within the same column indicate significant difference among groups (p<0.05)
Significant at the p< 0.05 level

4. DISCUSSION

Positive perceptions of the use of nursing diagnoses can increase the ability to identify patients' health problems and to plan nursing care, as well as to improve the quality of care (14). This study was conducted to determine Turkish nursing students' perceptions of nursing diagnoses, and the students' mean PND score was 2.46±051 out of 5. Inangil and Uzen-Cura (2020) reported the mean PND score of Turkish nursing students as 2.38±0.43, while Ozveren et al. (2019) reported the same score as 2.38±0.40 (14, 24). Abed El Rahman, Kaleldeh and Malek (2017) found that Jordanian nursing students' perceptions of nursing diagnoses were positive (15). Similar to our findings, other national and international

studies have found that perceptions of students on nursing diagnoses are positive and they had a positive perception of the use of nursing diagnoses (2,13,23,25,26).

In this study, most (86.4%) of the Turkish nursing students emphasized the necessity of nursing diagnoses for patient care. This is a welcome finding. Other studies have stated that nursing students' positive perceptions of nursing diagnoses affect how they are used, and also increase the quality of patient care by leading to more accurate diagnoses of patients' health problems and helping to better plan patient care (2,14,17). When nursing students have positive perceptions of nursing diagnoses, they are better able to use them to provide individualized care during clinical practice. Students who acquire the skill of making accurate nursing diagnoses during their nursing education are able to provide better-quality nursing care during their professional careers by continuing to use nursing diagnoses. However, the small number of students who stated that they did not believe in the necessity of nursing diagnoses should not be ignored. This is an important finding and should be taken into account by academics and nurse educators.

Clinical practice is a process in which nursing students think and theoretical knowledge into practice. In this process, nursing students make clinical decisions about the problems of the patients and transform these decisions into nursing diagnosis (27). Our findings showed that more than half of the nursing students found their knowledge about nursing diagnosis insufficient and had difficulties in determining appropriate nursing diagnoses during their clinical practice. The fact that most of the nursing students participating in this study wanted to receive training about nursing diagnoses also supports this finding. This finding is consistent with the results of previous studies conducted with nursing students. These studies also determined that the nursing students had difficulties in determining appropriate nursing diagnoses and distinguishing nursing diagnoses from medical diagnoses, misused nursing diagnoses, and expressed medical diagnosis, symptoms and findings as nursing diagnosis (3, 5,17,28-31). However, it is important for nursing students to determine nursing diagnoses correctly so that they can evaluate patients holistically, provide quality care, and achieve positive patient outcomes (32-34). Making incorrect nursing diagnoses may cause nursing students to prepare inappropriate care plans for their patients, and lead as a result to the implementation of non-patient-specific nursing interventions. This can give rise to negative consequences in the treatment of patients and endanger their safety (30,35). Therefore, it is the responsibility of nursing educators to ensure nursing students develop and maintain the skills required to make correct nursing diagnoses (36). Considering the students' positive perceptions of nursing diagnoses, it is suggested that they may also be open to educational interventions in this area. Educational interventions are an important factor in helping students to develop positive attitudes towards nursing diagnoses and improving their diagnostic skills (13,25,29,37-40). In this study, the perception of nursing diagnoses among students who reported that they had sufficient knowledge about nursing diagnoses was significantly more positive. This finding is consistent with the findings of Olivea et al. (2005), who stated that Brazilian nurses' attitudes towards nursing diagnoses were related to their degree of knowledge (41). It was an expected result that nursing students who believed they had sufficient knowledge about nursing diagnoses would have a positive perception of them. Therefore, nurse educators should aim to improve all nursing students' knowledge of nursing diagnoses and their ability to use these diagnoses.

Our findings showed that gender was a factor affecting the nursing students' perceptions of nursing diagnoses. The male students had a more negative perception for the sub-dimension of delineation and promotion of the nursing profession and whole PND scale compared to the female students. The number of studies investigating the role of gender in the use of nursing diagnoses and perceptions and attitudes towards them is limited. Unlike our findings, Lumillo-Gutierrez et al. (2018) reported that gender is not a factor affecting the use of nursing diagnoses and attitudes towards them (40). As a result of legal regulations in Turkey, nursing was for many years a profession only practiced by female individuals. Thanks to revisions made in the regulations since 2007, male nurses have also begun to slowly enter the profession. Thus, this situation may have caused that the concepts related to the role of caregiving, which is one of the professional responsibilities of nurses, to develop more slowly in male nurses. In various studies conducted with Turkish nursing students, female students were found to be better able to engage in professional behaviors than male students (42,43).

The scores of the nursing students for the subdimensions of the PND scale showed that their perceptions of the "clear representation of patient situation" subdimension were more negative. Some studies conducted with Turkish nursing students have similarly reported that the nursing students' perceptions of this subdimension were more negative (13,23,24). These findings suggest that nursing students have difficulty in making the nursing diagnoses that best identify the patients' problems. The finding that the perceptions of students who reported having difficulty in making nursing diagnoses were significantly more negative supports this idea. Ozveren et al. (2019) found that Turkish nursing students who stated that they could make nursing diagnoses without help had a more positive perception of the "clear representation of patient situation" subdimension (14). Inangil and Uzen-Cura (2020) reported that nursing students' perceptions of clinical decision-making were an effective and facilitating factor in making correct nursing diagnoses (24). Halverson et al. (2011) investigated the changes over time in the perceptions of nurses from Minnesota about nursing diagnoses (2). They reported that the negative perceptions of the "clear representation of patient situation" subdimension changed least. There is thus still a gap between nurses and nursing students with regard to understanding the meaning of nursing diagnoses, and this requires further attention and educational interventions. Karaca and Aslan (2018)

pointed out that training programs about nursing diagnoses can diminish the negative perceptions of nursing students regarding the "clear representation of patient situation" subdimension (13).

5. CONCLUSION

An accurate nursing diagnosis facilitates the resolution of the patients' problems and enables them to receive systematic and individualized care. The findings of this study have revealed that Turkish nursing students still have difficulty in making nursing diagnoses and thus sometimes feel incompetent in this respect. However, Turkish nursing students' perceptions of nursing diagnoses are positive and this is similar to other results in the literature. While having positive attitudes towards the necessity of nursing diagnoses and sufficient knowledge about such diagnoses, has a positive effect on the perception of them, the difficulties in using nursing diagnoses are associated with more negative perceptions.

In line with these results, were commended using effective teaching methods in teaching nursing diagnoses in fundamental nursing education, to give more importance to nursing diagnoses, to guide students in diagnosis stages, and to provide continuous feedback. Thus, understanding the importance of nursing diagnoses and developing a positive attitude regarding such diagnoses by nursing students can be ensured. Given the gender-based differences in students' perceptions of nursing diagnoses, planning gender-specific educational interventions may be beneficial in teaching concepts related to professional values in nursing and improving male students' perceptions of nursing diagnoses.

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Psychometric Properties of the Turkish Health Enhancement Lifestyle Profile-Screener Questionnaire (T-Help-Screener)

Özgu İnal[1],Fatma Nesrin Turan[2], Serkan Pekçetin[3],Eric Hwang[4]

[1] University of Health Sciences, Faculty of Gülhane Health Sciences, Department of Occupational Therapy, Ankara, Türkiye.
[2] Trakya University, Medicine Faculty, Department of Biostatistics, Edirne, Türkiye.
[3] University of Health Sciences, Faculty of Gülhane Health Sciences, Department of Occupational Therapy, Ankara, Türkiye.
[4] California State University, Dominguez Hills, College of Health, Human Services and Nursing, Department of Occupational Therapy, CA, USA.

Correspondence Author: Özgu İnal
E-mail: inalozgu@gmail.com

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ABSTRACT

Objective: Healthy ageing is associated with a healthy lifestyle. The aim of this study was to evaluate the validity, reliability, and cultural adaptation of the Turkish version of the Health Enhancement Lifestyle Profile Screener (T-HELP-Screener).

Methods: This study included 150 participants aged 65 years and above. Internal consistency of the T-HELP-Screener was measured using Kuder–Richardson. Test-retest reliability was performed with 66 of the 150 participants using intraclass correlation coefficient (ICC). Construct validity of the T-HELP-Screener was analyzed with the Healthy Lifestyle Behavior Scale-II (HLBS-II).

Results: This study showed a substantially reliable level (Kuder–Richardson=0.72). The ICC for each of the T-HELP-Screener items ranged from 0.750 (95% CI = 0.590–0.847) to 0.965 (95% CI = 0.942–0.978) indicating acceptable to good test-retest reliability. There was a significant moderate correlation between the T-HELP-Screener and the HLBS-II (r_s = 0.488; p =0.001).

Conclusion: This study supports the psychometric properties of the T-HELP-Screener. This tool can help quickly identify older adults who need a more comprehensive assessment of their health risk behaviours. Future studies should develop and validate a Turkish version of the comprehensive 56-item Health Enhancement Lifestyle Profile (HELP).

Keywords: aging, lifestyles, older adults, health promotion

1. INTRODUCTION

The world's elderly population is growing every day. By 2050, the population of people aged 65 and up is predicted to double (1). Older adults are at the highest risk for chronic diseases (2). The phenomena of changes in health status observed among individuals during ageing has led to theories on the causes of ageing as well as the conceptualization of "successful" or "healthy" ageing (3,4). These aging models depict the process by which older adults optimize their chances of improving and maintaining physical, cognitive, and social functions that can contribute to a healthy long life (5). Extension of both life span and health span has become the optimal outcome of health promotion measures of modern times (6).

Epidemiological studies have showed that a healthy lifestyle is linked to healthy aging and it has a significant role in the primary prevention of many diseases (7). Lifestyle factors such as smoking behaviour, alcohol consumption, physical activity and body weight are related to an individual's health and functionality (8, 9). Moreover, an unhealthy lifestyle has been accepted as the determinant of many diseases owing to which people have lost their lives to date. To provide the well-being of a rapidly growing elderly population, strategies must be in place to promote healthy lifestyles and prevent the occurrence of neurodegenerative and non-communicable diseases in the later stages of life (10). Therefore, measures aiming to enhance healthy lifestyle behaviours for the elderly have been of great interest in recent years (7).

The occupational therapy profession is focused on a holistic approach in analyzing and managing different lifestyle factors and vocations that can decide health and wellness, according to the literature (11,12). The screening version of the Health Enhancement Lifestyle Profile (HELP-Screener) is a 15-item self-report questionnaire that was designed as a time-efficient tool for screening health risk behaviours in older adults (13). The HELP-Screener uses a dichotomous scale: yes (1 point) or no (0 point), with a total score ranging from 0 to 15. Higher scores show a potentially healthier lifestyle. The established cut-off score (9 points) aids in

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identifying those who may benefit from a more in-depth evaluation with the 56-item Health Enhancement Lifestyle Profile (14,15). The development and adoption of a healthy lifestyle is critical in healthy ageing. Lifestyle measurements can guide occupational therapists in planning interventions or recommendations that could enhance healthy aging for individual clients or a community as a whole. In Turkey, there has been a lack of screening tools measuring lifestyle behaviours of older adults. Lengthy measurements have been found impractical for use as a routine screening in clinical settings with high numbers of elderly clients. The 15-item HELP-Screener (13) can potentially be a quick and easy lifestyle screening tool to use with older adults in various settings in Turkey.

The purpose of this study was to translate and adapt the 15-item HELP-Screener for its cross-cultural use in Turkey. Adapting an instrument developed within one cultural context for its use in another requires empirical scrutiny to ensure psychometric soundness (16). Several procedures were conducted in this study to ensure the linguistic and cultural appropriateness as well as to establish reliability and validity of the Turkish version of HELP-Screener (T-HELP-Screener).

2. METHODS

2.1. Participants

Criteria for the study participants were age over 65 years, no communication problems, and a Mini-Mental State Examination score of 24 or above (17). The necessary ethics committee approval was obtained for the study and written informed consent was received from all participants. In the validity and reliability studies, we decided to work with 150 people (15x10), 10 times the number of items, according to the application of 5-10 times the number of items We adopted the rule of thumb, the 10:1 ratio of respondents to items for determining our anticipated participant size of 150 (18). Permission to develop the Turkish version of HELP-Screener was granted by the original author.

2.2. Stages of the Study

The study comprised four parts: (1) translation and cultural adaptation of the T-HELP-Screener, (2) content validity, (3) internal consistency and reliability, and (4) construct validity.

2.2.1. Translation and Cultural Adaptation

Two bilingual Turkish experts translated the HELP-Screener from English to Turkish. Two translations were assessed and converted into one scale with consensus. Then, the scale was translated from Turkish to English by a native English-speaking expert who also speaks Turkish. The back translation was compared with the original version in a meeting by the translation team. Each item of the T-HELP-Screener was semantically the same as that in the original version. This initial T-HELP-Screener was reviewed and approved by the author of the original HELP-Screener. Subsequently, a pilot study was performed to evaluate the intelligibility of the Turkish version in the last stage. Thirty participants aged 65 years or above were invited to complete the initial T-HELP-Screener and to determine whether they faced difficulty in understanding the items due to possible language or cultural unfitness.

2.2.2. Content Validity

A panel of 10 experts comprising four occupational therapy practitioners, four physiotherapists, one dietitian, and one nurse were asked to contribute their opinions for content validity. A 4-point content validity index (CVI) was used by the experts to rate each T-HELP-Screener item (1 – not essential, 2 – somewhat essential but the item needs to be revised, 3 – essential but may need minor revision, and 4 – very essential). Lawshe's content validity ratio was used to determine content validity for each T-HELP-Screener item (19) (see Results).

2.2.3. Internal Consistency and Test-Retest Reliability

Internal consistency and test-retest reliability were assessed as reliability measures for the T-HELP-Screener. Internal consistency, which is the consistency of the results of the items in a test, was measured using Kuder Richardson. Test-retest reliability was determined by 66 participants who completed the T-HELP-Screener twice, with an interval of 2 weeks (20).

2.2.4. Construct Validity

The construct validity of the T-HELP-Screener was assessed by the correlation between the T-HELP-Screener and Healthy Lifestyle Behavior Scale II (HLBS-II) (21). The HLBS-II has been administered with individuals aged 19-92 years. In other words, the scale is used in the evaluation of adults, not exclusively for the elderly population. The HLBS-II measures health promoting behaviours through a total of 52 items in the six subscales: mental development, health responsibility, physical activity, nutrition, interpersonal relationships, and stress management. A 4-point Likert scale is used by the HLBS-II to indicate the frequency of health promoting behaviours (1 – never, 2 – sometimes, 3 – often, and 4 – regularly).

2.4. Data Analysis

Data was analyzed by using SPSS Statistics for Windows, Version 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). The internal consistency reliability of the T-HELP-Screener was assessed by calculating Kuder–Richardson coefficient. Both Kappa statistics and the intraclass correlation coefficient (ICC) were used to assess the test-retest reliability. To determine the construct validity, Spearman's rho correlation coefficient (r_s) was calculated to

analyze the correlations between the T-HELP-Screener and the HLBS-II. The following cut-offs of correlation coefficients were used to interpret the strength of the relationships: correlation coefficient (r_s) of 0 to 0.20 suggests a negligible correlation, 0.20 to 0.40 suggests a low correlation, 0.40 to 0.60 suggests a moderate correlation, 0.60 to 0.80 suggests a high correlation, and 0.80 to 1.00 suggests a very high correlation. A *p*-value of <0.05 was used to confirm statistical significance.

3. RESULTS

3.1. Participant Demographics

We recruited 150 study participants (81 females and 64 males) aged 65 years or above (67.33 ± 3.86 years). Five individuals were excluded from data analysis due to incomplete responses. Table 1 shows the demographics of the 145 participants. The majority were married (81%), with a university degree (57%), living in an urban area (92%), and retired (75%).

Table 1. *Descriptive characteristics of the individuals.*

Descriptive characteristics (N=145)		n (%)
Marital status	unmarried	1 (0.70)
	married	118 (81.40)
	widowed	26 (17.90)
Education	primary	24 (16.60)
	high school	39 (26.90)
	university and above	82 (56.60)
Living Place	village	4 (2.80)
	district	8 (5.50)
	city	133 (91.70)
Employment Status	never worked	18 (12.40)
	retired	108 (74.50)
	retired but I work	19 (13.10)

3.2. Translation and Cultural Adaptation

The results of the pilot study revealed that the 30 participants who completed the initial T-HELP-Screener considered the items, by and large, were understandable and culturally appropriate. However, some participants stated that they had difficulty understanding the word “sedentary” in Item 15. Therefore, the word “inactive” was added in parentheses next to “sedentary.”

3.3. Content validity

Minor changes were made in line with the recommendations of the 10 content validity experts, and the T-HELP-Screener was finalized. The mean CVI score by the experts was 3.47 ± 0.20. Table 2 shows the calculated content validity ratio (CVR) for each T-HELP-Screener item. The CVRs across the items ranged from 0.80 to 1.0, exceeding the critical value (0.62) set for the given numbers of our review experts (10) and test items (15) (19,22).

Table 2. *Content validity ratio (CVR) calculation table.*

Statement	n_e	N/2	$CVR = \left(\frac{n_e - (N/2)}{N/2}\right)$	CVR	Decision
Item 1	9	5	4/5	+0.80	Acceptance
Item 2	10	5	5/5	+1.00	Acceptance
Item 3	10	5	5/5	+1.00	Acceptance
Item 4	9	5	4/5	+0.80	Acceptance
Item 5	10	5	5/5	+1.00	Acceptance
Item 6	9	5	4/5	+0.80	Acceptance
Item 7	9	5	4/5	+0.80	Acceptance
Item 8	10	5	5/5	+1.00	Acceptance
Item 9	10	5	5/5	+1.00	Acceptance
Item 10	10	5	5/5	+1.00	Acceptance
Item 11	10	5	5/5	+1.00	Acceptance
Item 12	10	5	5/5	+1.00	Acceptance
Item 13	10	5	5/5	+1.00	Acceptance
Item 14	9	5	4/5	+0.80	Acceptance
Item 15	9	5	4/5	+0.80	Acceptance

ne = number of experts rating the item as “essential”
N= total number of experts

3.4. Internal Consistency and Test-Retest Reliability

The Kuder–Richardson reliability coefficient for the initial T-HELP-Screener was 0.66 and for the final T-HELP-Screener was 0.72. With the iteration process when the T-HELP-Screener items were deleted one by one, Kuder–Richardson reliability coefficients ranged from 0.623 to 0.657. All these results suggest a substantially reliable level of internal consistency for the T-HELP-Screener. The Kappa statistics at the item level ranged from 0.597 to 0.931, indicating moderate to excellent test-retest agreement across the T-HELP-Screener items. Similarly, the ICC values ranged from 0.750 (95% CI = 0.590 – 0.847) to 0.965 (95% CI = 0.942 – 0.978), suggesting that the test items demonstrate good to excellent test-retest agreement. Table 3 shows the ICC values for the 15 T-HELP-Screener items.

Table 3. *Test–retest evaluation of T-HELP-Screener test items.*

ITEM	ICC	95% CI
T-HELP-Screener Item 1	0.811	0.690–0.885
T-HELP-Screener Item 2	0.924	0.876–0.953
T-HELP-Screener Item 3	0.900	0.837–0.939
T-HELP-Screener Item 4	0.953	0.923–0.971
T-HELP-Screener Item 5	0.925	0.877–0.954
T-HELP-Screener Item 6	0.893	0.824–0.934
T-HELP-Screener Item 7	0.913	0.858–0.947
T-HELP-Screener Item 8	0.888	0.817–0.932
T-HELP-Screener Item 9	0.965	0.942–0.978
T-HELP-Screener Item 10	0.750	0.590–0.847
T-HELP-Screener Item 11	0.897	0.832–0.937
T-HELP-Screener Item 12	0.952	0.921–0.970
T-HELP-Screener Item 13	0.854	0.761–0.911
T-HELP-Screener Item 14	0.878	0.801–0.926
T-HELP-Screener Item 15	0.774	0.630–0.862

ICC: intraclass correlation coefficient
CI: confidence interval

3.5. Construct Validity

Table 4 shows the correlations between the T-HELP-Screener and the HLBS-II. Significant low to moderate correlations were found between the T-HELP-Screener and the six subscales of the HLBS-II (r_s = 0.368 – 0.459). There was a significant moderate correlation between the total scores of the two instruments (r = 0.488; p = 0.001).

Table 4. *Correlations between the T-HELP-Screener and Healthy Lifestyle Behavior Scale II (HLBS-II).*

	Spearman's rho (r_s)	*p*
HLBS-II: Health responsibility	0.376	0.001
HLBS-II: Physical activity	0.459	0.001
HLBS-II: Nutrition	0.368	<0.001
HLBS-II: Mental development	0.397	0.001
HLBS-II: Interpersonal relationships	0.387	0.001
HLBS-II: Stress management	0.412	0.001
HLBS-II Total	0.488	0.001

4. DISCUSSION

Occupational therapists and other healthcare professionals working in the geriatric field know the importance of measuring healthy lifestyle behaviours (14,15). In Turkey, there is no screening tool that provides information about healthy lifestyle behaviours of older adults. This study showed that the Turkish version of the Health Enhancement Lifestyle Profile – Screener (T-HELP-Screener) can help quickly identify older adults who need a more comprehensive assessment.

In the validity study of the original HELP-Screener, Hwang found that all the items, except one ("I consume a variety of healthy foods rich in protein, fibre, or calcium every day") fit the Rasch measurement model (13). Our study found this nutrition item had the lowest correlation with all other items of the T-HELP-Screener. This item was subject to scrutiny by the content review experts. As a result, the item was not deleted because 8 of 10 experts indicated that the item was suitable and essential.

Hwang (13) pointed out that, due to the all-inclusive feature of the HELP-Screener, a high level of internal consistency (homogeneity) within the scale was not expected. The original HELP-Screener's internal consistency of 0.74 was considered an acceptable level of reliability for the instrument (23). Our study has the similar findings: Kuder–Richardson coefficient of 0.66 for the initial T-HELP-Screener and 0.72 for the final version.

The test-retest reliability of the original HELP-Screener was good to excellent as the degree of agreement was supported through *k* statistic (*k*s = 0.76–0.96) (23). Similar findings were also found in our study. Most of the T-HELP-Screener demonstrate good to excellent test-retest reliability, except for Item 10 (avoid foods that are high in fact) that shows a Kappa score of 0.597. the T-HELP-Screener's test–retest reliability was confirmed between the test and retest scores of each item through the analyses of *k* statistic percentage of agreement. This study used a time interval of 2 weeks between testing and retest in recalling bias. The lowest Kappa score was 0.597 for Item 10 (avoid foods that are high in fat). Unknown factors that could altered eating habits of the participants (e.g., holiday events, personal binge or abstention) during the test-retest interval might call for further investigation.

The correlations between the T-HELP-Screener and the six subscales of the HLBS-II were, by and large, significant but low. This is not unanticipated because the T-HELP-Screener as a whole attempts to measure the all-inclusive lifestyle behaviours as opposed to each HLBS-II subscale that undertakes measure for only one specific aspect (e.g., physical activity, mental development, stress management). In turn, a significant moderate correlation (r_s = 0.488) was found between the total scores of the T-HELP-Screener and the HLBS-II. A high correlation was not seen possibly due to the difference in the response formats of the two instruments (binary versus Likert/ordinal scale) (24).

During the translation process of the HELP-Screener from English to Turkish, only one modification to Item 15 (avoid sedentary activities/behaviours) was made. The term "inactive" in parentheses was added beside the word "sedentary" because the latter term is not used in daily practice in the Turkish language. Given the universal feature and content of the health-related lifestyle behaviours included in the original HELP-Screener, the pilot study participants and the content review experts did not identify any need for cultural adaption for the translated T-HELP-Screener.

4.1. Limitations and Future Research

Despite the legitimate participant size according to the rule of thumb (Boateng et al, 2018), this study has a limitation related to the results of convenience sampling. For example, the majority of participants (57%) owned a bachelor's degree or higher. By contrast, only 11% of the population in Turkey are college graduates (25). Moreover, almost all participants (92%) were recruited from an urban area (city). It is unknown whether these disproportionate demographic characteristics as a whole might have skewed the resultant data.

Future normative studies with a larger participant size are needed to establish the cut-off score for the T-HELP-Screener and to examine other aspects of psychometric properties (e.g., known group validity, clinical utility). In addition, we hope to develop and validate a Turkey version of the comprehensive 56-item HELP (14).

5. CONCLUSION

The preliminary evidence is presented in this study for the T-HELP-Screener psychometric properties. The 15-item binary instrument can serve as a self-report questionnaire that is time-efficient and client-centred. All healthcare professionals including occupational therapists who work with elderly

clients in Turkey can adopt this tool in routine practice to monitor the clients' health promoting and risk behaviours. Each T-HELP-Screener item may indicate a specific aspect of health-related lifestyle behaviour. Before the cut-off norm of the T-HELP-Screen and its complete 56-item version come available, professionals can explore the client's response to each item and collaborate with them for identifying health risk behaviours as well as the corresponding remedial strategies.

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Clinical and Experimental Health Sciences

Evaluation of Nursing Functions In Home Health Services: A Retrospective Study

Şenay Şener[1], Dilek Cingil[2], Seda Göger[3]

[1] Sakarya University, Institute of Health Sciences Nursing Department, Sakarya, Türkiye.
[2] Necmettin Erbakan University, Faculty of Nursing Public Health Nursing Department, Konya, Türkiye.
[3] Sakarya University, Vocational School of Health Services, The Elderly Care Program, Sakarya, Türkiye.

Correspondence Author: Dilek Cingil
E-mail: ddayanircingil@gmail.com
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ABSTRACT

Objective: This study aimed to evaluate the nursing functions of home health services.

Methods: The study was planned retrospectively and descriptively in a state hospital's Home Health Care Unit. The study sample consists of all files of patients who received home health services between 16.01.2019 and 16-07.2019. Data Registration Form and Nursing Functions Evaluation Form were used in collecting the data. Kolmogorov-Smirnov test to test compliance with normal distribution in data analysis, descriptive statistics such as frequency, percentage, arithmetic mean, t-test and Mann Whitney U test in independent groups of 2, One-way ANOVA (Post-hoc Tamhane's T^2 test if the difference between groups was significant) and Kruskal-Wallis test were used in groups of 3.

Results: As a result of the data analysis, it was determined that there is a statistically significant difference between the independent, semi-dependent, and dependent function scores applied by nurses in patients with diabetes, recent operations, and heart failure($p<0.05$). Also, a statistically significant difference was found in the nursing function scores applied in individuals who need palliative care and dieticians, use medical devices or assistive devices, and are fed enterally by tube($p<0.05$).Asignificant positive relationship was found between the mean score of independent nursing functions and the frequency of home visits. ($rs=0.142$, $p<0.05$).

Conclusion: As a result of this study, it was determined that semi-dependent functions are used more for nursing practices. It is thought that the interventions implemented by nurses will be more visible with the development of nursing registration systems inhome health services.

Keywords: Home health care, nursing functions

1. INTRODUCTION

Home healthcare is an important healthcare model that is constantly growing (1). This model supports the elderly, physically or mentally disabled, chronically ill, or in the process of recovery in their environment and helping individuals adapt to social life and continue their lives happily and peacefully (2). In addition, within the scope of home health services, examination, test, analysis, treatment, medical care, and rehabilitation services are provided to individuals in their home environment. Training and consultancy services are provided on the care processes of the individual's disease and the correct use of medical devices and equipment that should be used at home. In this way, people can get the periodic care they need from a professional healthcare team (3).

Chronic or malignant diseases and dependency and disability due to these diseases increase the need for home healthcare services (4). Especially individuals with chronic diseases aged 65 and over constitute a group in which home healthcare services are predominantly provided, as they need long-term care (5). In a study conducted on the subject, it was found that 31.7% of the individuals who received home health care after intensive care were between the ages of 60-79, and 47.5% were 80 years old and above (6). In the study of Işık et al. (2016), it was found that 41.5% of the patients receiving home care were between the ages of 61-80, while 30.7% were 81 years old and above (7).Also, studies in the literature report that the most common chronic diseases in individuals receiving home healthcare services are cerebrovascular diseases, diabetes, hypertension, cardiovascular diseases, Alzheimer's, and malignancy (8,9,10).In this context, individuals who experience limitations in their daily life activities due to chronic diseases and treatment methods need nursing care (11).

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Nursing care is among the home health services that individuals and families need most (12). The home care nurse evaluates the individual with physical, emotional, social, and spiritual dimensions and provides home care services for the individual and family in line with the health-promoting, protective, therapeutic, and rehabilitative nursing diagnoses (13). Nursing diagnoses obtained by observation, interview, and physical evaluation methods are problems that nurses are authorized to diagnose and treat independently.In other words, they are independent functions used in nursing care. In addition, home care nurses also have semi-dependent and dependent functions based on physician directives (14).However, there are studies in the literature on the subjectthat describe health services such as urinary catheter care at home, pressure sore care, post-operative care, and the need for information (15,16,17,18,19), butthere is no study that addresses the nursing practices of nurses who have contributed to almost all of these services and have the most roles inhome care of the patient (20). by using their independent, semi-dependent and dependent functions. Therefore, it is thought that the study conducted on the subject will make significant contributions to the literature. Also, the study results are expected to reveal some points in planning home healthcare services, especially in determining the functions of nurses and job descriptions. This study was planned retrospectively and descriptively to evaluate nursing functions in home healthcare services.

Research Questions

-Is there a difference between independent, semi-dependent, and dependent nursing function scores according to the participants' illnesses?

– Is there a difference between independent, semi-dependent, and independent nursing function scores according to the participants' different care and support needs?

-Is there a relationship between home visit frequency, daily living activities, number of illnesses, age variables, and nursing function scores?

2. METHODS

This study aimed to evaluate the nursing functions in the delivery of home health services. The research was planned retrospectively and descriptively in the Home Health Care Unit of a state hospital. Completed in 2015, the hospital has a 72-bed capacity: three nurses and a physician work in the home healthcare unit. The unit makes approximately 12 home visits per day.The number of registered patients benefiting from home health services between 16.01.2019 and 16.07.2019 is 384. The files of all these patients were examined and included in the study.

Ethical Considerations

Before starting the research, necessary permissions were obtained from the Sakarya University Non-invasive Ethics Committee of a Medical Faculty (Number: 71522473/050.01.0433, Date: 03.07.2019).Ethical principles of the Declaration of Helsinki were taken into account in the study.

2.1. Participants and Sample

The universe of the study consists of the files of individuals who receive service from the home health services unit of the hospital where the research is conducted and who are registered in the electronic information system of the unit. The study sample consists of all patients who received home healthcare services in the last six months.According to the date they received the service, the patients were listed from the old to the latest date, and 384 files, which are the entire population, were included in the sampling.

2.2. Instruments

The data were collected with the Data Registration Form and the Nursing Functions Evaluation Form.

Data Registration Form

The data registration form was prepared using the hospital's data collection forms for the patient and family for home healthcare.There are a total of 21 questions in the data registration form about the patient's age, gender, date of the home visit, the date of the previous visit, how often the home visit was made, the reason for the application, the patient's illnesses, the patient's medical care, nursing service, rehabilitation care, social support, palliative care, psychological support, the need for a dietician, the drugs used, the post-op period, dependency in daily life activities, the use of any medical device, the evaluation of the nutritional status, who meets the personal needs of the patient and the reason why the patient is excluded from home health care follow-up.

Nursing Functions Evaluation Form

This questionnaire form has been prepared by using international and national sources (21,22,23) the Regulation on the Delivery of Home Care Services (2005), and the Nursing Regulation which prepared by experts in the field of nursing (2011) to group the independent, semi-dependent and dependent functions of nurses. In selecting the nursing functions included in the evaluation form, the interventions that nurses can implement within the scope of home health care and in the home environment were considered. A table was created by dividing nursing interventions applied at each home visit into independent (17), semi-dependent (7), and dependent function (4) groups. The records of each patient receiving service from home healthcare services were checked, interventions made by nurses during home visits

were marked on the form in any category of independent, semi-dependent and dependent functions. In each home visit, the function category of the interventions performed by the nurses was determined, and a score was given to each. After scoring, individual total numbers were calculated for each of the independent, semi-dependent, and dependent functions. Accordingly, a nurse's high score in any dependent, semi-dependent and independent nursing functions indicates that the nursing functions with high scores are more likely to be displayed in home care. The total number of nursing functions (dependent, semi-dependent and independent) is taken into account in this form. A score of zero indicates that no service is provided for the relevant nursing function.

2.3. Data Collection

The data were taken from the patient records registered in the electronic information system of the hospital's home health services unit. In collecting the data, a healthcare professional working in home health services opened the database of the hospital and was with the researcher until the data collection was completed. During the collection of data, attention has been paid to the protection of personal information.

2.4. Statistical Analysis

Licensed SPSS 20.0 (Statistical Package for Social Sciences) package program was used for statistical analysis. To test the conformity of the data to a normal distribution, the Kolmogorov-Smirnov p-value was required to be greater than 0.05, and the skewness and kurtosis values to be in the range (-2) and of (+2).In the analysis of the data, descriptive statistics such as frequency, percentage, arithmetic mean, t-test and Mann Whitney U test in independent groups of 2, One-way ANOVA in groups of 3 (Post-hoc Tamhane's T^2 test if the difference between groups is significant) and Kruskal-Wallis test were used $p<0.05$ was considered statistically significant. Spearman correlation analysis was performed to determine the relationship between variables.In terms of correlation coefficients, 0.00-0.19 was interpreted as no or negligible relationship, 0-0.24 no relationship/too weak relationship, 0.25-0.49 weak-moderate relationship, 0.50-0.74 strong relationship, 0.75-1.00very strong relationship .

3. RESULTS

3.1. Participant Characteristics

83.1% of the participants in the study are 65 years old, and above, 62% are women. 41.4% of home visits are made once a month. Those who receive care services; 33.6% applied to the home care unit for general examination, 1.7% periodic care, 12.3% blood collection, 12.2% dressing, 10.2% catheter change, 7.3% treatment, 19.8% education, 2% diaper report, 1% other reasons.

For individuals receiving home care services; 7.9% had stroke, 5% cancer, 9% Alzheimer's, 13.5% diabetes, 19.1% hypertension, 6.4% past operation, 13.2% heart failure, 2% kidney failure, 23.9% have other diseases, and 71.2% have more than one disease.

97.9% of the participants stated that they needed medical care, 99.7% nursing service, 34.7% rehabilitation, 9.5% social support, 8.2% palliative care, 17.5% psychological support, 5.4% dietician.14.1% of the people in the study were in the post-op period, 46.5% were semi-dependent on daily life activities, 94.9% were fed orally, 17% were using medical devices or assistive devices, and 70.2% were cared for by their family. 67.2% of those who were excluded from the home care follow-up system were excluded due to death.

3.2. Comparison of Nursing Functions Mean Scores

Table 1 shows the comparison of the mean scores of independent, semi-dependent, and dependent nursing functions according to the diseases of the participants. Accordingly, no statistically significant difference was found between the mean independent, semi-dependent, and dependent nursing function scores in stroke, cancer, Alzheimer's, hypertension, kidney failure, and other diseases ($p>0.05$). A statistically significant difference was found between the independent, semi-dependent and dependent function mean scores applied by nurses in patients with diabetes, recent operations, and heart failure ($p<0.05$). According to this result, the mean score of semi-dependent nursing functions in patients with diabetes is higher than in patients without diabetes, the mean score for independent and dependent nursing functions in patients who have recently undergone surgery is higher than in patients who have not undergone an operation, the mean score for dependent nursing functions is higher in patients with heart failurethan in patients without heart failure, and the difference between the means was found to be statistically significant ($p<0.05$) (Table 1).

In Table 2, a comparison of the mean scores of independent, semi-dependent, and dependent nursing functions according to some characteristics of the participants is presented. Accordingly, no statistically significant difference was found between the mean scores of independent, semi-dependent, and dependent nursing functions applied to patients needing medical care, nursing service, rehabilitation, social support, and psychological support ($p>005$). As a result of the analysis, it was found that the mean scores of independent and semi-dependent nursing functions applied to individuals in need of palliative care were higher than those who did not need palliative care, the mean score of independent nursing function in individuals who need a dietician is higher than those who do not need a dietician, the mean score of semi-dependent nursing function was higher in individuals using medical devices or assistive devices compared to those who did not use, the mean score of semi-dependent nursing function was higher in individuals fed enterally with tube than in individuals fed orally and the difference between the mean scores was found to be statistically significant ($p<0.05$) (Table 2).

Table 1. *Comparison of independent, semi-dependent and dependent nursing functions mean scores according to participants' diseases (n=384)*

	Independent Nursing Function Score X±SD/ $Q_2(Q_1-Q_3)$	Semi-Dependent Nursing Function Score X±SD/$Q_2(Q_1-Q_3)$	Dependent Nursing Function Score X±SD/ $Q_2(Q_1-Q_3)$
Stroke			
Yes	9.00(9.00-10.00)	0.00(0.00-1.00)	0.76±0.77
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.72
t/z	-1.958	-0.337	0.336
p-value	0.050	0.736	0.737
Cancer			
Yes	9.00(9.00-9.25)	0.00(0.00-1.00)	0.79±0.72
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.73
t/z	-1.898	-0.645	0.523
p-value	0.058	0.519	0.602
Alzheimer's			
Yes	9.00(9.00-10.00)	0.00(0.00-1.00)	0.84±0.77
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.70±0.72
t/z	-1.717	-0.332	1.454
p-value	0.086	0.740	0.147
Diabetes			
Yes	9.00(9.00-10.00)	0.00(0.00-1.00)	0.74±0.69
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.75
t/z	-0.365	-3.614	0.229
p-value	0.715	**0.000**	0.819
Hypertension			
Yes	9.00(9.00-10.00)	0.00(0.00-1.00)	0.70±0.67
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.75±0.77
t/z	-0.120	-1.020	-0.576
p-value	0.904	0.308	0.565
Past Operation			
Yes	10.00(10.00-10.50)	0.00(0.00-1.00)	0.40±0.63
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.78±0.73
t/z	-6.553	-1.350	-3.639
p-value	**0.000**	0.177	**0.000**
Heart failure			
Yes	9.00(9.00-10.00)	0.00(0.00-0.00)	0.85±0.73
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.68±0.73
t/z	-0.423	-0.177	2.122
p-value	0.672	0.859	**0.035**
Kidney failure			
Yes	9.00(9.00-9.50)	0.00(0.00-1.00)	0.65±0.61
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.73±0.74
t/z	-0.969	-1.186	-0.479
p-value	0.332	0.236	0.632
Other			
Yes	9.00(9.00-10.00)	0.00(0.00-0.00)	0.74±0.71
No	9.00(9.00-10.00)	0.00(0.00-1.00)	0.72±0.75
t/z	-0.327	-1.039	0.238
p-value	0.744	0.299	0.812

Only mean and standard deviation are given in parametric tests.

It was found that the mean score of the independent nursing function applied in patients who need post-op care was higher than those who did not need post-op care, and the mean score of dependent nursing function was lower, and a statistically significant difference was found between the mean scores ($p<0.05$) (Table 2).

A statistically significant difference was found between the independent and semi-dependent nursing functions score averages in the daily living activities of the individuals according to their dependency status. According to this result, it was found that individuals who are fully dependent on daily living activities have higher mean scores compared to individuals who are semi-dependent and independent, and it was found to be statistically significant ($p<0.05$) (Table 2).

Table 2. *Comparison of independent, semi-dependent and independent nursing functions mean scores according to different care and support needs of the participants (n=384)*

	Independent Nursing Function Score X±SD/Q_2(Q_1-Q_3)	Semi-Dependent Nursing Function Score X±SD/Q_2(Q_1-Q_3)	Dependent Nursing Function Score X±SD/Q_2(Q_1-Q_3)
Need for Medical Care			
Yes	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.72
No	9.00(9.00-9.75)	0.00(0.00-1.00)	1.00±1.20
U/t	-0.909	-0,855	-0.650
p-value	0.363	0.392	0.536
Need for Rehabilitation			
Yes	9.00(9.00-10.00)	0.00(0.00-0.00)	0.73±0.76
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.73±0.72
U/t	-0.079	-0.048	-0.082
p-value	0.937	0.962	0.935
Need for Social Support			
Yes	10.00(9.00-10.00)	0.00(0.00-0.50)	0.65±0.68
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.74±0.74
U/t	-1.320	-0.073	-0.713
p-value	0.187	0.941	0.476
Need for Palliative Care			
Yes	10.00(9.00-10.00)	0.00(0.00-1.00)	0.78±0.71
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.73±0.73
U/t	-2.204	-2.827	0.414
p-value	**0.028**	**0.005**	0.679
Need for Psychological Support			
Yes	9.50(9.00-10.00)	0.00(0.00-1.00)	0.78±0.69
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.74
U/t	-1.447	-0.564	0.613
p-value	0.148	0.573	0.540
Need for Dietitian			
Yes	10.00(9.00-10.00)	0.00(0.00-1.00)	0.95±0.74
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.73
U/t	-2.239	-0.959	1.437
p-value	**0.025**	0.338	0.151
Need for Post-op Care			
Yes	10.00(10.00-11.00)	0.00(0.00-0.00)	0.45±0.69
No	9.00(9.00-10.00)	0.00(0.00-1.00)	0.78±0.73
U/t	-7.222	-1.493	-3.054
p-value	**0.000**	0.135	**0.002**
Dependency Status in Activities of Daily Living			
Fully dependent[a]	10.00(9.00-10.00)	0.00(0.00-1.00)	0.76±0.78
Semi dependent[b]	9.00(9.00-10.00)	0.00(0.00-0.00)	0.71±0.74
Semi dependent[c]	9.00(9.00-9.50)	0.00(0.00-0.00)	0.73±0.61
KW/F	16.239/*a>b,c	22.056/*a>b,c	0.226
p-value	**0.000**	**0.000**	0.798
Use of Medical Devices or Assistive Devices			
Yes	9.00(9.00-10.00)	0.00(0.00-1.00)	0.80±0.79
No	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.72
U/t	-0.161	-2.511	0.891
p-value	0.872	**0.012**	0.373

	Independent Nursing Function Score X±SD/Q_2(Q_1-Q_3)	Semi-Dependent Nursing Function Score X±SD/Q_2(Q_1-Q_3)	Dependent Nursing Function Score X±SD/Q_2(Q_1-Q_3)
Evaluation of the Patient's Nutritional Status			
Oral	9.00(9.00-10.00)	0.00(0.00-0.00)	0.72±0.72
Enteral with tube	10.00(9.00-10.00)	0.00(1.00-1.00)	1.00±0.80
U/t	-1.714	-3.749	-1.702
p-value	0.087	**0.000**	0.089
The Person Who Meets Their Personal Needs			
Patient[a]	9.00(9.00-10.00)	0.00(0.00-0.00)	0.61±0.65
Family[b]	9.00(9.00-10.00)	0.00(0.00-0.50)	0.77±0.75
Caregiver[c]	9.00(9.00-10.00)	0.00(0.00-1.00)	0.74±0.73
KW/F	4.195	8.429/*a,b<c	1.830
p-value	0.123	**0.015**	0.162

*Only mean and standard deviation are given in parametric tests, U: Mann-Whitney U Test, t: t-test, F: Anova, KW: Kruskal-Wallis Test, *Post-Hoc Tamhane's T2 Test*

Table 3. *Correlations of home visiting frequency, activities of daily living, number of diseases, age variables and nursing functions mean scores (n=384)*

	Frequency of Home Visit	Dependency Status in Activities of Daily Living	Number of Diseases	Age
Independent Nursing Function Score				
r_s	0.142	-0.204	0.045	0.016
p-value	**0.005***	**0.000****	0.380	0.748
Semi-Dependent Nursing Function Score				
r_s	0.024	-0.216	0.065	0.026
p-value	0.640	**0.000****	0.201	0.616
Dependent Nursing Function Score				
r_s	0.029	-0.004	0.055	0.038
p-value	0.564	0.939	0.281	0.454

**p<0.05, **p<0.001*

A statistically significant difference was found between the mean scores of the semi-dependent nursing function applied according to the person who meets the participants' personal needs. Accordingly, it was found that the mean score of semi-dependent nursing functions applied to the person whose personal needs are met by the caregiver is higher than the individuals whose personal needs are met by himself and his family, and there was a statistically significant difference between the mean scores (p<0.05) (Table 2).

3.3. Correlations of Home Visit Frequency, Activities of Daily Living, Number of Diseases, Age Variables, and Nursing Function Scores

In the study, no significant relationship was found between the mean score of dependent nursing function and the frequency of home visits, daily living activities of the participants, the number of illnesses, and age variables (p>0.05). While a positive, too weak, and significant relationship was found between the mean score of independent nursing functions and the frequency of home visits, a negative, too weak, and statistically significant relationship was found with daily living activities (p<0.05). It was found that there is a negative, too weak and significant relationship between independent and semi-dependent nursing functions mean scores and the variable of addiction status in activities of daily living (p<0.05).

4. DISCUSSION

Nurses play a key role in providing home care services as effective healthcare team members (11). Nursing practices are very necessary and important in the home care of the individual in special periods of life such as old age, infancy, pregnancy, chronic diseases, after-surgery / post-discharge care and treatment, palliative care, ensuring that the daily life activities of addicted individuals are carried out and when there are health problems (24). As a result of the analysis made in this study, important findings were obtained that contribute to the development of strategies for determining the functions of nurses and increasing nursing practices in home health care.

The study found that nurses performed more semi-dependent functions in patients with diabetes than in patients without diabetes. Akaltun and Ersin (2016) emphasized the importance of educational activities, which are among the independent functions of nurses. Ergün and Sivrikaya (2012), on the other hand, reported the necessity of applying dependent and independent nursing functions by home care nurses to evaluate and support patients and caregivers in the process of diabetes control at home, such as causes of insulin use, correct insulin applications, appropriate site selection, dose adjustment and administration time, complications, individual monitoring of complications, providing appropriate storage conditions and exercise (26). O'Reilly (2005) emphasizes the importance of supporting patients with diabetes receiving home care more by preparing education, counseling, and patient-specific care plans, which are among the independent functions of nurses rather than semi-dependent functions (27).

The study found that independent and dependent nursing functions were applied more in patients who had a recent operation than those who did not have an operation. To develop a comprehensive nursing care plan for the home care of patients undergoing surgery; Determining postoperative complications, characteristics of incision wound, treatment, pain severity, limitations regarding daily life activities, special care needs, and nutritional status of the patient, additionally, training and counseling services, providing care and coordination of care are among the nursing functions (28). In this study, it was observed that nurses highlighted their dependent and independent functions during home visits to patients after surgery, such as wound care, administration of medications according to the treatment plan, vital signs follow-up, and helping patients in daily life activities.

The study determined that nurses used their dependent functions more in patients with heart failure, such as taking blood, administering the patient's medications according to the treatment plan, and making an appointment for the individual for examination, tests, and treatment planning for the transfer. In the literature, the importance of providing qualified nursing care for patients with heart failure is emphasized, and the importance of self-care of patients, symptom monitoring, treatment compliance, and patient and family education are emphasized (29,30). According to the study results, although it was determined that nurses use their dependent functions more in patients with heart failure, it is thought that the interventions performed are not fully documented due to the lack of registration.

The study determined that nurses use their independent and semi-dependent functions more in patients who need palliative care than those who do not need palliative care. Nurses are expected to manage acute symptoms such as pain and constipation in palliative care patients receiving home care services and provide wound care, parental medication, and psychosocial support to patients/relatives (31, 32). In the study of Schroeder and Lorenz (2018), the importance of providing patient-specific counseling and care coordination, symptom management, end-of-life care, medication, and managing the needs of daily life activities by the nurses who provide palliative care at home is emphasized. Sijabata et al. (2019) state that nurses who provide palliative care at home need to adopt a holistic and inclusive approach in terms of psychological, social, and spiritual aspects, but they are more interested in patients' physical ailments and treatments(33).

The study determined that individuals who receive home healthcare services need dependent and semi-dependent nursing interventions, especially for dietitians and nutritional problems. In the study of Işık et al. (2016), it was reported that the highest rate of dependency in daily living activities was nutrition activity with 48.6% (7). In the literature, it was found that nursing interventions related to education, mediation of patients / patient relatives and caregivers, assessment of nutritional status, enteral nutrition, feeding catheter problems, and catheter infections were applied to individuals receiving home care services (34,35,36,37).It is important that the home care nurse educates the patient receiving home care service or caregiver and that the patient is evaluated with a multidisciplinary approach at regular intervals under the coordination of the nurse.

The study found that 17% of the patients receiving home care were using medical devices or assistive devices, and semi-dependent functions for nurse practices were used more in these patients. The functions of home care nurses include helping and guiding individuals for the correct and safe use of devices or tools in care and treatment (38,39). In the literature, it is reported that nurses should evaluate the problems in patients in the intensive care class within the scope of home care by using the semi-dependent and independent functions related to the musculoskeletal system, the need for assistive devices such as crutches and wheelchairs, dependence on the bed and using an air mattress, and the problems experienced by the individual and the family (40).

A statistically significant relationship was found between the study's independent nursing function score and home visit frequency variables. Patients' care needs, dementia, depression, and recurrent acute diseases are thought to effectively determine the frequency of home visits (41). Nadarevic-Stefanec et al. (2011) reported that 30.6% of individuals receiving home care services received intensive care service and 54.2% received minimum health care, and the total number of visits per patient ranged from 4 to 720 (42). Öztürk and Toprak (2018) stated that a patient was visited an average of 6.54 times a year, as the age increased the number of visits by the home health unit increased to 4 and above, that there was no significant difference between patients with 4 or more visits by the home health unit and emergency visits and deaths; however, there is a significant relationship between diagnoses and frequency of visits (43). It is stated that while there are 3 or fewer visits in patients with Alzheimer's, 4 or more visits occur in those with essential hypertension and endocrine disorders. In the study of Bouman et al. (2007), it was reported that home

care nurses made eight visits to the elderly in 18 months and some social, psychological, physiological problems were detected in individuals and that they carried out counseling and training activities, which are among the independent functions of nurses, for these problems (44).

As a result of the study, a statistically significant difference was found between the mean scores of independent, semi-dependent and dependent functions applied by nurses in patients with diabetes, recent operations, and heart failure. A significant positive relationship was found between the mean score of independent nursing functions and the frequency of home visits and a negative relationship with daily living activities. Also, a statistically significant difference was found in the nursing functions of individuals in need of palliative care and dietician, using medical devices or assistive devices, and fed enterally by tube.

5. CONCLUSION

As a result of the study, it was observed that there was a statistically significant difference in the nursing functions applied to patients with diabetes, recent surgery, heart failure, palliative care and dietitian need, using medical devices or assistive devices, and tube-fed enterally. In addition ,it was determined that semi-dependent functions for nursing practices are used more. A positive correlation was found between the mean score of independent nursing functions and the frequency of home visits and a negative correlation with activities of daily living. Based on the results of the study, it can be suggested that nurses should use more independent functions such as training, counseling, mediation, orientation, and leadership. It is thought that this will have positive results on the prolongation of hospitalization, mortality, morbidity, and quality of life of the patient. It is considered important to rearrange the record-keeping systems for nursing interventions within the scope of home healthcare services because it is seen that independent nursing interventions are not included in the registries, and more records of dependent nursing practices are kept. It is thought that the interventions implemented by nurses will be more visible with the development of nursing registration systems in home healthcare services.

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Clinical and Experimental Health Sciences

Prevalence and Pattern of Stylohyoid Chain Complex on Panoramic Radiographs: A Retrospective Study

Ali Altindag[1], Hakan Eren[2], Muhammed Feyzi Kucukkalem[3], Omer Altindag[4]

[1] Necmettin Erbakan University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Konya, Türkiye.
[2] Canakkale Onsekiz Mart University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Canakkale, Türkiye.
[3] Ankara University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Ankara, Türkiye.
[4] Bilecik Seyh Edebali University, Science and Letters, Statistics and Computer Sciences Department, Bilecik, Türkiye.

Correspondence Author: Ali Altindag
E-mail: aaltindag@erbakan.edu.tr

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ABSTRACT

Objective: The elongated styloid process (SP) and calcified stylohyoid ligament can be evaluated radiologically and are associated with some symptoms and syndromes. This study aimed to define the incidence of different stylohyoid chain patterns and classify them.

Methods: Digital panoramic radiographs of 1217 patients were included in the study. The styloid chain patterns were analyzed according to MacDonald-Jankowski's study. Data analysis was performed using the IBM SPSS Statistics 21.0 (Statistical Package for Social Sciences) program. Descriptive values were analyzed by using descriptive statistics. The data were assessed by using Chi-squared tests. A probability level of less than .05 ($p < .05$) was considered significant.

Results: The prevalence of the stylohyoid chain complex patterns was higher for normal SP (86.5%), followed by elongated SP (7.1%), calcified stylohyoid ligament (2.2%), and absent stylohyoid chain (2.8%). Unclassified SP was detected in twenty individuals on the left (1.6%) and fourteen on the right (1.2%). In view of symmetry of the stylohyoid chain complex, 979 (80.4%) were symmetric, 238 (19.6%) were asymmetric and 14 (1.15%) of the asymmetrical ones were unilateral. There was no significant difference in gender for the type of stylohyoid chain complex pattern ($p > .05$). There was statistically significant difference between the stylohyoid complex pattern and age groups for the right and left sides ($p < .05$).

Conclusion: The authors believe that this study provides additional information about the frequency of the elongated styloid process and provides valuable knowledge of the anatomical variations of the SP. Current findings should be correlated with clinical symptoms in future studies.

Keywords: Styloid process, Digital panoramic radiography, Diagnostic imaging, Ossification, Eagle's syndrome

1. INTRODUCTION

The protrusion of the temporal bone extending down-front from the pars tympanica is referred to as styloid process (SP). The SP is a slender, cylindrical bone extension extending between the internal jugular vein and carotid arteries, posterior to the tonsillar fossa and anteromedial to the stylomastoid foramen. It measures approximately 2.5-3 cm in length and develops from the Reichert's cartilage in the second branchial arch. The styloglossus, the stylopharyngeus, and the stylohyoid muscle, and the stylohyoid ligament are attached to the distal part of the SP. The styloid ligament is located between the SP and lesser horn of the hyoid bone. The SP, the stylohyoid ligament, and lesser horn of hyoid bone form the stylohyoid complex (1-4).

Chronologically, the first elongation of SP and ossification of the styloid ligament was reported by Pietro Marchetti in 1652, and the first enrolled case of elongated SP in which Prof. Josef Weinlechner performed surgery in 1872, in Vienna (5, 6). In 1937 Dr. Watt W. Eagle, an otorhinolaryngologist, reported two symptomatic, mineralized SP cases. He noted the patients' complaints of "nagging or aching sensation in the throat similar to chronic pharyngitis" (7-10). Eagle was also the first to put forth the features of the syndrome in detail, and correlated it to the ossified styloid ligament and elongated SP, which is today known as Eagle's Syndrome. The SP is referred to as elongated when it exceeds 30 mm. Eagle' Syndrome determined that only around 4% of the population had an elongated SP and a calcified stylohyoid ligament, and only between 4-10.3% of these cases showed symptoms (8, 10-12). The clinical signs are foreign body sensation, ear pain, neck pain, facial pain, carotid pain, pain during tongue extension, discomfort when chewing, dizziness, headaches, and sore throats. The SP elongation is more common in women than men, and frequently with bilateral calcification

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(2, 13, 14). Some studies have reported that women have more symptoms than men and occur more frequently in older women, possibly due to menopause (2, 15).

The SP elongation can be determined as a coincidental finding frequently on panoramic radiographs during routine clinical examination in dentistry (2, 16). The imaging methods that detect the SP are panoramic radiography, Towne's view, lateral oblique mandible view, lateral cephalogram, posteroanterior skull view, computed tomography (CT), cone beam computed tomography (CBCT), and magnetic resonance imaging (16-18).

Diagnosis of this condition is not easy for the clinician because of its vague symptomatology and symptoms of the impacted third molar, temporomandibular disorders and dental diseases reflected in the relevant area. Therefore, dentists must have sufficient knowledge of the characteristics of SP to diagnose the cause of SP-related symptoms (19).

The purpose of this study was to investigate the incidence, and calcification patterns of elongated SP in a Turkish subpopulation using digital panoramic radiographs, and its relation, if any to subject sex and age.

2. METHODS

The Research Ethics Committee of Necmettin Erbakan University approved this study (approval date and number: 27.05.2021 and 2021/06-65) which was conducted according to the guidelines of the Declaration of Helsinki. All patients singned informed consent.

All images were obtained using a Morita Veraviewepocs 2D panoramic unit (J Morita MFG Corp., Kyoto, Japan) with parameters of 60-70 kVp, 5-7 mA, and 6-8 s exposure time, according to the manufacturer's recommended protocol.

Radiographs that do not contain information about the age and sex of the patients, patients with tonsillectomy, head trauma, and panoramic images with poor quality, and/or do not show SP, having positioning and magnification errors constitute the exclusion criteria. This study used only diagnostically acceptable images. The study was performed on 1217 radiographs with inclusion criteria out of 1450 digitally archived panoramic radiographs at Necmettin Erbakan University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology. Data were evaluated on an LCD monitor in ambient light by two maxillofacial radiologists with more than three and eight years of experience in ten sessions at 5-day intervals to rest the eyes. The final classification and radiographic status was recorded after inter-observer consensus.

The stylohyoid chain complex patterns were classified according to MacDonald-Jankowski's study (20). The SP patterns recorded bilaterally and pattern of calcification was described according to the center of calcification involved: Region 1, tympanohyal (the base of the SP); Region 2, stylohyal (forms the major portion of the SP); Region 3, ceratohyal (forms the stylohyoid ligament); Region 4, hypohyal (forms the minor horn of the hyoid bone). In Figure 1, the 12 patterns are detailed from the minor calcification pattern and whether the zones are continuous or distinct.

The patients' stylohyoid patterns have been grouped as normal (N), elongated (E) and calcified (C) samples coequal with the MacDonald-Jankowski's (20) method. In this process, patients who have absent or unclassified stylohyoid complex (Patterns L or U) were excluded from the analysis. Then remaining patients were analyzed statistically to determine whether age or sex influences groups of stylohyoid patterns. For this purpose, the age range of the patients have been divided into three groups: 18-30 (young adults), 31-50 (middle-aged adults), and 51+ (older adults).

All statistical analyses were performed with SPSS 21.00 software (SPSS, Chicago, IL, USA). In addition to descriptive statistics, the Chi-square test was used to evaluate statistical significance, and the significance level was set at α=.05.

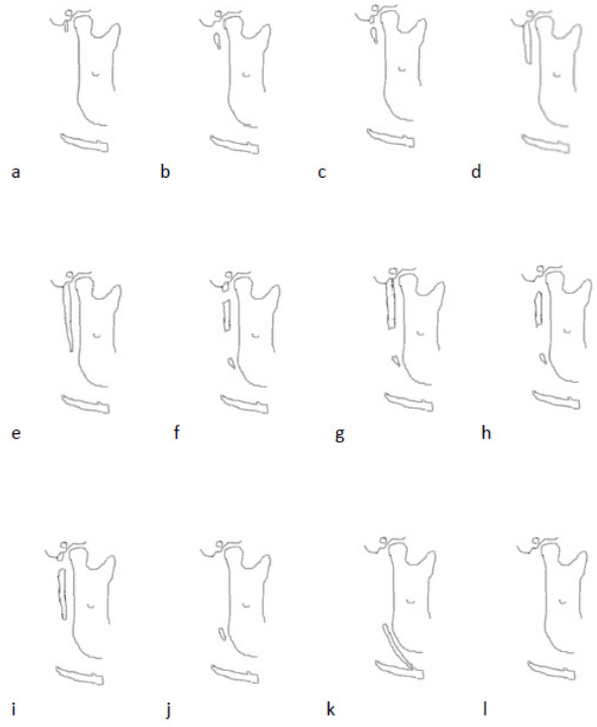

Figure 1. *The 12 patterns of calcification of stylohyoid complex used in this study. Pattern: (a) Region 1=tympanohyal alone: (b) Region 2 stylohyal alone: (c) Region 1 and 2, separate: (d) Regions 1 and 2, continuous: (e) Regions 1, 2 and 3, continuous: (f) Regions 1, 2 and 3, separate: (g) Regions 1 and 2, continuous, but separate from 3: (h) Regions 2 and 3, separate: (i) Regions 2 and 3, continuous, but separate from 1: (j) Region 3 alone: (k) Region 3 and 4, continuous (may include calcification in one other region): (l) No styloid process visible. Regions 1, 2, 3 and 4 coincide with the centres of calcification. Patterns A-D are normal styloid processes. Pattern E is an elongated styloid process. Patterns F-K are calcified stylohyoid ligaments. Pattern L is absent stylohyoid chain complex.*

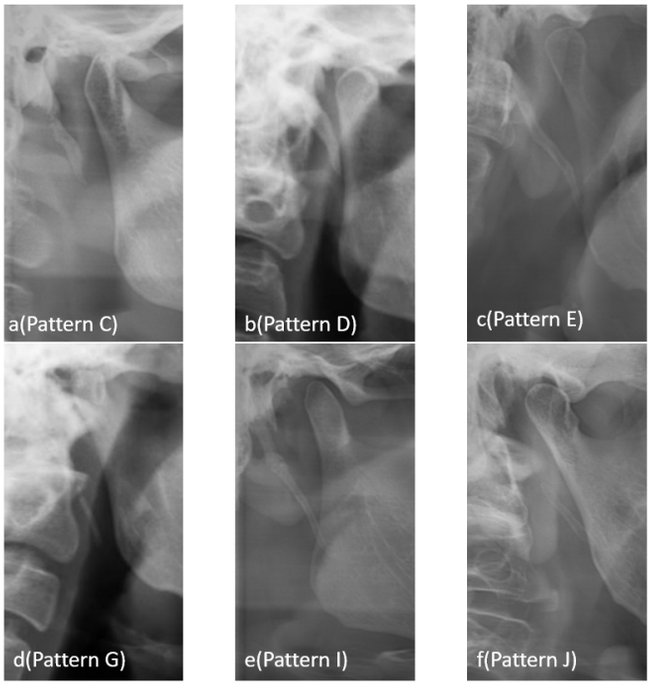

Figure 2. *The different types of stylohyoid chain complex patterns evaluated in this study. a Pattern C, b Pattern D, c Pattern E, d Pattern G, e Pattern I, f Pattern J*

3. RESULTS

The mean age and standard deviation in 1217 patients (745 female, 472 male) were 41.53 (±16.13, min-max:18-91). The mean age for female and male patients with standard deviation were 40.74±15.66 and 42.79±16.79 years, respectively.

The distribution of the twelve patterns of MacDonald-Jankowski's (20) classification was presented in Table 1. Of all cases, 86.5% were normal SP (Patterns A, B, C, and D) on both right and left sides (Fig. 2a-b). Elongated SP (Pattern E) and calcified stylohyoid ligament (Patterns F, G, H, I, J, and K) were 7.1% (Fig. 2c) and 2.2% (Fig. 2d-f), respectively. Approximately, 83.8% of elongated SPs were detected bilaterally, and 16.19% of individuals were unilateral. The absent stylohyoid chain (Pattern L) was 2.8%. Unclassified stylohyoid chain complex pattern was observed in 1.4% of cases. The frequency distribution of patterns was homogeneously on both sides according to sex. Also, 80.4% of the cases have the stylohyoid chain complex symmetry. In 79.2% of females and 82.3% of males, symmetry was found. However, there was no significant association between the sex and the symmetry of the stylohyoid chain ($p > .05$). Cross tabulations were presented in Table 2 and 3 for age groups and sex, respectively. A statistically significant difference was found only between age groups and the affected side.

Table 1. *Patterns of the stylohyoid chain complex according to sex*

Pattern	Side	Male	Female	Total
		n (%)	n (%)	n (%)
A	R	84 (6.9)	130 (10.7)	214 (17.6)
	L	87 (7.1)	133 (10.9)	220 (18.1)
B	R	15 (1.2)	31 (2.5)	46 (3.8)
	L	13 (1.1)	23 (1.9)	36 (3.0)
C	R	26 (2.1)	40 (3.3)	66 (5.4)
	L	28 (2.3)	45 (3.7)	73 (6.0)
D	R	278 (22.8)	446 (36.6)	724 (59.5)
	L	280 (23.0)	445 (36.6)	725 (59.6)
E	R	39 (3.2)	48 (3.9)	87 (7.1)
	L	38 (3.1)	48 (3.9)	86 (7.1)
F	R	1 (0.1)	3 (0.2)	4 (0.3)
	L	2 (0.2)	2 (0.2)	4 (0.3)
G	R	6 (0.5)	9 (0.7)	15 (1.2)
	L	1 (0.1)	7 (0.6)	8 (0.7)
H	R	0 (0)	2 (0.2)	2 (0.2)
	L	0 (0)	1 (0.1)	1 (0.1)
I	R	2 (0.2)	4 (0.3)	6 (0.5)
	L	2 (0.2)	1 (0.1)	3 (0.2)
J	R	2 (0.2)	4 (0.3)	6 (0.5)
	L	2 (0.2)	2 (0.2)	4 (0.3)
K	R	1 (0.1)	0 (0)	1 (0.1)
	L	0 (0)	0 (0)	0 (0)
L	R	11 (0.9)	21 (1.7)	32 (2.6)
	L	12 (1.0)	25 (2.1)	37 (3.0)
U	R	7 (0.6)	7 (0.6)	14 (1.2)
	L	7 (0.6)	13 (1.1)	20 (1.6)

Patterns A-D are normal styloid prcesses. Pattern E is an elongated styloid process. Patterns F-K are calcified stylohyoid ligaments. Pattern L is absent stylohyoid chain complex. Pattern U is unclassicified stylohyoid chain complex .R, right; L, left.

Table 2. *Distribution of the styloid complex according to age groups and side*

Age Group (years)	Right				Left			
	N n (%)	E n (%)	C n (%)	Total n	N n (%)	E n (%)	C n (%)	Total n
18-30	364 (92.2)	17 (4.3)	14 (3.5)	395	363 (92.8)	18 (4.6)	10 (2.6)	391
31-50	357 (87.6)	35 (8.7)	10 (2.9)	402	365 (91.0)	29 (7.2)	7 (1.7)	401
51+	324 (88.5)	34 (9.2)	8 (2.2)	366	323 (88.7)	38 (10.4)	3 (0.8)	364
Total	1045 (89.9)	86 (7.4)	32 (2.8)	1163	1051 (90.9)	85 (7.4)	20 (1.7)	1156

χ^2 (right)=9.599, df=2, p=.048; χ^2 (left)=2.368, df=2, p=.015. N, normal; E, elongated; C, calcified; n number of cases, % frequency

Table 3. *Prevalence of the styloid complex according to gender and side*

Gender	Right				Left			
	N n (%)	E n (%)	C n (%)	Total n	N n (%)	E n (%)	C n (%)	Total n
Male	402 (88.9)	38 (8.4)	12 (2.7)	452	406 (90.2)	37 (8.2)	7 (1.6)	450
Female	643 (90.4)	48 (6.8)	20 (2.8)	711	645 (91.4)	48 (6.8)	13 (1.8)	706
Total	1045 (89.9)	86 (7.4)	32 (2.8)	1163	1051 (90.9)	85 (7.4)	20 (1.7)	1156

X^2 (right)=1.119, df=2, p=.572; χ^2 (left)=.926, df=2, p=.629. N, normal; E, elongated; C, calcified; n number of cases, % frequency

4. DISCUSSION

The styloid complex, which develops embryologically from Reichert's cartilage, consists of the SP, the stylohyoid ligament, and the lesser horn of the hyoid bone (21, 22). The stylohyoid ligament is normally composed of dense fibrous connective tissue and may show partial or complete ossification which is not fully understood (13, 23-25).

Clinical diagnosis of the elongated SP is often difficult (23), and confirmation by radiological imaging is necessary (26, 27). Dentists can detect an elongated SP on panoramic radiographs, widely used in dental clinics (2, 28).

Although panoramic radiography has advantages such as availability, low cost, diagnostic performance, and low radiation dose, magnification and distortion limit the accurate measurement of SP (27, 29), it is not always possible to detect the SP due to the superposition of anatomical structures such as anteriorly by mandible, and posteriorly by cervical vertebra. Also, the variability of patient positioning could affect the measurements. Therefore, misdiagnosis may also occur (1).

There are many classification models in the literature (30-33). To be in accordance with the previous studies, O'Carroll's (33) classification was used which is preferred in the recent studies (20, 30, 31).

In addition, advanced imaging techniques were preferred in some studies. Öztunç et al. (34) and Andrei et al. (35) used CBCT in Turkish and Romanian samples, respectively. Gözil et al. (36) and Başekim et al. (37) used CT to estimate the SP elongation in various samples of the Turkish population. Three-dimensional systems effectivelly appraise SP angulations, length, and other morphological features (37). In these studies, two methods were preferred for the determination of elongated SP: I.İlgüy et al. (8) and Eagle W. (10) evaluated the SP as elongated if it was more than 30 mm; II. Rodriguez et al. (21) and Omami (38) assessed the SP as elongated when the SP's tip extends below the mandibular foramen. Consistent with previous studies, we refrained from measuring the lengths of SPs due to technical (panoramic radiography machine differences, magnifications, manual versus digital measurement and calibration) and epidemiological (ethnic and genetic variety) circumstances (36-39). Therefore, the types of SP were compared according to MacDonald-Jankowski's study (20).

Our results for the stylohyoid complex pattern were (both sides independent of each other): the normal pattern 86.5%; the elongated pattern 7.1%; the calcified pattern 2.2%. This reveals that the frequency of the elongated type is higher than the calcified type, consistent with the study of MacDonald-Jankowski (20). The prevalence of calcified type was higher for both sides in the young adult group (18-30 year-old). The presence of elongated SP was associated with increasing age for both sides. The findings cited above are consistent with the results of MacDonald-Jankowski (20) but contradictory to Bagga et al. (40). These different results between the studies may be caused by sample size, techniques used, age range, and ethnic/racial variety (30, 31).

In our research, symmetry was detected between the left and right sides (80.4%) in most cases. This result was consistent with previous studies (2, 20, 30).

Rizatti-Barbosa et al. (41) evaluated the stylohyoid complex calcification on panoramic images of 2252 patients in an adult and partially edentulous Brazilian population of both sexes. A calcified pattern was detected in 451 of the 2252 patients. Most of these calcified patterns were bilateral (n = 248, 54.9%). Although a complex anatomical variant of the stylohyoid ligament is more common in older women, this abnormality occurs in both men and women. The abnormality

was more common in patients aged 60–79. Rizatti-Barbosa et al's (41) findings differ from those of the present study. The inconsistency in results may be due to differences in ethnicity, as noted by previous researchers (20, 31).

In the study of Jankowski (20), which included radiographs of 1662 patients from different races, it was determined that there was no statistical difference in the prevalence of elongated SP (8.6% in Hong Kong Chinese and 7.8% in Londoners). Also, no significant difference was found in age for an elongated SP, contrary to the results of our study.

According to our findings, the prevalence of elongated SP (7.1%) was lower than some similar studies. However, there was a wide range for the elongated SP frequency of 1.4 to 83.6% in the literature (23, 34, 40, 45-47). Bruno et al. (47) observed the SP as a 66.6% normal and 33.40% elongated type. 56.2% of the elongated SP were bilateral, while 43.28% were unilateral. In the present study, elongated SP was lower than the results of Bruno et al., and 16.19% were unilateral. Taşsöker et al. (48), in a study they conducted on the Turkish population, measured the length of the SP on panoramic radiographs. They found that the SP differed according to age groups and gender, and the rate of being elongated was 16.3%. In another study on Turkish population conducted by Öztaş et al. (2), stylohyoid ligament calcification was evaluated according to the O'Carrol classification in panoramic images of 2000 patients. It has been observed that stylohyoid ligament calcification is more common in women than men, and the most common age group is 50-59 years. Mağat et al. (49) evaluated the styloid process in panoramic images according to the MacDonald-Jankowski classification. Patterns D and E were observed for both sides at approximately 42% and 32%, respectively. In the present study, the most observed patterns were D (59.5%), A (17.8%), and E (7.1%), respectively. A significant difference was found only for right SP morphological calcification types as to age groups in both genders ($p < .05$). In the present study, while a statistically significant difference was observed between both sides ($p < .05$) for the right side and the left side, Table 2) and age groups, it was not observed for gender. Alpoz et al. (30) evaluated styloid process patterns according to the MacDonald-Jankowski classification of panoramic images. Similar to our results, the authors concluded a high number of cases was found to be symmetrical for the SP (%83.3). A normal pattern was observed in 68.3%, an elongated pattern in 27.1%, and a calcified pattern in 1.7%. In our study, normal, elongated, and calcified patterns were found to be 86.5%, 7.1%, and 2.2%, respectively. Although it was studied in the same population, the results may be due to different age groups and sample sizes.

Since the retrospective design of this study, and the relationship between the radiological and clinical findings could not be evaluated. However, different types of SPs could be detected due to the large sample size, and a homogeneous distribution was achieved.

5. CONCLUSION

The present study highlighted the patterns of the elongated SP and calcification/ossification of the stylohyoid ligament. The SP patterns could be detected incidentally on panoramic radiographs in routine dental practice. Knowing the radiological findindgs of the SP variations may be beneficial for radiologists, surgeons, and neurologists to make more accurate diagnoses and avoid misinterpretation.

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Clinical and Experimental Health Sciences

Investigation of the Effects of Obesity on Physical Function and Quality of Life in Elderly Women

Tahir Keskin, Zeliha Baskurt, Ferdi Baskurt

Suleyman Demirel University Faculty of Health Sciences Physical Therapy and Rehabilitation Department, Isparta, Türkiye.

Correspondence Author: Tahir Keskin
E-mail: hndnozcn@hotmail.com
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ABSTRACT

Objective: The aim of this study was to determine the effects of obesity on physical function and quality of life in elderly women.

Methods: The sample of the study consisted of 90 elderly women living in Isparta provinence. The subjects included in the study were divided into 3 groups according to their body mass index (Group I: normal, Group II: overweight, Group III: obese).The physical activity scale for the elderly, short physical performance test battery, SF-12 quality of life questionnaire, timed up and go test and a scale that questions the difficulties of individuals experience in daily living activities were used as the evaluation methods.

Results: Physical Activity Scale for the Elderly and short physical performance test battery scores in group I were higher than that of the other groups, and group II were higher than group III; while timed up and go test scores were vice versa ($p<0.05$). Functional limitation scores were higher in Group III than Groups I and II ($p<0.05$). Body mass index showed a strong negative correlation with physical performance (r: −0.591), moderate negative correlation with the Physical Activity Scale for Elderly scores (r: −0.427) and moderate positive correlation with the timed up and go test value (r: 0.418) and functional limitation (r: 0.335) ($p<0.01$).

Conclusion: In elderly women; physical activity, functional mobility levels, physical performance decrease with increasing body mass index and functional limitation level increases in parallel with the body mass index. In addition, it was determined that the quality of life scores did not differ according to the body mass index.

Keywords: Elderly Women, Functional Limitation, Obesity, Physical Function, Quality of Life

1. INTRODUCTION

Decreasing birth rates, advances in health and technology and improvements in the treatment of chronic diseases have increased the average life expectancy in today's world as well as the pace of ageing population and rate of the elderly population (1). This increase has introduced the concept of 'active ageing'. Active ageing aims to improve the quality of life of the elderly in society and enable them to maintain a more active and social life during this period and perform daily activities independently (1,2).

Ageing brings about changes in the body composition, such as a progressive increase in the percentage of body fat mass, increasing the risk of developing obesity in the elderly population. This age-related increase in the fat mass is particularly higher in women than in men. These age-related changes lead to cardio metabolic complications and functional limitations in the elderly as well as impairment of the quality of life (2).

The decline in the physical activity levels and resting metabolic rate with increasing age causes a decrease in the total energy expenditure, resulting in a positive energy balance. Positive energy balance has an important role in increasing lean body mass and results in increased obesity rates in the elderly population. Reduced physical activity owing to inadequate engagement of elderly obese individuals in physical activities causes a decrease in physical function levels, functional mobility and independence in daily life activities (3). When the literature was reviewed, it was observed that elderly individuals who have low body mass index (BMI) values and adequate physical activity levels, live healthier and are at a lower risk of chronic diseases, and those with high physical performance have a higher scores of life quality (4).

According to the prevalence of the obesity in older adults, higher rates are observed in women. Furthermore, physical function levels in women are reported to be lower than

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those in men (4). Therefore, evaluation of the relationships among obesity, physical function and quality of life in elderly women becomes more important. In addition, it is important to determine the effect of obesity on physical function and quality of life, since one of the key factors of healthy aging is to keep physical function levels high. In light of this information, the aim of this study was to investigate the effect of obesity on the physical function and quality of life in elderly women.

2. METHODS

This study, with the aim of examining the effect of obesity on the physical function and quality of life in elderly women, was a descriptive study carried out in a cross sectional manner and conducted with 90 elderly women aged between 65 and 85 years in November 2017 and May 2018.

2.1. Study Population

The population of the study was determined as female individuals over the age of 65 years, who were residing in Davraz district of Isparta province. The study sample was determined using the snowball sampling method. In total, 112 elderly women over 65 years of age were enrolled in the study by this method. Eighteen people withdrew from the study on their own volition after being informed in detail about the study, whereas four people were withdrawn from the study because they felt unwell while performing the study procedures, and the study was completed with a total of 90 elderly women.

Age of ≥65 years, voluntariness to participate in the study, being independently mobile, absence of serious systemic disease, absence of serious hearing and visual loss, Geriatric Depression Scale Short Form (GDS-SF) score of ≥5 and Mini Mental State Test score of ≥24 points constituted the inclusion criteria. Individuals who were in the process of recovery from an acute disease, those who had serious diseases that would pose a contraindication for physical activity and those who experienced change in their health status while performing study procedures were excluded from the study.

A sociodemographic data form that was created by researchers based on literature review was initially administered to the participants, and then BMI was calculated by dividing body weight by the square of height. Participants were divided into the following three groups: normal (Group I) individuals with BMI between 20 kg/m² and 24.9 kg/m², overweight (Group II) individuals with BMI between 25.0 kg/m² and 29.9 kg/m² and obese (Group III) individuals with BMI of >30 kg/m² (5).

2.2. Physical Performance

The physical performance level of the participants was evaluated by short physical performance test battery (SPPB), the reliability and validity of which were proven in the elderly population (6). The test comprised three parts: gait speed, standing balance and chair stand. In standing balance section, participants were asked to maintain their feet together in a standing position as well as in tandem and semi-tandem positions for 10 seconds. In gait speed section of the test, participants were asked to walk 4 metres at the usual pace. In the chair stand section, participants were asked to quickly stand up five times from a backed chair of standard height without using their hands, while folding their arms on their chests. All three physical performance measures are scored between 0 and 4 according to the duration of the activity. The scores of the three tests are added to give a total score between 0 (minimum) and 12 (maximum). High scores indicated high levels of physical performance (6).

2.3. Physical Activity

The elderly individuals' physical activity levels were evaluated with the Physical Activity Scale for Elderly (PASE), which was developed to determine the levels of work, entertainment and physical activity of elderly individuals, and the Turkish reliability and validity study of this test has been performed (7). PASE is a scale evaluating physical activities that people have done in the last 7 days in a multidimensional manner. The PASE score is calculated by multiplying activity frequencies and activity weights. High scores indicate high physical activity levels (7).

2.4. Quality of Life

The level of the quality of life of the participants was evaluated by SF-12, which was created by shortening and simplifying the SF-36 questionnaire, allowing the evaluation of physical and mental status separately. The total physical health score consisted of physical functionality, general health, bodily pain and role-physical sub-dimensions; mental health total score is obtained from mental health, social functionality, role-emotional, and energy sub-dimensions. A score of 0–100 can be obtained from both the physical and mental health, high scores indicate better health status (8).

2.5. Functional Mobility

The functional mobility of the participants was evaluated with Timed Up and Go Test (TUG). TUG is a test used frequently to evaluate older adults' functional mobility (9). During the test, participants were asked to stand up from a chair without using the arms of the chair, walk 3 meters and turn back without touching anything and walk back to and sit back on the chair. Participants were asked to wear the shoes they regularly wear to walk at a normal pace, and walking aids were allowed. The time elapsed between the participants getting up from the chair and sitting back was recorded in second.

2.6. Functional Limitation

The functional limitation level of the participants was determined with a seven-item scale inquiring the difficulties faced in certain daily life activities, such as climbing or going down 15 stairs without pausing, walking outside the house

for 15 minutes, undressing, getting up from and sitting on a chair, cutting your own toenails, taking a bath or a shower and using a private vehicle or public transportation. On this scale, activities are scored between 0 and 4 according to the degree of difficulty experienced in daily life activities, and high scores indicate advanced functional limitation levels (10).

2.7. Cognitive Evaluation

The cognitive level of the subjects was determined using the Standardized Mini Mental Test (SMMT). In total, 30 points can be obtained in this test: 0–9 points indicate severe cognitive impairment, 10–19 points indicate moderate cognitive disorder, 20–23 points indicate mild cognitive disorder and 24–30 points indicate no cognitive impairment (11).

2.8. Depression

GDS short form was used to evaluate the depression levels of the participants. GDS-SF short form is a short and easily applicable form of GDS, consisting of 15 questions inquiring the patient's mood. Answers are given based on the feelings in the last week with yes-no questions. A score of ≥5 points indicates the presence of depression (12).

2.9. Statistical Analysis

IBM SPSS Statistics 20.0 software was used for the Statistical analysis of the data. Socio demographic characteristics of the participants were expressed as mean, standard deviation, percentage and rates. The distribution of the data was evaluated with the Kolmogorov-Smirnov test and it was determined that the data showed a normal distribution. One-Way Anova (with Tukey post-hoc analyse) were used to analyse the differences between the group averages. The relationships among the parameters were evaluated by Pearson's correlation coefficient. A p value of < 0.05 was considered to be statistically significant.

2.10. Ethical considerations

This study was carried out in accordance with the guidelines of the Declaration of Helsinki. The ethical approval of this study was obtained by the Presidency of Clinical Research Ethics Committee of Süleyman Demirel University Faculty of Medicine (receipt no. 204). All participants gave written informed consent.

3. RESULTS

The mean age of all cases was 69.15±4.37 years. The comparison of sociodemographic characteristics of the groups are shown in Table 1. The sociodemographic characteristics were not significantly different between groups except for weight and BMI (Table 1).

The mean SMMT score was 27.4±1.8 points. The mean SMMT score was significantly higher in Group II than in Group I ($p<0.05$). GDS scores did not differ significantly between the groups ($p>0.05$) (Table 2).

Table 1. *Physical properties of cases*

		Group I n=30	Group II n=30	Group III n=30	p
Age (years) $\bar{x}$ ± SD		70.6±4.3	68.0±3.9	68.8±4.5	0.063*
Height (m) $\bar{x}$ ± SD		1.56±0.7	1.58±0.7	1.57±0.6	0.587*
Weight (kg) $\bar{x}$ ± SD		56.7±5.9	71.0±7.6	85.1±8.8	<0.001*
BMI (kg/m²) $\bar{x}$ ± SD		23.0±1.5	27.9±1.2	34.4±3.5	<0.001*
Presence of Chronic Illness n/(%)		30 (100%)	28(93%)	30(100%)	0.129⸸
Living Situation n/(%)	Alone	16(53.3%)	14(46.7%)	12(40%)	0.276⸸
	With family members	14(46.7%)	16(53.3%)	18(60%)	
Education level n/(%)	Low	9(30%)	19(63.3%)	2(6.7%)	0.359⸸
	Medium	3(10%)	25(83.3%)	2(6.7%)	
	High	8(26.7%)	21(70%)	1(3.3%)	

*x̄: Mean, SD: Standart Deviation, n: number of cases, BMI: Body Mass Index, kg: kilogram, m: meter, m²: meters per square, *: One Way Anova test, ⸸: Chi square test*

Table 2. *Mini mental test and geriatric depression scale scores of cases*

		$\bar{x}$	SD	p*
Mini Mental Test	Group I	26.77[a]	0.32	**.025**
	Group II	28.03[b]	0.35	
	Group III	26.43[ab]	0.29	
Geriatric Depression Scale	Group I	2.73[a]	1.22	.447
	Group II	2.60[a]	1.35	
	Group III	3.00[a]	1.11	

x̄: Mean, SD: Standart Deviation, p: One Way Anova test. a, b,c: Values followed by different letters in columns differ significantly.*

Table 3. *PASE, SPPB, TUG, Functional Limitation and SF-12 Scores of Cases*

	Group I $\bar{x}$ ± SD	Group II $\bar{x}$ ± SD	Group III $\bar{x}$ ± SD	p*
PASE	127.82[a] ±6.80	102.35[b] ±9.20	76.21[c] ±5.28	**<0.001**
SPPB	11.06[a] ±0.69	9.70[b] ±1.14	8.30[c] ±1.78	**<0.001**
TUG	8.24[a] ±0.34	10.19[b] ±0.56	11.96[c] ±0.56	**<0.001**
Functional Limitation	1.90[a] ±1.21	4.63[b] ±4.03	5.56[bc] ±3.97	**<0.001**
SF-12 Physical	49.22[a] ±4.16	64.96 [a] ±16.58	41.35[a] ±4.62	.256
SF-12 Mental	59.85[a] ±3.67	65,28[a] ±3,85	62.55[a] ±3,70	.592

PASE: Physical Activity Scale for Elderly, SPPB: Short Physical Performance test Battery, TUG: Timed Up and Go Test, SF-12: Short Form 12, x̄: Mean, SD: Standart Deviation, p: One Way Anova test. a,b,c: Values followed by different letters in lines differ significantly.*

PASE and SPPB scores were significantly higher in Group I than in Groups II and III and significantly higher in Group II than in

Group III (p<0.05). Conversely, TUG scores were significantly lower in Group I than in Groups II and III and significantly lower in Group II than in Group III (p<0.05). Functional limitation scores were significantly higher in Groups II and III than in Group I (p<0.05). There was no significant difference between the groups in terms of their scores on SF-12 Physical and Mental subscales (p<0.05) (Table 3).

When the relationships between BMI and physical performance, SMMT, GDS, TUG and functional limitation parameters were investigated, BMI showed a strong negative correlation with physical performance (r :−0.591), moderate negative correlation with PASE (r:−0.427) and moderate positive correlation with TUG (r:0.418) and functional limitation (r: 0.335). There was no significant correlation between BMI and SF-12 Physical and Mental subscales (p>0.05) (Table 4).

Table 4. *The Relationships between BMI and PASE, SPPB, TUG, Functional Limitation*

		BMI	PASE	SPPB	TUG	Functional Limitation	SF-12 Physical	SF-12 Mental
BMI	r	1	-.427**	-.591**	.418**	.335**	.124	.099
	p		**<0.001**	**<0.001**	**<0.001**	**.001**	.243	.351
PASE	r	-.427**	1	.322**	-.213*	-.268*	.074	.117
	p	**<0.001**		.002	.044	.011	.488	.271
SPPB	r	-.591**	.322**	1	.-646**	-.432**	.044	.055
	p	**<0.001**	.002		.000	.000	.677	.610
TUG	r	.418**	-.213*	-.646**	1	.568**	-.124	.047
	p	**<0.001**	.044	<0.001		<0.001	.243	.660
Functional Limitation	r	.335**	-.268*	-.432**	.568**	1	-.202	.285**
	p	**<0.001**	.011	.000	.000		.056	.006
SF-12 Physical	r	.124	-.117	.164	-.063	-.164	1	.306**
	p	.243	.273	.122	.553	.123		.003
SF-12 Mental	r	.099	-.038	-.188	.116	.303**	.306**	1
	p	.351	.723	.076	.276	.004	.003	

*PASE: Physical Activity Scale for Elderly, SPPB: Short Physical Performance test Battery, TUG: Timed Up and Go Test, SF-12: Short Form 12, r: pearson correlation analysis, *: p<0.05 level is significant, **: p<0.01 level is significant.*

4. DISCUSSION

According to the current study; the levels of physical activity, physical performance and functional mobility decreased and that of functional limitation increased with increasing BMI in the elderly. Quality of life scores did not differ according to BMI.

Physical performance reaches maximum levels in the second and third decades of life, and its level tends to decline after this period owing to the physiological changes that occur with increasing age, and the decline in physical performance reaches serious levels when patients are in their 70s. Obesity in old age accelerates this decline and increases the rate of mortality. In fact, studies show that weight loss in the elderly improves physical function, obesity affects physical function negatively and abdominal obesity is associated with low physical activity and function in the elderly (14,15). Consistent with the literature, the results of this study showed that physical activity level decreased as BMI increased.

In their prospective study, Brach et al. observed that overweight and obese individuals had significantly lower physical function levels than normal-weight individuals and that there was no difference between overweight and obese individuals in terms of physical function (15). Chang et al. reported that the levels of physical performance of obese elderly individuals were lower than those of their non-obese peers (16). Ling et al. reported that individuals with a BMI of ≥40 kg/m² had lower TUG scores than those with a BMI of 26–35 kg/m² (16). Previous studies show that physical function and functional mobility levels decrease owing to increased body mass (15-17). Although we use different methods for evaluation; consistent with the literature, physical function and functional mobility levels decreased as BMI increased in our study.

In their cross-sectional study, Barbosa et al. found a significant decrease in all physical performance tests with increasing age, regardless of gender (18). Mickle et al. conducted a study on 312 elderly individuals and reported that the likelihood of lower extremity-related functional limitation was higher in obese individuals than in non-obese individuals (19). Different tests and questionnaires have been used in the literature to determine functional limitations. Most of these are based on performance measurements. Studies show that physical function decreases with increasing age and weight. Consistent with the literature, we found that

functional limitation levels increased as BMI values increased. Functional capacity decreases with age. In the case of obesity accompanying old age, functional capacity decreases further and causes difficulties in daily life activities. In our study, individuals in the obese group stated that they had difficulty in climbing stairs, getting up from and sitting on a chair as well as walking.

When the literature was examined, different studies showing the relationship between obesity and quality of life in the elderly were found. Fjeldstad et al. reported that health-related quality of life was low in the obese elderly (20). Conversely, Sach et al. reported that some health-related quality of life parameters were low in obese women, and the opposite was true in men (21). In our study, quality of life scores did not change according to BMI. We believe that this difference is owing to the different questionnaires used to evaluate quality of life and owing to low quality of life as a result of high percentage of chronic diseases in all three groups.

High BMI results in performance-based measurements to be completed in longer times. Studies show that BMI adversely affects physical activity and physical function, decreases functional mobility levels and increases functional limitation levels (13, 14, 17, 19, 22). In our study, high BMI values were associated with low physical activity and physical performance. Furthermore, individuals with high BMI were found to have high TUG and functional limitation scores.

There are certain limitations to this study. It was conducted on participants living in a specific geographical region and city. In addition, the study was conducted with only female participants; therefore, no comparison could be made according to gender.

5. CONCLUSION

In conclusion, it was determined that obesity causes a decrease in physical function and functional mobility levels and causes functional limitation in elderly women and that quality of life scores are not affected by BMI. In this context, necessary measures should be taken to prevent obesity in the elderly population, and it should be ensured that regular physical activity and exercise becomes a lifestyle not only in old age but also throughout life because it is an important determinant of health and physical function.

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Clinical and Experimental Health Sciences

Effect of Different Mouthwashing Regimens on Adhesion of a Universal Adhesive:A Microshear Bond Strength and Scaning Electron Microscopy Evaluation

Ece Meral, Cansu Atalay, Esra Ergin

Hacettepe University, Faculty of Dentistry, Department of Restorative Dentistry, Ankara, Türkiye.

Correspondence Author: Ece Meral
E-mail: e.yildz@gmail.com

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ABSTRACT

Objective: To evaluate the effect of probiotic or chlorhexidine-based mouthwashes and coconut oil pulling therapy on microshear bond strength of a universal adhesive, used with two application modes.

Method: Ninety-six enamel specimens were prepared using bovine incisors and the surfaces were grounded. Then the specimens were randomly divided into 4groups and each group were subjected to a mouth washing regimen with one of three agents-chlorhexidine mouthwash, probiotic-based mouthwash, coconut oil pulling – or stored in artificial saliva(control) for 7 days(n=24). After the procedure, all groups were divided into 2 subgroups, and a universal adhesive was applied with etch-and-rinse or self-etch mode(n=12). Composite micro-cylinders were bonded to the enamel surfaces and micro-shear-bond strength was measured after 24hours water storage. Failure modes were determined using a stereomicroscope and SEM analysis was also performed. The data were analyzed using Mann-Whitney-U and Kruskal-Wallis tests.

Results: No significant differences were observed between the different mouthwash groups, regardless of application modes($p > .05$). There were no significant differences in microshear-bond strength, within the same mouthwash groups, between self-etch or etch-and-rinse modes, except for oil pulling group. Etch-and-rinse group showed higher bond strength than self-etch group in specimens subjected to oil pulling($p < .05$).

Conclusion: Etch-and-rinse mode might be preferable on patients who practice oil pulling.

Keywords: oil pulling, universal adhesive, shear-bond strength, mouthwash

1. INTRODUCTION

Recently, with the increasing consciousness about oral health, people started to use various products to enhance their oral hygiene routines. Among these products, mouthwashes are commonly used to reduce plaque accumulation and to combat caries. There are numerous types of mouthwashes containing different active agents in the dental market. Chlorhexidine gluconate (CHX) is considered as one of the most effective agents in plaque control (1). Despite their indisputable antibacterial and antiplaque efficacy, CHX containing mouthwashes, have some serious drawbacks, such as staining of teeth and restorative materials, which limit their use for a certain time (2). Another effective anti-plaque agent used in mouthwashes are probiotics, described as living bacteria that have various benefits to general health, if used in adequate amount (3). In recent years, their effect on oral health is gained importance and a number of studies reported different effects from reduction of mutans streptococci(4) to reduction of halitosis (5).

Along with the commercial mouth rinsing products, some people incline traditional medicine and home-made remedies. Oil pulling, is an Ayurvedic therapy, which is performed by whisking a tablespoon of oil in the mouth for about 20 minutes, with an empty stomach (6). But since the long application time can be tiring, 5 to 10 minutes application is considered adequate. The oils used in this practice are edible oils that are commonly available in the household, which makes this therapy achievable.

As the use of different mouth rinsing techniques become widespread, the knowledge of their interaction with restorative materials grows in importance. Universal or multimode adhesives are a newer type of adhesive, which can be used in etch-and-rinse, self-etch or the combination of two – selective etch – strategies. Because of requiring relatively less technical sensitivity and allowing flexible use, Universal adhesives' popularity have been increasing since the day they have first launched. While their practicality

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provides a large user base, there are some points to be emphasized regarding universal adhesives. When these materials used with self-etch strategy on enamel, their bond strength were shown to be lower due to their lower aggressiveness causing incapability to fully demineralize enamel (7). However, in several studies, it was reported that when etch-and-rinse strategy was used, enamel bond strength values were significantly increased (8-10). On the other hand, there are also clinical studies that reported there were no significant differences in terms of retention between application strategies on enamel (10, 11). As controversial findings on bond strength of universal adhesives utilized with different strategies are included in the literature, there are no clear data on the effect of different mouth washing agents on bond strength of universal adhesives.

Therefore, the purpose of this in-vitro study is to evaluate the effect of CHX or probiotic based mouthwashes and oil pulling therapy on microshear bond strength of a universal adhesive used with two application modes.

The null hypotheses tested were;

1. There would be no difference in microshear bond strength between the specimens exposed to different mouthwashes, regardless of adhesive application mode.
2. There would be no difference between the microshear bond strength of different adhesive application modes, regardless of the mouthwash used.

2 .METHODS

Materials used in this study were presented in Table 1. Ninety-six bovine enamel specimens were prepared from freshly extracted bovine incisors collected from slaughterhouse as a product of regular cattle slaughtering for human consumption. The bovine teeth were stored in 0.1% thymol solution for one month and cleaned with pumice using a rubber cap.

After the teeth were cleaned, enamel blocks (4mm×4mm×4mm) were prepared by cutting from the middle third of the buccal surfaces with a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. The enamel blocks were then, embedded in acrylic resin (Meliodent, Bayer Dental, Berkshire, UK) blocks. The enamel surfaces were ground flat using 600 grit SiC paper. After that, the specimens were randomly divided into 4 groups (n=24).

Group I: The specimens were stored in artificial saliva during the whole test period to act as control group.

Group II: The specimens were immersed in a CHX based mouthwash (Kloroben, Drogsan, Ankara, Turkey) for 1 min, twice a day for one-week period. Between the immersion periods, the specimens were stored in artificial saliva and the saliva was refreshed every day.

Group III: The specimens were immersed in a probiotic based mouthwash, using the same procedure with Group II. For the preparation of probiotic based mouthwash; a probiotic sachet (Quadbiotic, MCG Pharma, Ankara, Turkey) was dissolved in 100 ml distilled water.

Group IV: The specimens were immersed in coconut oil (The Life Co., Istanbul, Turkey) for 5 min two times a day for one week and stored in artificial saliva in intervals.

At the end of the immersion periods, all four groups were subdivided into two groups according to the adhesive procedure accomplished;

Sub-Group ER: A universal adhesive (Tetric-N-Bond Universal, Ivoclar Vivadent) was applied using etch-and-rinse mode. A 37% orthophosphoric acid (Panora, Imicryl, Turkey) was applied for 30 s on enamel surface, rinsed off for 15 s and air dried. The adhesive was applied with scrubbing motion for 20 s and dispersed with air until an immobile film layer was observed. Then the adhesive was polymerized using a light-curing device (Henry Schein, HS-LED Light 1200, NY, USA) for 20 s.

Sub-Group SE: The same universal adhesive was applied using self-etch mode. For this procedure, the adhesive was applied with scrubbing motion for 20 s, dispersed with air and light-cured for 20 s.

After the adhesive was applied, resin composite (Tetric-N-Ceram, Ivoclar Vivadent, Zurich, Switzerland) micro-cylinders with 0.7 mm diameter were bonded to enamel specimens using plastic tubes. After removing the tubes with a scalpel, the microshear bond strength testing was performed. The shear load was applied by a thin metal wire, placed at the adhesive interface with a crosshead speed of 0.5 mm/s until the failure occurred using a test machine (LRX, Lloyd Instruments, Chicago, USA). The microshear bond strength values were expressed in MPa after measuring the cross-sectional area at the site of fracture with digital calipers. After testing, modes of failure were examined using a stereomicroscope under 30x magnification and categorized as adhesive failure, cohesive failure and mixed failure.

2.1. Scanning Electron Microscopic Evaluation

One representative specimen from each group was prepared for SEM evaluation. Each sample was cut in half perpendicular to the bonded interface, exposing the adhesive interface at the center of the tooth surface. The exposed adhesive interfaces were then polished with 1000 grit silicon carbid paper and then with diamond paste, under water cooling. For decontamination, the samples were soaked in 10% neutral buffered formalin solution for 8 hours. The specimens were fixed on metal stubs and then gold sputtered (one cycle of 120 s) in a vacuum chamber, using a sputtering device (MED 010, Balzers Union, Balzers, Liechtenstein). The surfaces were examined by scanning electron microscopy (Tescan GAIA 3) to evaluate the enamel-resin interface.

2.2. Statistical Analysis

Statistical analysis was performed with SPSS 21.0, Inc., (SPSS, Inc., Chicago, IL, USA) for Windows. All data sets were subjected to normality testing using the Kolmogorov–Smirnov test. Since the data were failed the normality test, the means microshear bond strength values of the groups were compared by Kruskal Wallis test. Multiple comparisons were done using Mann Whitney U test. The degree of significance was defined as p= .05.

Table 1. *Materials used in the study.*

Materials	Type	Manufacturer	Composition
Kloroben	Mouthwash	Drogsan, Ankara, Turkey	Clorhexidine gluconate, benzydamine hydrochloride
Quadbiotic	Mouthwash	MCG Pharma, Ankara, Turkey	Saccharomyces Boulardii, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium longum
Coconut Oil	Mouthwash	The Life Co., İstanbul, Turkey	Coconut oil
Tetric-N-Bond Universal	Resin composite	Ivoclar Vivadent, Zurich, Switzerland	Ethanol, phosphonic acid acrylate, Bis-GMA, HEMA, UDMA, diphenyl (2,4,6 – trimethylbenzoyl) phosphine oxide
Tetric-N-ceram	Adhesive	Ivoclar Vivadent, Zurich, Switzerland	UDMA, Bis-GMA, Ethoxylated Bis-EMA, TEGDMA, Barium Glass, Yitterbium Triflouride, Silicon dioxide, Additives, Stabilizers, Catalysts
Phosphoric acid	Dental conditioning gel 37%.	Dentsply, Brazil	Phosphoric acid, colloidal silica, Surfactant, and pigment.

Abbreviations: Bis-GMA: bisphenol A-glycidyl methacrylate, HEMA: Hydroxyethyl Methacrylate, UDMA: Urethane Dimethacrylate, TEGDMA: Tetraethyleneglycol Dimethacrylate

Table 2. *Descriptive statistics for µSBS of self-etch and etch-and-rinse modes within each mouth washing group*

Groups	Application modes	Median	Minimum	Maximum	Z	*p*
Group I Control	Self-etch	9.77	4.76	17.19	-1.937	*.053*
	Etch-and-rinse	14.75	6.75	29.57		
Group II Chlorhexidine mouthwash	Self-etch	11.39	4.37	29.33	-0.624	*.533*
	Etch-and-rinse	16.90	8.23	24.34		
Group III Probiotic-based mouthwash	Self-etch	13.58	2.16	32.51	-0.0624	*.533*
	Etch-and-rinse	15.66	3.90	27.59		
Group IV Coconut oil pulling	Self-etch	7.83	2.35	18.66	-2.528	*.011**
	Etch-and-rinse	15.83	4.17	47.46		

** indicates significant difference (p<.05).*

3. RESULTS

No significant differences were observed between the different mouthwash groups, regardless of application modes (p= .375 for SE groups, p= .935 for ER groups). Also, no significant differences in microshear bond strength values were found, within the same mouthwash groups, between self-etch or etch-and-rinse modes, except for oil pulling group (p= .533 for CHX, p= .533 for probiotic, p= .053 for control) Etch-and-rinse group showed higher bond strength than self-etch group in specimens subjected to oil pulling. (p= .011) Although not significant, all other mouthwash groups showed higher microshear bond strength values, when the adhesive was applied in etch and rinse mode. (Table 2)

All mouth rinse groups showed mostly cohesive failures when the adhesive system was used in etch-and rinse mode. In self-etch mode, oil pulling group showed mostly adhesive failures (Figure 1).

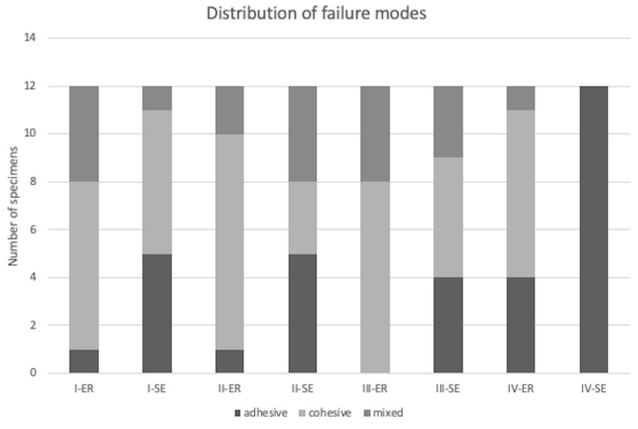

Figure 1. *Represents distribution of failure modes across all groups.*

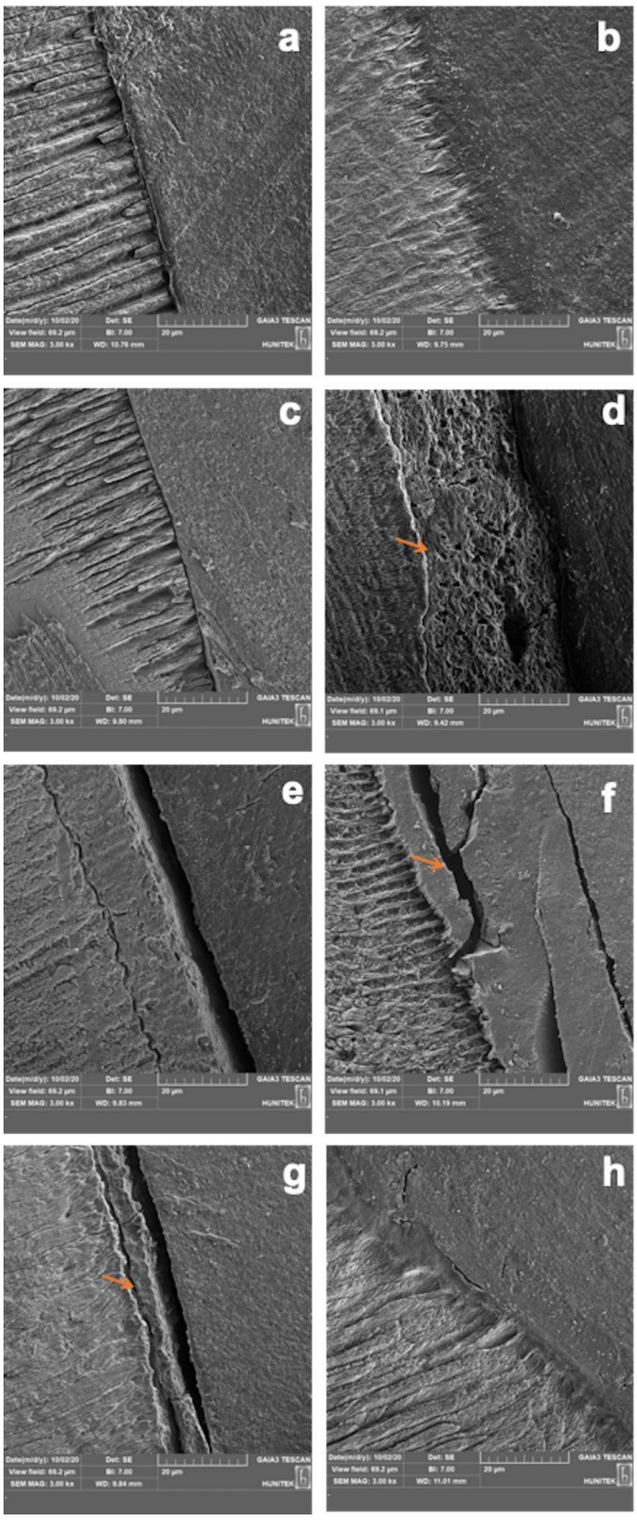

Figure 2. *SEM photos of representative samples from all groups. a: I-SE, b: I-ER, c:II-SE, d: II-ER, e:III-SE, f: III-ER, g: IV-SE, h: IV-ER. In all ER groups (b,d,f,h) it was observed that an intact adhesive interface and deep micro retention was occurred. In SE groups (a,c,e,g), however , the resin tags were shorter, and partial spacing were observed. In group II-ER, a very thick adhesive layer, possibly caused by adhesive ponding in sectioned area(arrow) was observed. In group III-ER, cracks in adhesive, possibly caused by the preparation of SEM process, can be observed. (arrow) In group IV-SE, a layer between adhesive and tooth tissue and also distinct spacing was observed.(arrow)*

Representative SEM images of adhesive-enamel interfaces are shown in Figure 2. In general, all etch-and rinse groups showed intact anchoring between the adhesive and enamel and deep penetration into demineralized enamel tissue forming well-defined, long resin tags; whereas in self-etch groups resin tags were shorter and partial disintegrated areas between adhesive and tooth tissue were present. Only in oil pulling group, distinct spacing between tooth tissue and adhesive was observed in self-etch mode where, remnants of coconut oil was also present between adhesive and tooth tissue. On the other hand, etch and rinse group revealed an even and reliable adhesive interface without any sign of residual coconut oil.

4. DISCUSSION

Individuals with good oral hygiene habits, frequently prefer minimally invasive methods and esthetic adhesive restorations when a restorative treatment is needed. Therefore, the knowledge of the effect of mouthwashes on the success of adhesive restorations can guide the clinicians through material choice. Thus, to fill a gap in the literature, the effect of current rinsing methods on the bonding efficacy of Tetric-N-Bond – one of the most preferred universal adhesives of the day – was evaluated in this study.

Shear bond strength test is a distinguishing tool to assess the bonding efficacy in the tooth restoration interface. Recently the microshear bond strength test, which defines as a shear bond strength test applied with a bonded cross-sectional area of 1mm2 or less, gained popularity. It is believed that the smaller bonded area leads to a more uniform stress distribution providing more precise measurements (12).

The present study compared the effect of probiotic/CHX mouthwashes and oil pulling on microshear bond strength of a universal adhesive used with two different application methods. According to the current results; there were no differences between the tested mouthwashes regardless of application modes. Therefore, the first null hypothesis had to be accepted.

CHX is a cationic bisbiguanide, which is commonly used to maintain oral hygiene and considered as the gold standard amongst the antiplaque agents (13). Sinha et al.(14) reported that the use of CHX as a dentin pretreatment agent increases the shear bond strength of resin composite to dentin. However in another in-vitro study, the use of CHX as dentin disinfectant was found to decrease the shear bond strength to dentin (15). As far as the authors' knowledge, there are a limited number of studies regarding the shear bond strength of adhesive materials to chlorhexidine-exposed enamel. In the present study, the use of chlorhexidine-based mouthwashes had no adverse effects on microshear bond strength of the tested universal adhesive with either of the two application modes. Frey et al.(16) evaluated the effect of CHX application on different concentrations to enamel prior to bracket bonding. They reported that the use of CHX based mouthwash didn't influence the shear bond strength,

which is in accordance with the results of the present study. Similarly, in two different in-vitro studies Bishara et al(17,18), reported that; the shear bond strength to enamel was not affected by application of CHX.

Recently, the use of probiotics for providing a balanced oral flora has become the main topic. The probiotics can compete with the harmful bacteria for adhering hard and soft tissues inside the oral cavity, thus and so may prevent the periodontal diseases and caries (19). There are several studies regarding the probiotics' effects in oral cavity (4, 13, 20-22). They have been shown to reduce, plaque accumulation (1) and streptococcus mutans levels in saliva (21). However, based on the authors' knowledge, there is no published data regarding the interaction of probiotic based mouthwashes exposed dental tissues with adhesive agents. In the present study, the use of probiotic mouthwash didn't affect the microshear bond strength of the universal adhesive to enamel regardless of different application modes, compared to control group. Previously, probiotic mouthwash was found to be more effective than CHX in reducing gingival inflammation (23, 24). Based on the present study's findings, it may be suggested to prefer probiotic mouthwashes over CHX, since probiotic mouthwashes don't have the certain drawbacks of CHX such as discoloration or alteration in taste. However, the reason why the probiotics not interfering with the bond strength in the present study, might be related to the type of adhesive agent used and its composition. Tetric-N-Bond Universal is a mild-etching adhesive with a pH level of 2.5-3.0. The matrix of Tetric-N-Bond Universal is a combination of hydrophobic, hydrophilic and intermediate natured monomers, which allows this material to successfully interact with both hydrophilic tooth, and hydrophobic resin restorative substrates (25). Different types of adhesives with different compositions might present results inconsistent with the present study. Thus, the study must be repeated with various types of adhesives to understand the nature of the interaction of probiotics and adhesives.

Oil pulling therapy has become popular among many people, that try to avoid the use of chemicals. As the healthy lifestyle becomes the new trend, the idea of synthetic therapeutics is toxic for the body, leads more and more people to use natural remedies. Therefore, the knowledge of the interaction between the oils used in this therapy and teeth tissues gain importance. In the present study, coconut oil pulling application didn't affect the microshear bond strength of the universal adhesive comparing to other groups. However, within the same group, application with self-etch mode revealed lower microshear bond strength values than etch-and-rinse mode. In the present study, no difference was observed between the application modes, regardless of the mouthwash applied, except for oil pulling group. Therefore, the second null hypothesis had to be partially rejected. This finding might be explained with the fact that the lauric acid exist in coconut oil, can react with the saliva components to form a soap like substance that reduces plaque and bacterial adhesion (26, 27). In parallel with that, in the present study, a similar layer between adhesive and tooth tissue was observed in SEM photos (Figure 2). The film layer formed on tooth tissue might have adversely affected the bond strength of universal adhesive used with self-etch mode, while the acid etching step in etch-and-rinse mode might have eliminated the film layer. Therefore, it might be suggested to use etch-and-rinse mode in patients who frequently performs oil pulling while performing adhesive restorations.

In control group, no significant differences were observed between etch-and-rinse and self-etch mode. Normally, the etch-and-rinse method was known to be the gold standard when working on enamel tissue. In previous in-vitro studies, etch-and-rinse method have shown higher shear bond strength values compared to self-etch method when applied on enamel tissue (28, 29). There might be several reasons that cause this situation. In the present study bovine teeth were used whereas human teeth were used in the mentioned studies. Although Bovine teeth has the most similar Ca:P rate to human enamel, it has some morphological differences in its crystalline structure which may have caused the contradiction with other previous studies (30). Also, the difference between the compositions or the pH levels of the adhesive used in the current study and the previously mentioned studies may have created the conflict. On the other hand, some clinical studies reported that; there were no significant differences in terms of retention rate between etch-and-rinse and self-etch modes of universal adhesive, supporting the present study's findings (11, 31). However, in these studies, universal adhesive was applied on non-carious cervical lesions that contains both enamel and dentin tissue. Since self-etch mode is the preferred bonding method in dentin tissue because of presenting higher bond strength values compared to etch-and-rinse method, it may have balanced the weak bonding to enamel tissue. Also, further follow-up periods may result in significant differences between retention rates. In any case, because of insufficient data on universal adhesives, it is hard to obtain an exact interpretation.

The findings of this in-vitro study provide a preliminary overview about the effects of some most frequently preferred rinsing methods on the adhesive performance of a popular universal adhesive system. Having some certain limitations due to the nature of all in-vitro studies, these results have to be interpreted carefully and it is recommended to perform further studies with larger groups of specimens to achieve more reliable and comprehensive data. Furthermore, further in-vitro and in-vivo studies must be performed with different mouthwashes and adhesive systems.

5. CONCLUSION

Based on the limitations of this study;

1. The mouth washing habits with different agents, didn't interfere with either etch-and rinse or self-etch application modes of the current universal adhesive.
2. Etch-and-rinse adhesive strategy might be more reliable on patients who performs oil pulling with coconut oil.

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Clinical and Experimental Health Sciences

A Novel Design to Optimize Biomechanical Properties of Dental Implant

Salih Eren Meral, Hakan Hifzi Tuz

Hacettepe University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Türkiye.

Correspondence Author: Salih Eren Meral
E-mail: erenmeral@gmail.com
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ABSTRACT

Objective: The main objective of this study is to evaluate a novel design to optimize dental implant biomechanics. According to this objective, evaluations of the resilient implant design which aimed to mimic biomechanical behaviors of natural tooth have been made and outcomes were compared with natural tooth and standard dental implants with using 3D hyper-elastic finite element analysis.

Methods: Models used in the study corresponding to conventional dental implant, natural tooth and resilient dental implant design. Hyper-elastic model analysis were performed for close presentment of mechanical behaviors of resilient materials like periodontal ligament and medical silicone. Top values of maximum principal stress, minimum principal stress of surrounding bone and displacement of each model were evaluated under axial and non-axial loading conditions with magnitude of 30N, 80N and 100N.

Results: Outcomes of finite element study showed reduction on maximum principal stress and minimum principal stress levels with the use of resilient dental implant comparing to the standard implant model. Standard implant model had been observed notably rigid in all loading conditions compared to the other models. Resilient implant model showed similar biomechanical characteristics with natural tooth model within the limitations of this study.

Conclusion: According to finite element analysis results; resilient implant design was able to resolve some biomechanical discrepancies and seem to have adequate biomechanical similarity with natural tooth under both axial and non-axial loading conditions.

Keywords: Resilient dental implant, dental implant biomechanics, tooth biomechanics, shock absorber, hyper elastic analysis

1. INTRODUCTION

Esthetically and functionally, replacement of missing organs, compatible with natural ones, is one of the main objectives of medicine and dentistry. In cases of replacement of missing teeth, dental implants seem to be fairly similar with natural teeth and bear many advantages and benefits compared to the other options. Nevertheless, there are some discrepancies with nature, especially in the field of biomechanics.

The biomechanical difference between a dental implant and a natural tooth is related to their attachment mechanisms. Healthy roots are normally covered with a specialized soft tissue called periodontal ligament, which makes connection between bone and root, provides biologic and biomechanical characteristics, such as shock absorption, force transmission, sensory and nutrition functions (1). On the other hand, a dental implant, which can be considered as a root analogue, makes rigid connection with bone via a mechanism called “osseointegration”(2). Although osseointegration with bone is a fundamental criterion of success in dental implantology, it creates the basis of the biomechanical differences with natural teeth.

Biomechanical disadvantages of dental implants are shown to be responsible for many undesirable situations, like microfractures and resorption of alveolar bone, loss of osseointegration, implant fracture, challenges in treatment planning of patients with partially edentulism. Additionally; current biomechanics of dental implants have been negatively affecting the healing period of immediate loaded implants (3).

The aim of this study is to evaluate a novel resilient dental implant modelling, mimicking biomechanical behavior of natural tooth. For this purpose, biomechanical comparison between natural tooth, conventional dental implant and resilient dental implant under axially and oblique loads have been conducted using finite element analysis (FEA).

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2. METHODS

2.1. 3D Models

A 3D section of phantom mandible without any malformation was derived from a computerized tomography in .stl data. The solid geometry of the mandible was reshaped from this data with a software: Mimics Innovation Suite (Materialise, Belgium). The surrounding cortical bone thickness was calibrated homogeneously as 2mm and cancellous bone dimensions were modelled 18mm width, 24,5mm depth and 20mm length. Conventional dental implant, natural tooth and resilient dental implant models are all embedded in the same bone structure.

2.1.1. Natural Tooth Model

Premolar (bicuspid) teeth with a single root was modelled with 14,5mm of root length and 8mm of crown height similar with average dimensions (4). Periodontal ligament tissue was modelled as 0,25mm width, homogeneously surrounding whole root surfaces (1). (Figure 1a,1b)

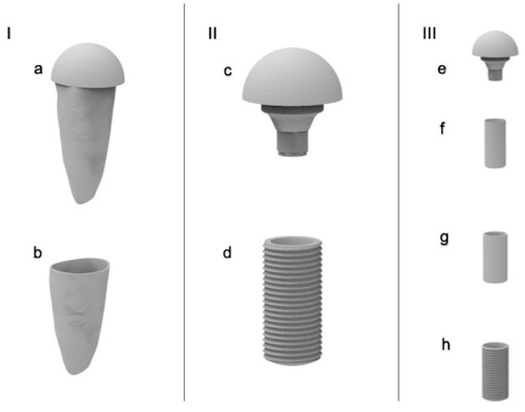

Figure 1. *I: Natural tooth model (a: root and hemisphere crown; b: periodontal ligament), II: Standard implant model (c: hemisphere crown and abutment, d: dental implant), III: Resilient implant model (e: hemisphere crown and abutment, f: internal titanium core, g: resilient layer, h: external titanium cylinder)*

2.1.2. Dental Implant Model

Cylindrical dental implant with symmetrical homogenous reverse buttress shaped grooves and dimensions of 4,8mm diameter and 10mm length was modelled. (Figure 1c,1d)

2.1.3. Resilient Implant Model

Morphologic features of the resilient implant were identical with the dental implant model. Micro motion and shock absorbing capacities of this design were planned to be provided by a resilient material, medical silicone, with thickness of 0,3mm that adopted homogeneously beneath the titanium surfaces, which are in direct contact with the bone. (Figure 1e,1f,g,h)

Coronal sections of the models were designed as a hemisphere with same dimensions and made of titanium for each model for standardization.

Modellings were transferred to a software; 3-matic (Materialise, Belgium), to create a three-dimensional mesh consisting mainly of tetrahedral nodes. All models assumed to be homogenous, linear elastic and isotropic; except for the periodontal ligament in tooth model and the medical silicone in resilient dental implant model. Hyper elastic model analysis were preferred for these materials.

Number of elements and nodes of each model were 74.261, 126.397 for standard implant model; 72.840, 127.919 for resilient implant model and 70,717, 114.377 for natural tooth model, respectively.

Mechanical properties of hyper elastic materials used in this study were C10: 0.04MPa, C01: 0.02MPa, d: 0.02 for PDL (5), C10: 0.14 MPa, C01:0.023MPa (6) for medical silicone. Mechanical properties of other elements used in this study are detailed in table1.

Table 1. *Material properties of finite element models.*

Component	Possion's ratio	Young modulus (MPa)
Titanium	0.35	110000
Dentin	0.31	18600
Cancellous bone	0.30	1370
Cortical bone	0.30	13700

2.2. Finite Element Analysis

Forces with magnitude of 30N,80N and 100N were applied on the most upper section of the hemisphere axially and non-axially with 45 degrees. Movement of the upper most section of each crown, maximum principal stresses and minimum principal stresses of surrounding peri implant bone were observed and compared between models. Since the models are created via a software and there has not been any material directly/indirectly gathered from an individual, approval of IRB was not found applicable.

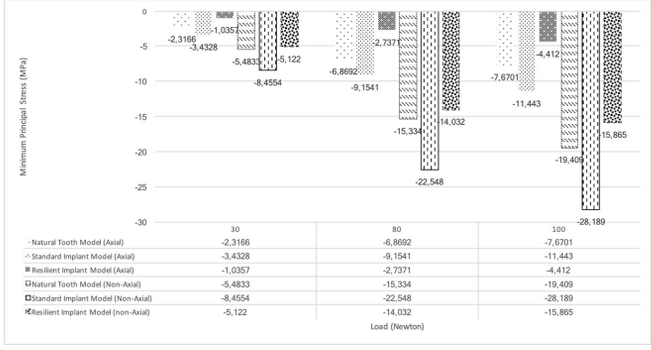

Figure 2. *Minimum principal stress of surrounding bone of each model, under 30N, 80N and 100N axial and non-axial loads presented in figure 2. Minimum principal stress of standard implant model concentrated at most coronal part of the bone with greater magnitudes.*

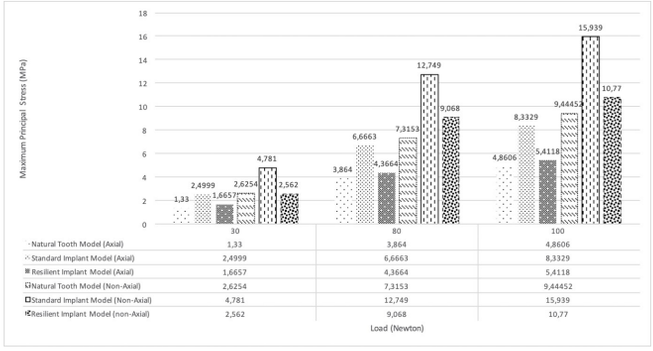

Figure 3. *Maximum principal stress of surrounding bone of each model under 30N, 80N and 100N axial and non-axial loads presented in figure 3. Maximum principal stress of standard implant model concentrated at most coronal part of the bone with greater magnitudes.*

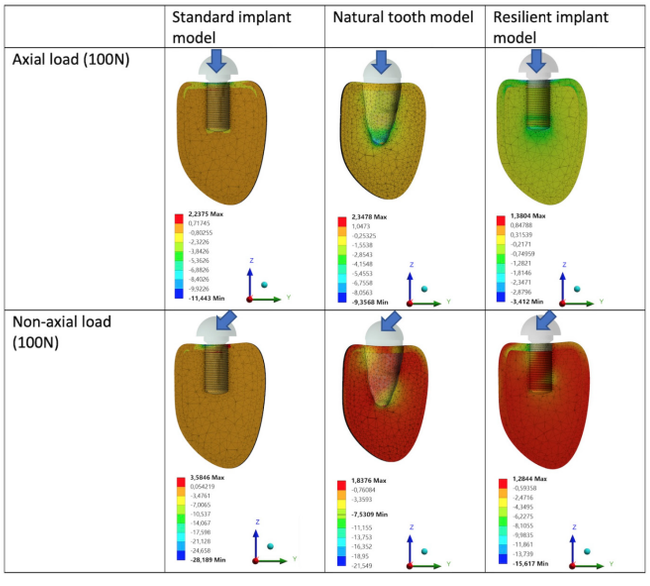

Figure 4. *Pattern of force transmission and accumulation area of minimum principal stresses on surrounding bone for each model under axial and non-axial load have been demonstrated in figure 4. Although amount of the stress had changed corresponding to the magnitude of applied forces, stress pattern remained same regardless from the amplitude of forces. The given color scales are specialized for each sample.*

3. RESULTS

For better traceability, the outcomes gathered from the finite element analysis of each model under axial and non-axial loads were summarized in figures. Models were compared in terms of vector and magnitude of applied forces. While, compared to the other models, maximum principal stress and minimum principal stress levels of standard implant model were observed to be higher and concentrated mostly around the superior part of the surrounding bone; resilient implant model and natural tooth model exhibited relatively similar stress distributions and magnitudes (Figure2-5).

Greater amount of displacement, higher maximum and minimum principal stresses were observed with non-axial loadings in all models. Natural tooth model and resilient implant model showed similar biomechanical behavior in all conditions. Higher levels of maximum principal stresses and minimum principal stresses were observed in standard implant model with both, axial and non-axial loading conditions. In resilient implant model and natural tooth model, the achieved maximum and minimum principal stress levels were similar and quite lower than standard implant model. Displacement of crown was also observed to be approximately similar in natural tooth model and resilient implant model. Moreover, these models showed a similar rate of increase in displacement correlated with increase in loading. On the other hand, standard implant model stayed notably rigid in all loading conditions.

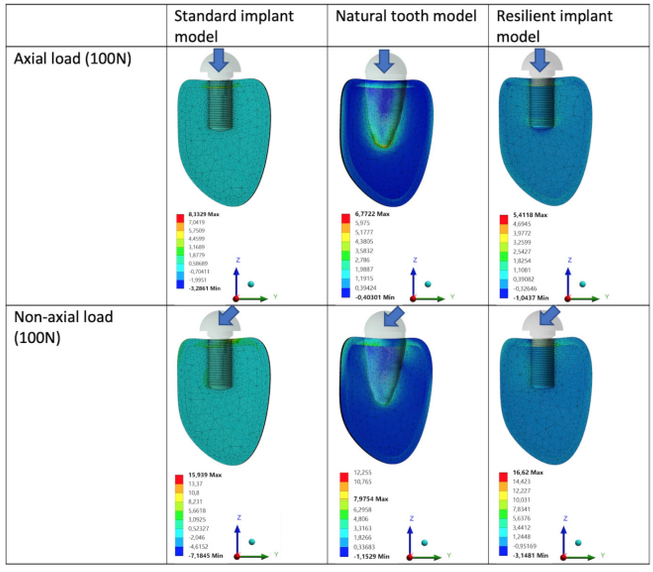

Figure 5. *Pattern of force transmission and accumulation area of maximum principal stresses on surrounding bone for each model under axial and non-axial load have been demonstrated in figure 5. Although amount of the stress had changed corresponding to the magnitude of applied forces, stress pattern remained same regardless from the amplitude of forces. The given color scales are specialized for each sample.*

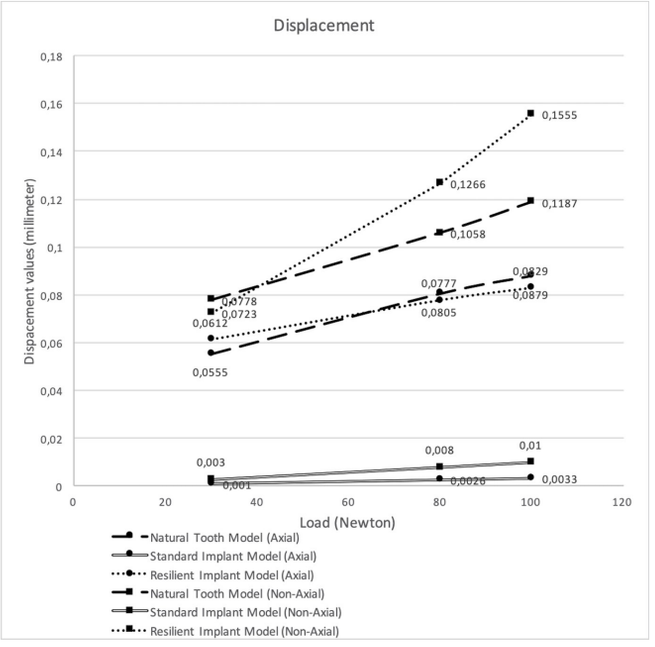

Figure 6. *Displacement values of the edge of each model is presented in the figure. Natural tooth model and resilient implant model showed relatively close micromovement patterns, whereas standard implant models were observed to be quite rigid under each circumstance.*

4. DISCUSSION

When it comes to rehabilitate tooth deficiencies, dental implants are the most popular treatment modality with high rate of success(7) . This popularity owes itself to many advantages such as transmitting occlusal forces, stimulating bone and preserving bone volume, achieving maximum bite force and chewing efficacy, working as a single unit, like natural teeth, if the prosthesis were designed separately from other teeth or implants (8). All in all, dental implants could be considered as an important contribution to dentistry.

Dental implants have taken the stage in the armamentarium of dentistry thanks to the mechanism called “osseointegration”. Osseointegration is defined as “a direct structural and functional connection between ordered living bone and the surface of load-covering implant “ (2) and is a master key for success. After an uneventful osseointegration period, implants act as a root analogue except for the inevitable rigid connection with bone. Unfortunately, this main success criteria, makes the basis of discrepancies with nature. Biomechanical discrepancies between tooth and dental implants are a result of the difference in attachment mechanisms; periodontal ligament and osseointegration. Beyond the role of attachment to the bone, PDL is responsible for micro-motion capability, absorption and distribution of forces.

The average amount of micro movement observed in natural teeth has been reported as 28-108 microns, and these values are shown as 3-5 microns in dental implants. (8). In addition to this, movement pattern of teeth can be observed in two phases. Non-linear and complicated initial movement occurs in PDL and after that a secondary movement occurs, as a result of elastic deformation of bone tissue. Dental implants are only capable of mimicking the secondary movement. A previous in-vivo study on measurement of tooth and implant mobility under physiological loadings also reported that, natural tooth has biphasic micromovement pattern which had ten-times greater magnitude compared to dental implants (9). Current data obtained from the standard implant model and the natural tooth model have shown significant consistency with previous studies in terms of the magnitude and behavior of the micro motion (9-11). Resilient implant model showed clear similarity with natural tooth model and previous data on biomechanics of natural teeth from other reports. Based on this similarity with the literature, it might be interpreted that, hyper-elastic model analysis also found as an effective method in accordance to intended use in both natural tooth and resilient implant model. Tooth to implant borne fixed prosthesis are also not desired because of this uncorrelated movement pattern, which has a hazardous effect on both implants and teeth. In some cases, these inharmonious two elements have been making the rehabilitations of patients more complicated, invasive and expensive.

Bone remodeling is related with mechanical inputs directed to the bone (12). It have been pointed out that the bone tissue continuously responds as apposition or resorption to the change in mechanical environment to maintain elastic deformation of bone (13). Dental implants transmit occlusal forces directly to the bone and stimulate and preserve the bone volume. Nevertheless, due to the rigid connection between bone and implant, the force is concentrated mostly on the coronal section of bone with higher levels and this situation has been reported as one of the reasons for bone resorption around dental implants (14, 15). On the other hand, PDL absorbs the forces and transmits homogeneously along the root surface, thereby bone resorption around healthy teeth are not commonly seen. In the present study the outcome of evaluation and comparison of dental implant model and standard implant model regarding the force concentration and transmission to the surrounding bone tissue were found to be similar with current literature (16-18). While relatively homogeneous force concentration on the bone tissue had been observed in natural tooth model, whole stresses were mostly concentrated on superior part of cortical bone around standard dental implant in all loading conditions. The difference of the amount of stresses on the surrounding bone have been became even more dramatic with non-axial forces. On the other hand, in all loading conditions, resilient implant model showed quite similar results with natural tooth model on behalf of force transmission and concentration. According to the outcome of this simulation, novel resilient implant design had been eliminated discrepancies on force transmission to and intense concentration points of the bone compared to the dental implant. In literature, the ultimate endurance of cortical bone tissue is shown to range from 72-26MPa

in tension and 140-170 MPa in compression zones (19). In all loading conditions maximum and minimum principal stress values were monitored below the ultimate endurance levels of bone. Nevertheless, maximum values of tensional and compressional stresses have been encountered with standard implant model in all loading conditions compared to the natural tooth and resilient implant models. On the other hand, the results of the resilient implant model demonstrated the success of cushioning ability of the implant design by eliminating intense force transfer on the surrounding bone tissue. Still, regardless from the mechanical features as resilient or conventional implants, none of the implants have bear neural capacity like proprioception. Due to the lack of proprioception, dental implants have shown to experience higher loads compared to the natural tooth (8, 20). Even though resilient implants cannot eliminate neural oriented parafunctional habits, it may enhance the capacity of toleration.

Although it was not evaluated in this study, another potential advantage of resilient implant might be encountered in the healing period of immediate loaded implants. During the osseointegration period, micromovement of dental implant could affect bone healing adversely and lead to undesirable fibrous healing(3). In immediate loading protocols micromotion at bone-implant interface above 50-150 microns, was proposed to be avoided due to possible failure of osseointegration (21). In this case, resilient dental implant may affect bone healing by suspending and absorbing forces directed to the implant in immediate loading protocols.

Biomaterials, such as dental implants, are dynamic products; their designs and mechanical properties are constantly evolving in order to meet the increasing demands with the help of developing technology. There are and will be some studies and designs that aimed to optimize and enhance the biomechanical properties of dental implant, like currently evaluated design(11, 22-30). Of these, IMZ system had already been used clinically many years until they lost their popularity due to frequent complications (26). Regardless of previous failures and inconclusiveness, with the help of increased technology and enhanced biomaterials, several designs and prototypes, which are aimed the same goal with currently presented design, have recently being proposed to overcome standard implants' handicaps (11, 30).

In the presented design; external titanium cylinder, resilient area, internal core are planned to be analogues of dense bone, PDL and root respectively. External titanium cylinder and internal core are planned to be made of titanium that is already in use in dental implantology. While considering the resilient area; medical silicone, which is already being used in medicine and has been proven to be biocompatible was preferred as the resilient material (31). Main advantages of this design is that, all the materials used in the design were proven to be available in the armamentarium of medical materials and the materials are well known.

Main limitations of this study are mostly based on limitations of finite element analysis. Some assumptions and simplifications have been made with regard to material properties and model generations. Although living tissues had shown to be anisotropic and inhomogeneous, the structures modelled in this study were assumed to be homogenous, isotropic and linear elastic, except PDL and medical silicone, which are the main elements for this study(32) . In this study, implant models are assumed as fully osseointegrated, on the other hand histomorphometric studies have revealed that the bone implant contact never reaches 100% (33). Modelled section of the mandible was composed of cancellous bone, surrounded by 2mm thickness cortical bone layer homogeneously, which is also hard to observe in nature as well. Although, the forces applied in this study are shown to be in functional limits, then again, more hazardous loading conditions may occur especially in implants, which are incapable of proprioception (20). Finite element analysis cannot be evaluated statistically.

5. CONCLUSION

Within the limitations of this study, resilient dental implant model showed great biomechanical resemblance to natural tooth model. With the use of resilient dental implant, biomechanical discrepancies may be resolved and many advantages may be obtained. Therefore, development of dental implants mimicking mechanical behavior of natuæral tooth can be considered as a major advancement in implant dentistry. However, these outcomes were based on FEA and needs to be valideted by in vitro studies.

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Conflict of Interest:

The authors report no conflicts of interest

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Clinical and Experimental Health Sciences

Evaluation of Perioperative Complications and Mortality in Covid-19 Patients Who Had Emergency Surgery

Hamide Ayben Korkmaz, Ilkay Ceylan

Bursa Training and Research Hospital, Department of Anesthesia and Reanimation, Bursa, Türkiye.

Correspondence Author: Hamide Ayben Korkmaz
E-mail: aybenkorkmaz73@gmail.com
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ABSTRACT

Objective: The risk for adverse outcomes in COVID-19 patients necessitates further scrutiny in Covid 19 patients in providing appropriate surgical indications and perioperative surgical safety precautions. In this study, we aimed to contribute to elective surgery resumption about infection with early and late postoperative complications and mortality in patients with RT-PCR (+) and clinically suspicious COVID-19 who underwent emergency surgery in our hospital.

Methods: A total of 86 patients who have been operated on in our institution for emergency surgery over the age of 18 who were diagnosed with SARS-CoV-2 infection seven days before or 30 days after surgery were enrolled in the study. In this retrospective study, the primary outcome has been established as mortality factors and survival within postoperative 30 days.

Results: Regarding the primary outcome as 30-day survival, every 1-year increase in age increased the risk of death by two folds. Patients with one or more comorbidities have an increased risk of death 13 times and those with two or more have an increased risk of death 23 times. Patients in intensive care units increase the risk of death by 8.5 times compared to those who are not hospitalized. On the contrary, an increase in hemoglobin level was shown to reduce the risk of death by 0.8 times.

Conclusion: The need for intensive care and mortality is high, especially after emergency surgery, in patients with COVID19 symptoms and more than one comorbidity. Surgical indications of such patients should be well investigated.

Keywords: COVID-19, PCR, emergency surgery, surgery complications, pandemic

1. INTRODUCTION

Coronavirus disease 2019 *(COVID-19)* was first detected in Wuhan City, China. COVID-19 was caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 *(SARS-CoV-2)* and has caused a worldwide pandemic *(1)*. Although most patients survive the disease with mild to moderate symptoms, almost one-third of the individuals are at risk of developing acute respiratory distress syndrome *(ARDS)* due to COVID-19. This group of severe patients may require mechanical ventilation *(MV)*, intensive care, and their diseases may even result in death *(2,3)*.

The rapidly spreading epidemic has caused severe difficulties in the effectiveness and sustainability of health systems. There was a significant increase in admissions to emergency services, hospitalization to inpatient services, and admissions to intensive care units. Coronavirus disease 2019 *(COVID-19)* can lead to severe viral pneumonia. Characteristic computerized tomography *(CT)* findings in affected patients include bilateral, multilobar ground-glass opacities *(GGO)* and peripheral and posterior dispersed consolidations *(4,5)*. In addition to common symptoms such as dry cough, fever, myalgia, and/or fatigue, recent studies have identified severe secondary complications such as acute respiratory distress syndrome *(ARDS)*, acute kidney or heart injury, secondary infection, and liver dysfunction *(6,7)*.

According to most health care professionals, elective surgeries and endoscopic procedures other than emergency and oncological surgeries should be postponed to a later date during the COVID-19 pandemic. Although there is no sufficient scientific evidence yet, surgical procedures to be performed on patients diagnosed with COVID-19 involve a high risk in the postoperative period *(8)*. It is convenient to proceed with a safe non-operative treatment approach that should be considered in patients *(9)*.

Although the utilization of resources, such as hospital and intensive care unit *(ICU)* admission, is well documented in patients with COVID-19, data on surgical needs and outcomes are limited. The unexpectedly high rate of postoperative mortality in patients infected with SARS COV-2 in the global

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literatüre requires perioperative surgical safety measures like evaluating the necessity and emergency of surgery, assuring the personal protective equipment and isolated operation rooms, lastly extubating the patients in the COVID-ICUs *(10)*.

Viral pneumonia is a condition that requires treatment, but infected patients may require emergency surgery. Coronavirus infection is associated with a marked inflammatory and prothrombotic state and cytokine storm plays a vital role in critical patient groups with SARS-CoV-2 infection *(11,12)*. The inflammatory and prothrombotic process in severe COVID-19 infection may be exacerbated by surgery and immobilization, complicating postoperative recovery. It is essential to document the necessity and diversity of surgical procedures in this population in estimating risk for patients *(10,11)*.

1.2. Study Hypothesis

The risk for adverse outcomes in the perioperative period in individuals infected with SARS-CoV-2 remains uncertain. Some studies have published that the perioperative risk is high in COVID-19 patients, while some studies have no difference. It necessitates further scrutiny in Covid 19 patients to provide appropriate surgical indications and perioperative surgical safety precautions *(13)*.

In this study, we aimed to contribute to the resumption of elective surgery following hospital resources, service and ICU beds by investigating the relationship of infection with early and late postoperative complications and mortality in patients with COVID-19 who underwent emergency surgery in our hospital. The primary outcome has been established as mortality factors within 30 days postoperatively, and the secondary outcome was pneumonia, acute respiratory distress syndrome, or pulmonary complications requiring postoperative mechanical ventilation.

2. METHODS

A total of 86 patients who applied to Bursa Training and Research Hospital between 1st of April 2020 to 31st of March 2021 and who have been operated in our institution as emergency surgery via general, regional or local anesthesia were evaluated in the scope of this research. Subjects over 18 diagnosed or suspected of SARS-CoV-2 infection seven days before or 30 days after surgery were enrolled in the study. SBU Bursa Training and Research Hospital Ethics Committee approval was granted in 2021 *(protocol number: 2011-KAEK-25 2021/04-02)* and informed consent was not obtained from patients.

The study had a retrospective nature. PCR could not be evaluated in all patients. Emergency patients were operated without waiting for test results. Those with clinical and radiological findings were considered suspicious. Age, gender, and American Society of Anesthesiologists *(ASA)* physical status classification have been interpreted as the demographic variables of the study population. The time from the PCR positivity or the onset of symptoms to surgery was recorded.

The patient's preoperative symptoms *(fever, respiratory distress, etc.),* baseline characteristics such as preoperative oxygen saturation or requirement for invasive mechanical ventilation, previous medication *(antiviral agents, anticoagulants, steroids, antibiotics)* have been investigated via the hospital database. Surgery type, surgical urgency, initial laboratory values, initial radiological findings, preoperative Sequential Organ Failure Assessment *(SOFA)* score, and intraoperative variables were obtained from the patient files.

Primary outcome parameter was recorded 30-day survival and secondary outcomes were classified as postoperative respiratory complications *(atelectasis, pneumonia, Acute Respiratory Distress Syndrome [ARDS] and pulmonary aspiration),* non-pulmonary infectious complications, acute kidney injury, thrombosis-related complications (pulmonary embolism, myocardial infarction, stroke, and cardiac arrest). Duration of hospital stay, 30-day mechanical ventilation-free days, 30-day organ dysfunction-free days, and any new ICU admissions were also recorded.

The data set has interpreted the preoperative, operative and postoperative information of 96 procedures conducted between 1 April 2020 and 31 March 2021. Ten individuals were operated on twice for various reasons, and of these patients, the procedure closest to the diagnosis of SARS-CoV-2 infection was evaluated.

2.1. Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences *(SPSS)* for Windows 23.0 *(IBM Corp., Armonk, NY)* package program. Frequency and percentage were given for categorical data and median, minimum and maximum descriptive values for continuous data. "Mann Whitney-U Test" was utilized for comparisons between groups, *"Chi-Square Test"* was used for comparison of categorical variables, and *"Cox Regression Analysis"* was used for examining risk factors affecting survival. The results were considered statistically significant when the *p*-value was less than 0.05.

3. RESULTS

A total of 86 patients were enrolled in the study, and the 30-day postoperative information of all patients, except for one, was obtained. A patient whose data information could not be retrieved was transferred to another center because there was no room in intensive care units of our hospital. The study population consisted of 4 *(5%)* children, 52 men *(60%)* and 30 *(35%)* women.

We could not reach 11 PCR results due to the loss of the samples. Thirty-nine patients were negative for Sars-Cov-2 PCR. Thirty patients were positive preoperatively and six were positive postoperativly.

Table 1. *Baseline demographics and clinical features of the patients*

Characteristics (n=86)	Alive (n=61) Median (Min-Max) or n (%)	Exitus (n=25) Median (Min-Max) or n (%)	P-value
Age (years)	51 (1-92)	69 (36-90)	<0.001
<29	15 (24.6)	0 (0.0)	0.005
30-49	14 (23.0)	2 (8.0)	
50-69	18 (29.5)	12 (48.0)	
>70	14 (23.0)	11 (44.0)	
Gender			0.923
Female	22 (36.1)	10 (40.0)	
Male	39 (63.9)	15 (60.0)	
Smoking	5 (8.2)	3 (12.0)	0.686
PCR positive	38 (62.3)	13 (52.0)	0.522
ICU admission	23 (37.7)	22 (88.0)	<0.001
Duration of hospital stay (days)	7 (1-66)	13 (1-58)	0.095
Time to surgery from diagnosis	5 (1-55)	3 (1-40)	0.599
COVID-19 adherent radiology	33 (54.1)	23 (92.0)	0.002
Number of Comorbidities			<0.001
None	31 (50.8)	1 (4.0)	
1	14 (23.0)	8 (32.0)	
2 or more	16 (26.2)	16 (64.0)	
Concomitant Disease			
Hypertension	17 (27.9)	13 (52.0)	0.060
Heart Failure	5 (8.2)	4 (16.0)	0.438
Atrial Fibrillation	4 (6.6)	3 (12.0)	0.409
Coronary Artery Disease	9 (14.8)	6 (24.0)	0.353
Diabetes Mellitus	8 (13.1)	8 (32.0)	0.065
Neurologic Diseases	5 (8.2)	5 (20.0)	0.146
COPD	1 (1.6)	2 (8.0)	0.201
Malignity	5 (8.2)	3 (12.0)	0.686
Obesity	1 (1.6)	1 (4.0)	0.499
Thyroid Disease	1 (1.6)	2 (8.0)	0.201
Chronic Renal Failure	2 (3.3)	3 (12.0)	0.145
Deep Vein Thrombosis	2 (3.3)	1 (4.0)	1.000
ASA Score			<0.001
1	24 (39.3)	0 (0.0)	
2	20 (32.8)	4 (16.0)	
3	16 (26.2)	12 (48.0)	
4	1 (1.6)	9 (36.0)	
Chronic Medication	31 (50.8)	23 (92.0)	0.001
Hemoglobin g/dL	12.7 (7.5-18.4)	10.8 (6.7-14.8)	0.006
White Blood Cells 10^3/mL	11.21 (2.4-35.3)	12.99 (4.09-59.55)	0.351
Lymphocytes 10^3/mL	1.41 (0.14-13.72)	0.9 (0.16-5.06)	0.061
Plateles 10^3/mL	240 (68-612)	250 (108-637)	0.326
Neutrophil 10^3/mL	8.43 (2.22-213.00)	9.65 (2.25-57.41)	0.219
CRP mg/L	19.5 (3.11-278.0)	77 (9-493)	0.003
Fibrinogen mg/dl	494 (104-778)	466.5 (292-812)	0.914
Ferritin ng/mL	192.19 (19.91-637.00)	843 (20.5-2000)	0.013
LDH U/L	254 (100-1145)	403 (229-862)	0.104
ALT U/L	14.5 (4.0-472)	19 (5-109)	0.522
AST U/L	22 (8-611)	23 (10-196)	0.333
BUN mg/dL	15.89 (5.84-73.32)	29.77 (12.9-111.0)	<0.001
Creatinine mg/dL	0.87 (0.36-5.19)	1.08 (0.31-11.72)	0.040
INR kU/L	0.98 (0.87-11.6)	1.05 (0.1-1.23)	0.107
Hemoglobin g/dL	3.61 (0.25-80.0)	5.43 (0.98-25.82)	0.303

The overall mortality rate was 29.6% and most of these originated due to respiratory complications were 75.86%. The distribution of demographic and clinical findings of the participants within the scope of the study was given in *Table 1*. When the table is examined, there were a statistically significant difference between alive and deceased individuals in terms of age, duration of stay in intensive care, presence of radiology compatible with COVID-19, number of comorbidities, ASA score, chronic drug use, hemoglobin, CRP, ferritin, urea and creatinine counts. It was determined that there was a statistically significant relationship *(p<0.05)*. ICU admission was a strong denominator and observed in 88% of the deceased patients compared to alive subjects *(p<0.001)*. The number of comorbid diseases was another significant predictor of survival, and the deterioration has increased with the number of comorbid conditions *(p<0.001)*. On the other hand, none of the concomitant diseases had any clinically significant effect on the 30-day survival.

Table 2 indicates the distribution of the participants' preoperative evaluation results. When the table was examined, it was seen that there was a statistically significant relationship between the two groups in terms of preoperative COVID-19 symptoms such as fatigue/myalgia, respiratory distress, and encephalopathy *(p<0.005, p<0.031 and p<0.022, respectively)*. It was determined that there was a significant relationship between survival status and anesthesia type and intraoperative variables *(p<0.025)* and the majority of the deceased subjects were operated via general anesthesia *(76%)*. The intraoperative status of the patient *(stable or unstable)* has been significant as 76% of the deceased individuals were unstable cases by no means. However, no statistical significance has been observed regarding the type of surgery.

Postoperative complications were also a significant determinant of mortality. The 88% of the deceased cases had respiratory *(px-thorax tube)* problems *(p<0.001)*, 84% had infection *(p<0.001),* 80% had acute renal injury *(p<0.001)* and 96% has acute thrombotic disease *(p<0.001) (Table 3).*

Individuals in intensive care units increase the risk of death by 8.5 times compared to those who are not admitted to ICU. A COVID-19 adherent radiological findings elevated the risk of death by 7.5 times compared to those who do not *(p<0.006) (Table 4)*.

Table 2. *Distribution of participants pre-operational evaluation results*

Characteristics (n=86)	Alive (n=61)	Exitus (n=25)	P-value
	Median (Min-Max) or n (%)	Median (Min-Max) or n (%)	
Pre-operative COVID-19 Symptoms			
Headache	2 (3.3)	1 (4.0)	1.000
Fatigue/myalgia	10 (16.4)	12 (48.0)	0.005
Cough	2 (3.3)	1 (4.0)	1.000
Respiratory Distress	7 (11.5)	8 (32.0)	0.031
Fever	6 (9.8)	4 (16.0)	0.467
Diarrhea	2 (3.3)	0 (0.0)	1.000
Encephalopathy	0 (0.0)	3 (12.0)	0.022
COVID-19 Treatment	31 (50.8)	20 (80.0)	0.024
Surgery Type			0.688
Minor	19 (31.1)	6 (24.0)	
Major	42 (68.9)	19 (76.0)	
Anesthesia Type			0.025
Spinal	27 (44.3)	4 (16.0)	
Local + Sedatives	1 (1.6)	2 (8.0)	
General	33 (54.1)	19 (76.0)	
Intraoperative Status			<0.001
Stabile	45 (73.8)	6 (24.0)	
Unstabile	16 (26.2)	19 (76.0)	

Chronic medication enhanced the risk of death 8.7 times compared to non-users. On the other hand, it has been determined that those who received COVID-19 treatment had 3.4 higher risk of death times compared to those who did not *(p<0.003) (Table 4)*.

Table 3. *Distribution of post-operational complications*

Characteristics (n=86)	Alive (n=61)	Exitus (n=25)	P-value
	Median (Min-Max) or n (%)	Median (Min-Max) or n (%)	
Postoperative complications			
Respiratory (px-thorax tube)	7 (11.5)	22 (88.0)	<0.001
Infection	7 (11.5)	21 (84.0)	<0.001
Acute Renal Injury	5 (8.2)	20 (80.0)	<0.001
Acute Trombotic Disease	7 (11.5)	24 (96.0)	<0.001

Fatigue/myalgia, which was one of the preoperative symptoms, increased the risk of death 3.6 times *(p<0.001)* compared to those who did not have, and 2.8 times the risk of death compared to those who did not have respiratory distress *(p<0.017) (Table 4)*.

Table 4. *Analysis of risk factors affecting mortality*

Characteristics (n=86)	Mortality	
	Odds ratio (%95 GA)	P-value
Age (years)	1.04 (1.02-1.06)	0.001
PCR positive		
No	1.0	-
Yes	0.75 (0.34-1.65)	0.477
Number of Comorbidities		
None	1.0	-
1	13.23 (1.65-105.82)	0.015
2 or more	23.03 (3.05-173.87)	0.002
ICU admission		
No	1.0	-
Yes	8.54 (2.55-28.58)	0.001
COVID-19 adherent radiology		
No	1,0	-
Yes	7.49 (1.76-31.80)	0.006
Chronic Medication		
No	1.0	-
Yes	8.66 (2.04-36.75)	0.003
Pre-op COVID-19 Symptoms		
Fatigue/myalgia		
No	1.0	-
Yes	3.60 (1.63-7.90)	0.001
Respiratory Distress		
No	1.0	-
Yes	2.81 (1.21-6.53)	0.017
COVID Treatment		
No	1.0	-
Yes	3.35 (1.26-8.94)	0.016
Surgery Type		
Minor	1.0	-
Major	1.34 (0.53-3.35)	0.535
Hemoglobin g/dL	0.80 (0.66-0.95)	0.012
CRP mg/L	1.01 (1.00-1.02)	0.003
Ferritin ng/mL	1.001 (1.00-1.01)	0.007
BUN mg/dL	1.02 (1.01-1.04)	0.001
Creatinine mg/dL	1.24 (1.07-1.43)	0.004
INR kU/L	0.58 (0.03-7.58)	0.578
D-dimer microg/mL	0.98 (0.94-1.03)	0.449

An increase in hemoglobin level was shown to reduce the risk of death by 0.8 times *(p<0.012)*. On the contrary, it was observed that each increase in CRP *(p<0.003)*, ferritin *(p<0.007)*, urea *(p<0.001),* and creatinine *(p<0.004)* levels deteriorated the risk of death approximately by two folds *(Table 4)*.

Table 4 and figure 1 denoted the Cox Regression Analysis results conducted the risk factors affecting the 30-day survival of individuals. According to the results every increase, in age increased the risk of death by two folds. Individuals with one or more comorbidities have an increased risk of death 13 times compared to those without any, and those with two or more have an increased risk of death 23 times compared to those without.

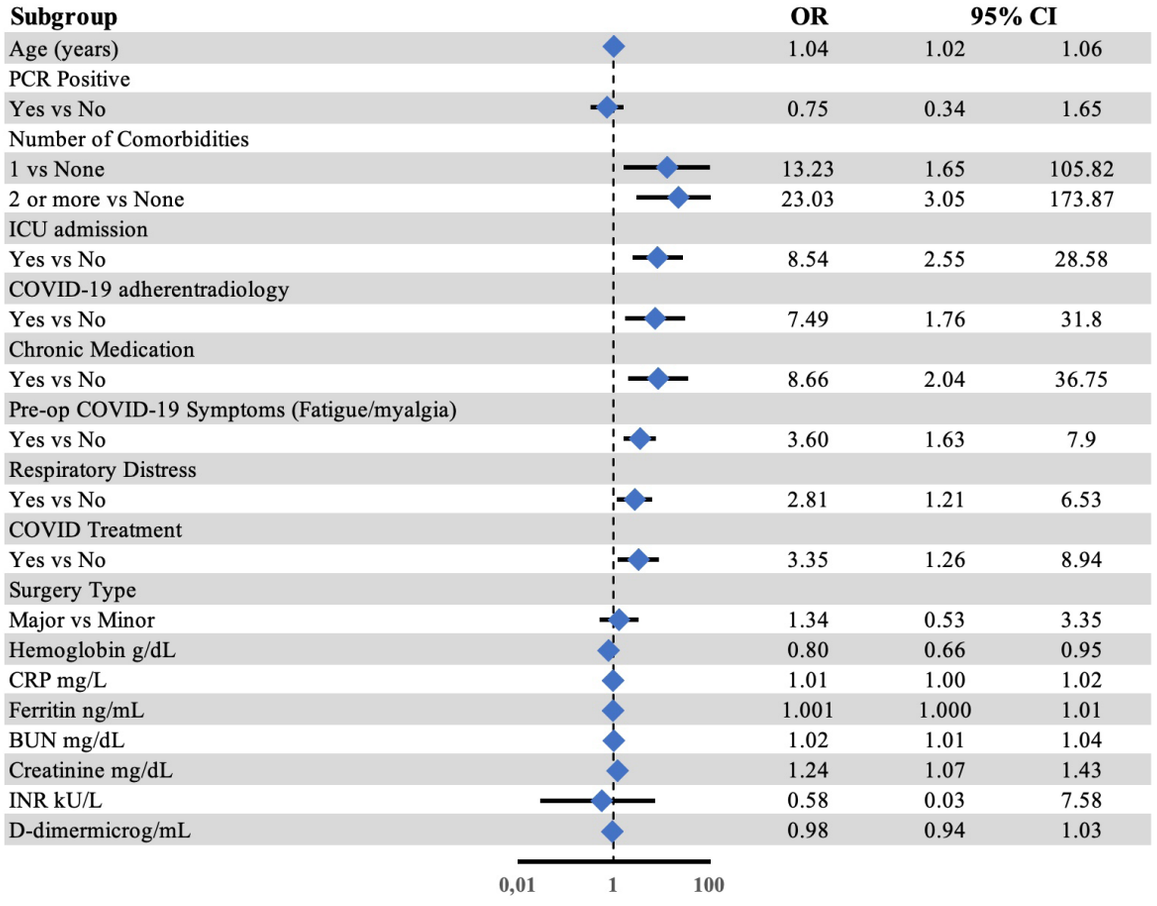

Figure 1. *Analysis of Risk Factors Affecting Mortality – Odds ratio graphic*

4. DISCUSSION

In this study, the need for intensive care is higher in advanced elderly patients who have symptoms of COVID-19 and require emergency surgery, and the mortality of these patients was found to be higher. The frightening aspect of COVID 19 infection is the lack of proven treatment and the unknowns about the disease. The global literature's high postoperative mortality rate in patients infected with SARS COV-2 warrants further scrutiny in providing appropriate surgical indications and perioperative surgical safety measures in this vulnerable patient cohort. During the COVID-19 epidemic, elective surgeries were canceled as a health management policy, Hospital organizations were urgently changed, and notable in-hospital routes and special operating rooms for COVID-19 patients were created. These practices are essential for using hospital bed capacity, providing personal protective equipment (PPE), and protecting patients and healthcare workers. The COVIDSurg Collaborative has created a predictive model regarding these pandemic originated conditions and stated that the three-month cancellation rate was 72.3% *(14)*. Most of these cancellations were situations *(90.2%) (14)*.

According to the results of COVID Surg collaborative study, the 30-day mortality rate was 23.8% for all patient groups.

The breakdown of all-cause mortality has been elaborated as 18.9% in elective operations, 25.6% in emergency patients, 16.3% in minor surgery and 26.9% in major surgery *(13)*. In the current study, we have focused on the factors affecting and found that every 1-year increase in age increased the risk of death by two folds. Individuals with one or more comorbidities have an increased risk of death 13 times compared to those without any, and those with two or more have an increased risk of death 23 times compared to those without. Individuals in intensive care units increase the risk of death by 8.5 times compared to those who were not admitted to ICU. On the contrary, an increase in hemoglobin level was shown to reduce the risk of death by 0.8 times. Nevertheless, the overall mortality rate was 29.6%, in line with the results of the COVIDSurg Collaborative international cohort study and a majority of these originated due to respiratory complications were 75.86%.

The main reason for mortality in SARS-CoV-2-infected patients in the postoperative period was pulmonary complications, accounting for 50% of the cases *(13)*. This rate of mortality was considerably high when compared to the pre-pandemic healthcare environment. The POPULAR study conducted in 211 institutions from 28 European Union Countries reported that the mortality rate due to pulmonary complications was 8% *(15)*. ARDS has been observed 50% among postoperative pulmonary complications mortality in COVIDSurg Collaborative international cohort study. The 30-day mortality was deferred in subjects with postoperative pulmonary complications *(38% versus 23.8%) (13)*. This rate has been 20% higher than the results surgical Outcomes Study published results-by Biccard et al. *(2018)* in pre-pandemic conditions *(16)*. This study found that 88% of the deceased cases had respiratory *(px-thorax tube)* problems as postoperative complications. Respiratory distress was also a statistically significant predictive factor affecting mortality. This rate was quite similar to the one of COVIDSurg Collaborative, which was 83% *(13)*.

Infection stands as another game-changing player in the streamline of SARS-CoV-2 positive individuals. COVIDSurg Collaborative international cohort study declared that 71.5% of the infections were proclaimed in the postoperative period. Only one-third of them were detected in the preoperative period as GGO in the CT scan *(13)*. In this study, the mortality rate *(84% – p<0.001)* due to infection was statistically significant for mortality. One can derive these data that perioperative SARS-CoV-2 infection is associated with high mortality. The remaining postoperative complications could be elaborated as infection, acute renal injury and acute thrombotic disease.

Nashon et al. (2020) have reviewed for COVID-19 patients asymptomatic or not tested in the preoperative period. The postoperative mortality rate of these studies has been pooled as 27.5% *(17)*. Lei et al. *(2020)* published that the postoperative death rate was 20.5%, ICU admission was 44.1%, and pulmonary complications were 100% among 34 asymptomatic COVID-19 patients who underwent elective surgery. All patients who were inadvertently scheduled for elective surgery in the incubation period of COVID-19 developed COVID-19 pneumonia with abnormal findings on computed tomography scans in the early postoperative period. *(18)*. In an Italian study by Luca et al. (2020), they reported that 32% of the cases had ICU admissions, 33.8% had postoperative pulmonary complications and 22% had acute respiratory distress in a retrospective analysis. The emergency and oncological surgeries postoperative mortality rate were 14.7%, considerably low compared to prospective data *(19)*. In our study, six patients had PCR positivity after the surgery. Four of these patients were admitted to ICU and dead in ICU. This study observed that the mortality of the patients who underwent surgery during the incubation period was high. However, this situation cannot be directly attributed to this because the comorbidities and surgeries of these patients are different.

The SARS-CoV-2 infection is associated with high rate of mortality over the globe. A majority of the severely ill patients die due to this disease. At this stage, the surgery decision should be taken by a multidisciplinary approach and conducted in benign, not postponable diseases or emergency operations. It was recommended to follow a conservative approach as delaying surgery in most cases. Thus the pandemic was an extraordinary situation and had caused multifactorial obstacles for the health institutions via limiting resources. The medical team should evaluate each case separately and conduct the surgery if the medical urgency is life-threatening such as perforation, trauma, intestinal ischemia etc.

Canceling or delaying oncological surgeries worsens patients' condition should remain throughout the pandemic. When the rate of COVID-19 infection decreases, the resumption of elective surgeries should be considered if hospital resources, service and ICU beds are sufficient to meet the demand.

The main limitation of the study could be attributed to its retrospective nature. Unfortunately, a randomized prospective study could not be conducted due to the emerging health conditions and increased patient burden with scarce sources.

5. CONCLUSION

It may be appropriate to postpone elective surgical procedures in patients with COVID19 symptoms or PCR positivity. It should be kept in mind that complications that may develop in the postoperative period and the symptoms of COVID19 may worsen, especially in patients who require urgent interventions. The urgency of the operation should be reconsidered, especially in those with the additional disease. As no one knows the exact date of COVID-19 infection eradication, this subject will require further studies and the establishment of guidelines.

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Competing interests

The authors declare that they have no competing interests.

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Clinical and Experimental Health Sciences

Foot Muscle Strength, Muscle Shortness, Balance, and Shoe Preferences in Different Foot Postures

Banu Unver[1], Hanifegul Taskiran[2], Ahmet Cuneyt Akgol[3]

[1] Lokman Hekim University, Faculty of Health Science, Department of Physiotherapy and Rehabilitation, Ankara, Türkiye.
[2] Istanbul Aydin University, Faculty of Health Science, Department of Physiotherapy and Rehabilitation, Aydin, Türkiye.
[3] Istanbul Okan University, Faculty of Health Science, Department of Physiotherapy and Rehabilitation, Istanbul, Türkiye.

Correspondence Author: Banu Unver
E-mail: banuukarahan@yahoo.com
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ABSTRACT

Objective: The aim of this study was to investigate the foot muscle strength, muscle shortness, tibialis posterior endurance, balance, and the shoe preference differences between the neutral and pronated foot posture.

Methods: Forty-nine participants consisting of 23 women and 26 men, and age of between 18 and 45 years were participated in the study. Foot posture, medial longitudinal arch height, height, gastrocnemius and hamstring muscle shortness, foot and ankle muscle strength, tibialis posterior muscle endurance, static balance, and shoe preferences of the participants were evaluated. Subjects were recruited into two groups according to their foot posture evaluated with Foot Posture Index: as those with neutral and pronated foot posture.

Results: Navicular drop, gastrocnemius, and hamstring muscle shortness were significantly higher in participants with pronated foot posture compared to those with neutral foot ($p<0.05$). There were no significant differences in terms of tibialis posterior, tibialis anterior, peroneal, and gastrocnemius muscle strength; tibialis posterior muscle endurance, balance, and shoe preferences between two groups ($p>0.05$).

Conclusion: Flexibility of gastrocnemius and hamstring muscles were reduced, but foot muscle strength, tibialis posterior muscle endurance, and balance remained unaffected in young individuals with excessive foot pronation. Moreover, shoe preferences may not affect the foot posture in young people. Although all age-related biomechanical effects of foot pronation are not well known yet, muscle shortness seems to arise earlier than muscle weakness and reduced balance in pronated foot posture.

Keywords: foot posture, tibialis posterior muscle, balance, shoe preference

1. INTRODUCTION

The foot provides ground contact, shock absorption, adaptation to different grounds and generates momentum for push-off in weight-bearing activities (1). Foot problems are very common in the general population. It has been reported that the rate of foot problems is ranged from 13% to 36% in adult population (2). Excessive foot pronation is characterized by flattening of the medial longitudinal arch (MLA), valgus of the rearfoot and abduction of the forefoot (3). Excessive foot pronation causes impaired load distribution in the gait, increased stresses in the foot and ankle joints, shearing forces in the knee joint, and internal rotation in the hip joint. Hallux valgus, plantar fasciitis, tibialis posterior dysfunction, tarsal tunnel syndrome, and patellofemoral pain syndrome are known to be associated with excessive foot pronation (3-6).

During standing and walking, active stabilization of MLA is provided by the extrinsic and intrinsic muscles of the foot. Excessive foot pronation is commonly associated with dysfunction of the tibialis posterior muscle, which plays a primary role in the dynamic stabilization of the rearfoot and MLA. The tibialis posterior attempts to compensate for the supportive task of the elongated plantar connective tissues in excessive foot pronation. Muscle fatigue and overuse injuries may occur as a result (4). Gastrocnemius muscle shortness is also known to be associated with foot pronation as a factor that increases rearfoot valgus (6).

Lower MLA was demonstrated to be associated with poor postural control especially in unipedal standing (7). Lower MLA with tibialis posterior muscle weakness or dysfunction leads to reduced structural stability of the foot and finally impaired postural stability (8, 9). Besides, tibialis posterior muscle fatigue alters the dynamic foot function, which may reduce postural stability (10).

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An appropriate shoe protects and supports the foot, improves the function, and controls the foot deformities and musculoskeletal injuries. Shoes with poor characteristics are associated with foot, knee and low back pain, foot deformities, falls, ulcerations and amputations in various age and disease groups or in healthy adults. It is important to reveal the risk factors caused by different shoes, to prevent or treat possible shoe related pathologies (11).

There are several studies investigating the relationship between excessive foot pronation and the biomechanical properties of the lower limb and its effect on balance (7, 8, 12, 13). Unver et al. (13) indicated that pronated foot may lead to increased pelvic inclination and low back pain. Kabak et al. (7) revealed that athletes with bilateral pes planus had reduced postural stability in one leg standing with the dominant side. Besides, there is some evidence about the effects of shoe preferences on foot-related pathologies (14, 15). Buldt and Menz (16) indicated that ill-fitted shoes were associated with foot pain and foot disorders. However, the literature is limited about the relationship between shoe preference and foot posture and the effect of tibialis posterior fatigue on balance in different foot postures. Additionally, there are limited studies investigating the relationship between shoe preference and foot posture and the effect of tibialis posterior fatigue on balance in different foot postures. The purpose of this study was to investigate the foot muscle strength, lower extremity muscle shortness, tibialis posterior endurance, balance, and the shoe preference differences between the healthy young individuals with neutral and pronated foot posture.

2. METHODS

This was a cross-sectional study conducted between April 2016 and September 2017 at İstanbul Aydın University University. Forty-nine participants consisting of 23 women and 26 men were recruited in the study. Asymptomatic sedentary individuals who do not participate in a regular sporting activity or exercise program and aged between 18 and 45 years included. The exclusion criteria were systemic, neurologic, rheumatologic, or orthopedic diseases, and history of surgery on the lower extremities. Before the study was conducted, the required permission was obtained from the Clinical Research Ethics Committee of İstanbul Aydın University (Date:17.02.2016, Number: 2016-06). All participants were informed about the study, and an informed consent form was signed by each participant.

Demographic data of the participants were recorded, and the dominant foot of each participant was determined. Foot posture, MLA height, gastrocnemius and hamstring muscle shortness, foot and ankle muscle strength, tibialis posterior muscle endurance, static balance and footwear preferences of the participants were evaluated once. According to the Foot Posture Index (FPI) results, the participants were divided into two groups: those with a neutral and pronated foot posture.

2.1. Foot Posture

FPI was used to evaluate the foot posture. This assessment was conducted while the participants were in a relaxed standing position. Talar head palpation, curves above and below the lateral malleolus, inversion/eversion of the calcaneus, talonavicular joint prominence, MLA height, and abduction/ adduction of the forefoot were evaluated. Each of these criteria got scores between – 2 and +2, and total score was obtained. A score between 0 and 5 indicated neutral, above 6 indicated pronated and negative scores indicated supinated foot (17). FPI was found to be a valid and reliable tool to evaluate static foot posture (18).

2.2. MLA Height

MLA height was evaluated by navicular drop (ND) test. The difference of the navicular height in between the weight-bearing standing and the non-weight-bearing sitting positions in mm was taken as the navicular drop (19).

2.3. Muscle Shortness

The shortness of the gastrocnemius and hamstring muscles were measured using a universal goniometer. Measurements were made while the participant was in the supine position. For the hamstring muscle shortness measurement; the assessor flexed passively one hip as much as possible keeping the knee extended, while the other lower extremity was on the table with the hip in a neutral position and the knee extended. The hip flexion angle was measured with the goniometer at the last degree of flexion that the hip joint could reach. The axis of the goniometer was aligned with the hip joint, the stationary arm was parallel to the trunk, and the moveable arm was parallel to the longitudinal axis of the femur. The angular value measured in the goniometer was subtracted from 90 degrees and recorded as the amount of shortness. This method has been shown to be a reliable method for measuring hamstring muscle shortness. For the gastrocnemius muscle shortness measurement; the assessor dorsiflexed passively one ankle as much as possible keeping the knee extended while the participant was in the supine position. The ankle dorsiflexion angle was measured with the goniometer at the last degree of dorsiflexion that the ankle joint could reach. The axis of the goniometer was aligned with the lateral malleolus, the stationary arm was parallel to the lateral midline of the fibula, and the moveable arm was parallel to the lateral aspect of the calcaneus. The angular value obtained by subtracting 90 degrees from the value measured with the goniometer was recorded as the amount of shortness (20, 21).

2.4. Muscle Strength

The isometric strength of tibialis anterior, tibialis posterior, gastrocnemius and peroneal muscles were measured using “Nicholas Manual Muscle Tester” (model 01160, The Lafayette Instrument Company, Lafayette, Indiana).

Measurements were made in a sitting position with the back support (with hips flexed and knees flexed 10 degrees with rigid roll under the popliteal fold). The feet were positioned outside the bed. Pad placement of the dynamometer was as follows: for dorsiflexors at the level of the metatarsal heads in the dorsum of the feet, for plantar flexors at the base of the metatarsal heads on the foot sole, for invertors just below the first metatarsal head in the medial line of the forefoot, and for evertors just below the fifth metatarsal head in the lateral line of the forefoot. The participants were asked to maintain the maximum contraction for 3 to 5 seconds, while the assessor fixed the dynamometer. "Make test" was used for the assessments, to avoid the participants overpowering the assessor. Measurements were performed 3 times for each muscle group, and the highest value was recorded (22, 23, 24).

2.5. Tibialis Posterior Muscle Endurance

The single-limb heel rise was used to evaluate tibialis posterior muscle endurance. The participant was in a standing position on a single leg, with a completely extended knee, while the contralateral leg was lifted maintaining the knee flexed. Each participant was asked to repeatedly lift the heel as high as possible until exhaustion. The fingertips of the participants were lightly placed on the assessor's hands for balance support during the test. The test was completed when the participant fails to reach a consistent height of each heel rise or makes compensation. Maximum number of heel rise repetitions and the spent time was recorded for each participant (25). Maximum repetitions test of singe-limb heel rise was found to have acceptable test-retest reliability (26).

2.6. Static Balance

Static balance of the participants was evaluated by Balance Error Scoring System (BESS). Evaluations were performed on a medium-hard foam pad. The subjects were evaluated in the single leg (on each left and right foot) and tandem position respectively, with the hands-on the waist, eyes closed. In the original BESS, evaluations are performed in three different positions: single-leg stance, double-leg stance, and tandem stance. However, since this study was conducted with healthy young people, there would be no balance impairment in double-leg stance position, so that position was not used in the current study. The observer monitored the subjects for 20 seconds at each position and recorded the number of standard balance errors determined in the test. The balance errors are as follows: lifting hands off of the iliac crest, eye opening, stepping, stumbling, or falling, moving the hip into more than 30 degrees of flexion or abduction, lifting the forefoot or heel, and remaining out of the testing position for more than 5 seconds. The total number of errors in each position was recorded as the balance error score (27). Participants were evaluated twice with this system; initially once, and once after tibialis posterior muscle fatigue, which was created by single-limb heel rise test (25).

2.7. Shoe Preferences

Participants were asked to choose 5 shoe models which they frequently used in the last 5 years from a catalog with 152 shoe models with different features and models. Each shoe selected was scored according to the adequacy of its sole, heel, and upper sections, and its stabilization and shock absorption features. These parameters used in scoring were established based on previous literature that developed shoe evaluation tools (11, 28). Shoes that provide the mentioned features exactly were scored 2, those with insufficiency in one or two of these features were scored 1, those with insufficiency in three or more were scored 0 points. The total score of the 5 selected shoes was recorded. This shoe catalog and scoring system were developed by the authors of the current study.

2.8. Statistical Analysis

Data were evaluated using the Statistical Package for Social Science 18 (SPSS Inc, Chicago, IL) program for Windows. The significance level was set to $p<0.05$. Normality tests (visual and analytical) were conducted. Since the continuous variables were not normally distributed, Mann–Whitney U test was used to compare age, body mass index, ND, muscle shortness, muscle strength, muscle endurance, balance, and shoe preferences data between the two groups with the neutral and pronated foot. Chi-square test was used to compare the sex ratio between two groups.

3. RESULTS

Forty-nine participants were included in the study. According to the FPI results, the participants were divided into two groups; 33 had neutral, 16 had pronated foot posture. Demographic data and FPI results of the participants were presented in Table 1. There were no significant differences between the demographic features of the two groups ($p>0.05$).

***Table 1**. Demographic data and FPI scores of the participants*

	NFPG (n=33)	PFPG (n=16)	p
Age (mean ± SD)	21.55± 2.57	21.88±3.46	0.96
BMI (mean ± SD)	22.14± 2.30	23.36±4.41	0.89
Gender (F/M)	17/16	6/10	0.35
FPI (mean ± SD)			
left	2.42±1.56	7.87±1.92	**<0.001**
right	2.06±1.76	7.31±1.99	**<0.001**

NFPG, neutral foot posture group; PFPG, pronated foot posture group; BMI, body mass index; F, female; M, male; FPI, foot posture index; SD, standard deviation.

Navicular drop, gastrocnemius, and hamstring muscle shortness were significantly higher in participants with pronated foot posture compared to those with neutral foot ($p<0.05$). There were no significant differences in terms of tibialis posterior, tibialis anterior, peroneal, and

gastrocnemius muscle strength between two groups (p>0.05) (Table 2).

Table 2. *Comparison of ND, muscle shortness and muscle strength outcomes between neutral and pronated foot posture groups*

	NFPG (n=33) (mean ± SD)	PFPG (n=16) (mean ± SD)	p
ND (mm)			
left	6.33±2.21	10.50±3.09	**<0.001**
right	6.75±2.09	10.93±3.23	**<0.001**
Tibialis posterior muscle strength (N)	25.54±5.11	27.02±7.62	0.67
left	26.55±5.94	31.05±8.39	0.09
right			
Tibialis anterior muscle strength (N)			
left	27.26±6.04	30.22±8.90	0.26
right	27.98±6.12	30.28±8.96	0.63
Peroneal muscle strength (N)			
left	25.09±5.87	27.18±9.15	0.65
right	25.38±5.85	27.44±8.01	0.38
Gastrocnemius muscle strength (N)			
left	33.29±7.38	33.22±9.50	0.71
right	33.29±8.23	34.18±10.76	0.68
Gastrocnemius muscle shortness (°)			
left	3.87±14.96	7.62±12.87	**0.037**
right	4.45±14.25	8.18±13.29	**0.009**
Hamstring muscle shortness (°)			
left	4.75±29.43	15.93±17.02	**0.007**
right	5.66±26.59	11.25±15.23	**0.017**

NFPG, neutral foot posture group; PFPG, pronated foot posture group; SD, standard deviation; ND, navicular drop.

Table 3. *Comparison of single-limb heel rise and BESS scores between neutral and pronated foot posture groups*

	NFPG (n=33) (mean ± SD)	PFPG (n=16) (mean ± SD)	p
Single-limb heel rise score			
Repetition			
left	29.13±10.23	33.56±12.81	0.23
right	29.54±10.35	35.31±16.49	0.17
Time			
left (sec)	27.72±8.67	36.70±17.43	0.06
right (sec)	29.00±12.96	38.81±22.87	0.17
BESS score			
Initial			
left limb	8.42±3.82	9.75±6.07	0.54
right limb	7.72±4.27	8.00±4.01	0.75
tandem	4.00±3.89	5.06±4.94	0.53
BESS score			
After TP fatigue			
left limb	7.78±5.38	11.12±6.98	0.10
right limb	9.24±5.64	9.68±6.40	0.91
tandem	4.45±4.25	4.18±4.24	0.82

NFPG, neutral foot posture group; PFPG, pronated foot posture group, SD, standard deviation; BESS, Balance Error Scoring System; TP, tibialis posterior.

Table 3 presents the single-limb heel rise and BESS outcomes of the groups. There were no significant differences in terms of single-limb heel rise and BESS scores between the participants with neutral and pronated foot posture (p>0.05).

Comparison of five-year shoe preference scores of the groups with neutral and pronated foot posture indicated no significant difference between the groups (p>0.05) (Table 4).

Table 4. *Comparison of five-year shoe preference scores between neutral and pronated foot posture groups*

	NFPG (n=33) (mean ± SD)	PFPG (n=16) (mean ± SD)	p
Shoe preferences score	5.57±1.82	5.53±2.06	0.86

NFPG, neutral foot posture group; PFPG, pronated foot posture group, SD, standard deviation.

4. DISCUSSION

This study was conducted to reveal foot muscle strength, lower extremity muscle shortness, tibialis posterior endurance, balance, and the shoe preferences between the neutral and pronated foot posture. Current results indicated that gastrocnemius and hamstring muscle shortness was higher in pronated foot posture compared to neutral foot posture. Foot muscle strength, tibialis posterior endurance, balance, and shoe preferences were similar in neutral and pronated foot posture.

It has been reported that 25% of individuals with excessive foot pronation have gastrocnemius and soleus shortness which leads to pain and functional limitation (29). Kızılcı et al. (30) demonstrated that gastrocnemius flexibility is less in healthy young men with pes planus than those without. However, Unver et al. (13) revealed that the gastrocnemius, soleus, and hamstring muscle flexibility were similar in both pes planus and neutral foot posture. Nevertheless, the current study revealed that excessive foot pronation was associated with gastrocnemius and hamstring muscle shortness in healthy young individuals. Although it is accepted that there is a strong relationship between pes planus and plantar flexor muscle shortness, it is not clear whether the plantar flexor muscle shortness is the cause or the result of the pes planus (31). Hindfoot valgus associated with pes planus and pronated foot posture is thought to lead to functional shortening of the gastrocnemius-soleus complex (32). On the other hand, reduced dorsiflexion during the stance phase of the gait due to plantar flexor muscle shortness may compensate with hindfoot pronation leading to pronated foot posture (31).

Excessive foot pronation is known to be associated with the weakness of tibialis posterior and intrinsic foot muscles (4,12). In excessive foot pronation, plantar intrinsic muscles and tibialis posterior activation are more needed to support MLA and stabilize the foot in the weight-bearing activities, resulting in muscle fatigue and insufficiency (4). In the current study, the reason for similar foot muscle strength and tibialis posterior muscle endurance in neutral and pronated foot posture groups may be because the participants were young and did not have high activity levels (like athletes). Snook (33) found a decreased isokinetic concentric plantarflexion

strength in individuals with pronated foot posture, compared to normal. In that study mean ND of the participants with pronated foot posture was 15.1 mm. On the other hand, the pronated foot posture group had ND mean of 10.9 mm in the current study. This indicates that the participants in our study had mild ND. The fact that the participants with the pronated foot posture in our study had less ND may also explain the unaffected muscle strength and endurance.

Pronated foot posture with lower MLA and tibialis posterior dysfunction lead to reduced postural stabilization due to impaired foot stability (8,10). However, according to current results, static balance scores were similar in neutral and pronated foot posture groups at baseline and after tibialis posterior fatigue was created. Considering that one of the most important stabilizers of the foot is the tibialis posterior muscle; this result may be related to the unaffected tibialis posterior muscle strength and endurance of the group with pronated foot posture. Harrison and Littlewood (34) exhibited that static postural stability decreased as the severity of the foot pronation increased. Besides, it has been reported that foot deformities, foot pain, and gait alterations caused by foot pronation can negatively affect postural stability in older individuals (35). Based on the results of our study, it can be suggested that mild pronated foot posture does not affect static balance in young individuals.

The results of the present study revealed that the features of the shoes chosen by the neutral and pronated foot posture groups in the last five years were similar. The shoe properties are known to be related to several foot pathologies (36, 37). It has been revealed that inappropriate shoes lead to forefoot deformities and foot pain in older individuals (38). Foot pain and deformities were not evaluated in the current study, but our results showed that the preference for shoes in healthy young individuals may not affect the foot posture.

This study has some limitations. Although only healthy young individuals were included in our study, studies involving different age groups are needed to investigate the relationship of foot posture with biomechanical properties, balance, and shoe preferences. The sample size was not calculated and a small sample size included in the current study. Besides, the numbers of participants with neutral and pronated feet were not similar. The foot posture of a randomly selected 49 young adults was evaluated instead of starting with two groups with neutral and pronated feet. Since the current shoe evaluation tools were developed to evaluate the present shoe of the subject, we used a method that we developed in order to evaluate the shoe preferences of the participants in the last 5 years. Therefore, the validity and reliability study of the catalog and scoring system we use for this purpose has not been conducted. Finally, more accurate results might be obtained if the balance was evaluated using a more objective method that could detect displacements in the center of mass.

5. CONCLUSION

The current results revealed that flexibility of gastrocnemius and hamstring muscles were reduced, but foot muscle strength, tibialis posterior muscle endurance, and balance remained unaffected in young individuals with excessive foot pronation. Besides, shoe preferences may not affect the foot posture in young people. Although there is a need for follow-up studies that investigate how muscle strength, shortness, and balance can be affected during the advancing ages in excessive foot pronation; it can be reported that muscle shortness might begin earlier than muscle weakness and reduced balance in pronated foot posture.

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Clinical and Experimental Health Sciences

Effects of Heat and Massage Applications to the Lumbosacral Area on Duration of Delivery and Perception of Labor Pain: A Randomized Controlled Experimental Trial

Aysenur Durmus[1], Gulsen Eryilmaz[2]

[1] Kastamonu University, Faculty of Health Sciences,Department of Midwifery, Kastamonu, Türkiye.
[2] Atatürk University, Faculty of Nursing, Department of Obstetric and Gynecology Nursing, Erzurum, Türkiye.

Correspondence Author: Aysenur Durmus
E-mail: aysenurdurmus@kastamonu.edu.tr
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ABSTRACT

Objective: This study was carried out to determine the effects of massage and hot-pack applications in the first stage of labor on perceptions of labor pain and duration of delivery.

Methods: This randomized controlled experimental trial was conducted in an obstetrics and pediatrics hospital. The research sample comprised 120 pregnant women, 40 of whom were in the massage group, 40 of whom were in the hot-pack application group, and 40 of whom were in the control group. Patient identification forms, labor process monitoring forms, and a visual analogue scale (VAS) were used to collect data.

Results: It was determined that the massage and hot-pack applications shortened the durations of the active and transition phases of labor. The massage and hot-pack applications also reduced perceptions of pain in the active and transition phases. Mean VAS scores of the massage and hot-pack application groups were lower than those of the control group ($p<0.001$).

Conclusion: Massage and hot-pack applications decreased the duration of the active and transition phases of labor and the perceptions of pain in those phases. These results demonstrate that massage and hot-pack applications are effective nursing interventions for the treatment of labor pain and can be used confidently.

Keywords: Labor pain, pain perception, pregnant women, massage, heat application

1. INTRODUCTION

The act of delivery is one of the most important life experiences for expectant mothers, and it is expected to conclude in a problem-free way. For this reason, it is very important for the health of both the mother and the baby that the process of childbirth be remembered as a healthy and successful experience. One of the aspects of labor that women are most frightened by is pain, as the acute pain of childbirth is one of the most severe types of pain known. However, this pain is also an expected part of a natural process, it takes place in a limited period of time, it involves periods of rest and also entails a preparation period, and the mother willingly endures it for her baby. These factors separate it from other types of pain (1,2).

Nonpharmacological methods that provide sufficient pain control have various advantages when compared to pharmacological methods. Nonpharmacological methods are harmless for both the mother and the fetus and do not slow down the process of labor, and there are no risks of adverse effects or allergies (2,3). Reducing perceptions of pain during childbirth is one of the objectives of basic nursing practices. The pain perceptions of women in labor can be reduced with topical therapy methods (massage or hot-pack applications), which are among the nonpharmacological intervention methods. The effects of massage and heat applications on pain in childbirth have been explained with the gate control theory. Massage and heat applications are thought to reduce perceptions of pain by activating large-diameter fibers transmitting physical sensations, which inhibits or overpowers the small-diameter fibers that carry messages of pain (1,2). Hot-pack or massage applications during childbirth oxygenate the region to which they are applied and remove waste materials from that region as a result of vasodilation of peripheral blood vessels. They also increase the local blood flow, reduce ischemia, and increase the release of endorphins. These applications help to stimulate the sympathetic nervous system, loosen the skeletal muscles, and alleviate skeletal pain (3). Reduced perceptions of labor

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pain with heat applications occur as endorphin levels and blood flow are increased, muscle relaxation is achieved, and the cellular metabolites that stimulate painful impulses are inhibited (3-5).

There are many studies in the literature investigating the effects of nonpharmacological methods on labor pain (6-10). In previous studies, the impact of massage applied to the waist, sacrum, and hip regions on pain in the first stage of labor was investigated (11-14). Other studies have considered the effect on labor pain of applying heat to the sacral region (15-17). In another study, the effect of applying hot water and ice packs to the waist and sacral region on the severity of labor pain was evaluated (18). There are also studies in the literature that delve into the impact of massage alone or massage coupled with acupressure in relieving labor pain and shortening labor time (19,20). However, no study has been found that assesses the influence of heat applications together with massage on the duration of labor and perceptions of labor pain. Therefore, in the current study, the effects of hot-pack and massage applications on the duration of delivery and pain perception were evaluated together.

Objective and Hypotheses

This study was planned to determine the effects of massage and hot-pack applications to the lumbosacral region on perceptions of labor pain and the duration of labor.

H1. Lumbosacral hot-pack application during the active or transition phase of childbirth shortens the durations of these phases.

H2. Lumbosacral massage during the active or transition phases of childbirth shortens the durations of these phases.

H.3. Lumbosacral hot-pack application during the active or transition phases of childbirth reduces the perception of labor pain in these phases.

H.4. Lumbosacral massage during the active or transition phases of childbirth reduces the perception of labor pain in these phases.

2. METHODS

2.1. Participants and Setting

The study was designed as a randomized controlled experimental trial and was conducted in the Maternity Ward and Postpartum Clinic of the Ministry of Health an Obstetrics and Pediatrics Hospital in Turkey. The universe of the study comprised primiparous pregnant women who had applied to the hospital to give birth for the first time. Inclusion criteria were being between the ages of 18 and 35, being primiparous with a full-term pregnancy, having a single fetus in vertex presentation, presenting for a spontaneous vaginal birth, not having a risky pregnancy, not taking any painkillers or receiving any analgesic treatment, and agreeing to participate in the study. A total of 120 pregnant women were included in the study, with 40 women in the control group, 40 in the massage group, and 40 in the hot-pack application group.

2.2. Sample Size and Randomization

The strength of the study was calculated in Power 3.1 to determine the minimum sampling size. As a result of power analysis, it was concluded that at least 38 women should be included in each group for a minimal total of 114 women at the 5% significance level with effect size of 0.81 and power (1-β) of 0.88. Thus, a total of 120 pregnant women were included, with 40 women in each of the three study groups.

Envelopes were numbered consecutively for the total study sample of 120 participants. Thereafter, based on the order of enrollment, participants were assigned to one of the three groups by way of simple randomization method using a computer. The first two participating pregnant women were assigned to the control group, the next one to the massage group, the next two to the hot-pack application group, and so on.

The groups to which the women were thus assigned were written inside the envelopes and the envelopes were sealed. These opaque envelopes, numbered according to the order of enrollment, were given to the women as they arrived for delivery. A researcher opened each woman's envelope to learn that woman's assigned group, and participants underwent applications according to the group to which they were assigned. Upon being assigned to the groups, the participants were blinded and did not know to which group they belonged. They were also unaware of the applications being performed for the other groups. However, by virtue of the nature of the interventions, the researchers could not be blinded. This process of randomization was performed in compliance with the CONSORT 2010 guidelines (Fig. 1).

2.3. Data Collection Tools

The data of the study were collected with patient identification forms, labor process monitoring forms, and a visual analogue scale (VAS).

2.3.1. Patient Information Form

This form included 13 questions related to the sociodemographic and obstetric characteristics of the pregnant women, the course of the pregnancy, prenatal check-ups, and other such information.

2.3.2. Labor Process Monitoring Forms

This form included sections about the time of onset of labor, cervical dilatation and effacement findings, vital signs of the mother, fetal heart rate, the starting time of experimental applications for relieving labor pain, the durations of the stages of labor, time of delivery, and time of separation of the placenta. A separate form was used for each participant.

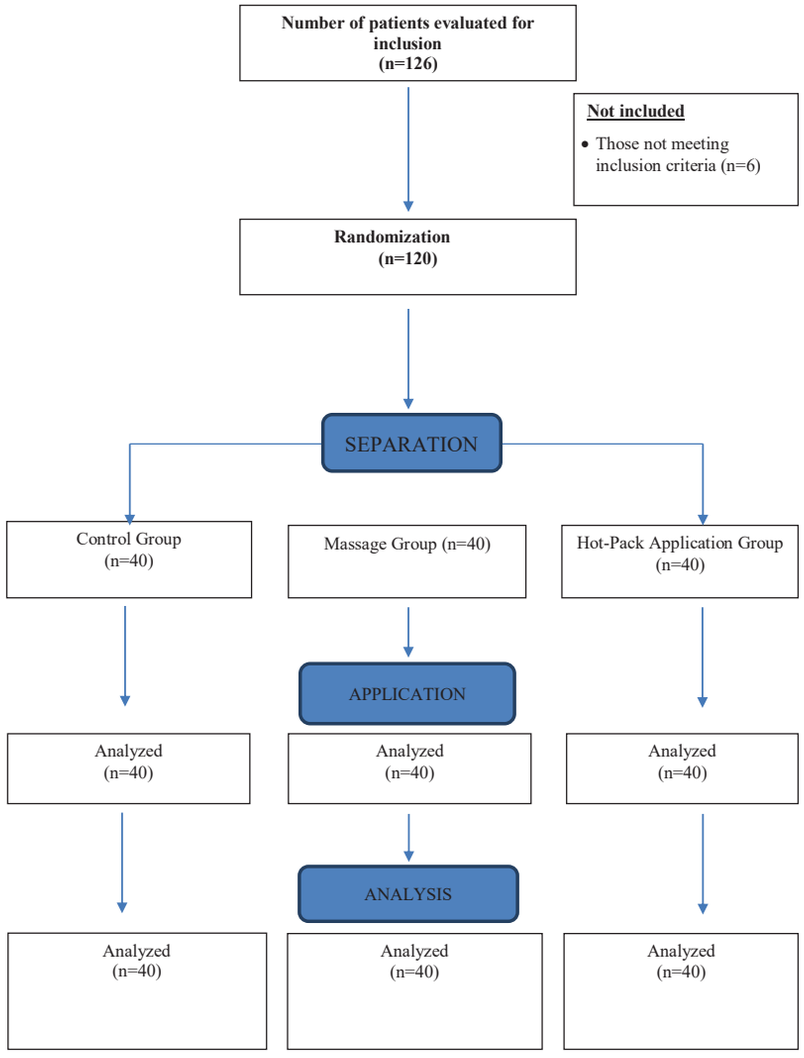

Figure 1. *CONSORT diagram*

2.3.3. Visual Analogue Scale (VAS)

A VAS is a one-dimensional measurement tool most commonly used to determine pain severity. It gives fast results, evaluates pain in a subjective way, and is sensitive and reliable in measuring pain severity. This approach was first developed by Bond and Pilowsky in 1966, and its sensitivity and validity in Turkey were determined by Aslan in 1998 (21). The scale consists of a 10-cm line upon which the severity of pain is marked, with "No Pain" at one end and "Unbearable Pain" at the other end. A VAS thus resembles a ruler of 0-10 cm and can be used vertically or horizontally. The distance between the spot indicated by the participants and the "No Pain" point was measured in centimeters and recorded. Since it is stated in the literature that vertical applications of the VAS are better understood by patients, a vertical form was used in the present study. A higher score indicates more pain.

2.4. Collection of Data for Experimental Applications

In addition to the routine clinical procedures, the pregnant women in the experimental groups were administered either hot-pack or massage applications by a researcher. In the health institution where the study was conducted, the same dose of oxytocin is routinely administered to primiparous pregnant women in the course of delivery. To evaluate the health of the mother and the fetus, the number of fetal heart beats was recorded at 15-minute intervals and the vital signs of the mother were evaluated at 1-hour intervals. Participants in all groups were asked to mark their pain at the most appropriate point on the VAS before any treatment was administered in the latent phase (VAS I), and that response was measured and recorded on the labor process monitoring form.

2.4.1. Hot-Pack Application Procedure

Materials used for hot-pack application

Hot packs: The hot packs contained special compounds such as silicon dioxide and silicate gel that absorb heat and retain the heat in the hot pack for 30 minutes. A total of 10 hot packs of 33 × 26 cm were used in the study. Hot packs were heated in a boiler with temperature set to 70-80 °C and they were kept in the boiler when not in use.

Hot-pack boiler: The stainless-steel electrical boiler had double walls, a thermostat, and automatic heat control with a capacity of 10 hot packs.

Towels: Towels of 50 × 90 cm in size were used to wrap the hot packs during hot-pack applications and a separate towel was used for each patient. Hot packs were applied after being wrapped in a towel. The temperature of the hot packs as felt through the towel was 40-42 °C.

The hot packs were applied to the area of the lumbosacral vertebrae while the women were sitting or lying on their left sides. Hot-pack application was begun in the active phase of labor (cervical dilatation of 4-7 cm) between contractions and continued uninterruptedly for 20 minutes. There was an average of 5 contractions in the active phase during this period. After the hot-pack application was concluded and at least one more contraction was experienced, pain severity was measured for the second time between contractions time (VAS II). In the transition phase, where labor progresses and cervical dilatation reaches 8-10 cm, the hot-pack application was begun again between contractions and continued to be applied for 20 minutes without interruption. There was an average of 10 contractions during this time. Pain severity was measured for the third time (VAS III) between contractions after the hot-pack application was concluded in the transition phase and at least one further contraction was experienced.

2.4.2. Massage Application Procedure

Materials used for massage application

Liquid Vaseline: Liquid Vaseline that did not contain any active substances was used in the massage application. With the patient sitting or lying on her left side, liquid Vaseline was applied to the area where the lumbosacral vertebrae were located (lower back region) and the right and left lateral parts of the midline (4-5 cm) were massaged for 30 minutes with effleurage and friction techniques. The massage was first begun between contractions in the course of the active phase of labor (cervical dilatation of 4-7 cm). After 30 minutes of massage and at least one contraction, pain severity was measured for the second time (VAS II) between contractions. In the transition phase, where labor progresses and cervical dilatation reaches 8-10 cm, massage was begun again between contractions and continued to be applied for 30 minutes without interruption. After 30 minutes and at least one contraction, pain severity was measured for the third time (VAS III) between contractions.

2.4.3. Procedure for the Control Group

In the control group, only the routine clinical procedures were applied. Pain levels of the participants were measured between contractions in the active (VAS II) and transition (VAS III) phases of labor. Furthermore, the pain levels of all mothers included in the study were measured postpartum in the service ward without any painkillers within the first 2 hours of leaving the maternity ward (VAS IV).

2.5. Statistical Analyses

The data obtained in this study were evaluated using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The results were evaluated in the 95% confidence range and at a statistical significance level of 0.05%. Percentages, means, standard deviations, and chi-square tests were used for comparisons between groups. Comparisons of cervical dilatation, effacement, fetal heart rate, vital signs of mothers, duration of labor, and VAS pain scores were performed by one-way analysis of variance. When a difference was determined between the groups, the post hoc least significant difference (LSD) test was used to determine from which group(s) the difference originated.

2.6. Ethical Considerations

Before the study was begun, the approval of the Ethics Committee of the Atatürk University Institute of Health Sciences was obtained (date: 24.07.2012, number: 2012.3.1/21). Subsequently, written permission was obtained from the a Provincial Health Directorate, where the hospital in which the study was conducted was located. Written consent forms were signed by all pregnant women who met the inclusion criteria of the study and agreed to participate. The purpose, duration, and procedures to be applied were explained to all women who agreed to participate in the study, and they were informed that they could leave the study at any time they wanted and that the ethical principles of consent, volunteerism, and respect for life would be observed.

3. RESULTS

The sociodemographic and obstetric characteristics of the pregnant women in the hot-pack application group, massage application group, and control group were statistically similar (Table 1). Vital signs (systolic and diastolic blood pressure, pulse rate, body temperature, and respiratory rate) of the pregnant women in the active and transition phases of labor were also statistically similar among the three groups ($p>0.05$), as was fetal heart rate ($F=0.108$, $p>0.05$ and $F=1.321$, $p>0.05$, respectively). In addition, hot-pack application and massage during labor were not found to affect cervical dilatation or effacement. Cervical dilatation and effacement rates in the active and transition phases of labor were similar among the three groups ($F=0.096$, $p>0.05$; $F=1.626$, $p>0.05$ and $F=2.583$, $p>0.05$; $F=1.321$, $p>0.05$, respectively).

For the duration of the latent phase of labor of the women in all three groups, no significant difference was observed. However, the average durations of the active and transition phases of labor in the hot-pack and massage application groups were shorter than those of the control group. While a significant difference was found between the groups for the duration of the active phase of labor (F=5.571, p<0.005), the difference observed for the transition phase was not significant. As a consequence of the post hoc LSD test, it was determined that the difference in the durations of the active phase of labor was due to the control group. While the durations of the second and third stages of labor were shorter in the hot-pack and massage application groups, these differences were not significant (Table 2).

The mean pain scores measured in the latent phase of labor (VAS I) were similar among the three groups with no statistical significance (p>0.05). The mean pain scores in the active phase of labor (VAS II) were lower in the hot-pack and massage groups than the control group with statistical significance (p<0.001). In the transition phase of labor (VAS III), mean pain scores were lower in the hot-pack and massage groups than in the control group, and this difference was statistically significant (p<0.001). The pain levels measured 2 hours after leaving the maternity ward (VAS IV) were similar among the three groups (p>0.05) (Table 3).

Table 1. *Sociodemographic and obstetric characteristics of participants*

	Hot-pack application group		Massage application group		Control group		Test value	p
Characteristics	Mean±SD		Mean±SD		Mean±SD			
Age	23.95±3.83		23.45±3.82		23.45±3.06			
Prenatal examinations	13.00±4.86		11.73±4.32		11.40±4.68		F=1.336	0.267
	n	**%**	**n**	**%**	**n**	**%**		
Educational level								
Primary school	23	57.5	26	65.0	21	52.5	χ^2=5.417	0.247
High school	12	30.0	9	22.5	15	37.5		
University	5	12.5	5	12.5	4	10.0		
Employment status								
Unemployed	33	82.5	38	95	38	95	χ^2=5.004	0.082
Employed	7	17.5	2	5.0	2	5.0		
Abortion history								
No previous abortion	34	85.0	37	92.5	37	92.5	χ^2=1.667	0.435
Previous abortion	6	15.0	3	7.5	3	7.5		
Knowledge about delivery obtained in prenatal appointments								
No	35	87.5	37	92.5	32	80.0	χ^2=2.740	0.254
Yes	5	12.5	3	7.5	8	20.0		
From whom that knowledge was obtained								
Nurse/midwife	0	-	0	-	4	10.0	χ^2=5.333	0.069
Doctor	5	12.5	3	7.5	4	10.0		

Table 2. *Comparison of the mean delivery durations of the participants*

	Patient groups					
Durations of first phase of labor (min)	**Massage application group (n=40)**	**Control group (n=40)**	**Hot-pack application group (n=40)**	**Test p**		
	Mean±SD	**Mean±SD**	**Mean±SD**	**F**	**p**	**Difference**
Latent phase duration	235.25±149.20	196.25±131.34	199.88±145.40	0.919	0.402	–
Active phase duration	209.63±74.55	223.88±79.44	173.38±52.29	5.571	0.005	1, 3 < 2
Transition phase duration	72.28±41.68	83.75±56.23	66.75±42.68	1.343	0.265	–
Duration of the second phase of labor (min)	52.38±23.78	58.13±33.98	51.75±27.26	0.602	0.550	–
Duration of the third phase of labor (min)	8.75±3.88	10.00±4.08	8.63±3.75	1.514	0.224	–
Total labor duration	578.28±198.69	572.00±196.92	500.38±160.30	2.162	0.120	–

Table 3. *Comparison of participants' mean VAS scores during labor*

	Patient groups					
Mean VAS Scores in the 1st Phase Delivery (min)	**Massage application group (n=40)**	**Control group (n=40)**	**Hot-pack application group (n=40)**			
	Mean±SD	**Mean±SD**	**Mean±SD**	**F**	**p**	**Difference**
VAS I (pain level measured in latent phase)	2.70±1.83	3.63±2.10	2.85±1.94	0.570	0.081	-
VAS II (pain level measured in active phase)	6.43±1.47	8.20±1.28	4.95±1.63	4.916	0.000	1 > 3 2 > 1, 3
VAS III (pain level measured in transition phase)	8.63±1.84	9.75±1.59	7.60±1.06	6.406	0.000	1 > 3 2 > 1, 3
VAS IV (pain level measured 2 hours after leaving maternity ward)	1.05±0.50	1.13±0.69	0.73±0.55	5.257	0.007	
Total mean pain score	18.80±2.73	22.70±3.73	16.13±4.07	37.755	0.000	1 > 3 2 >1, 3

4. DISCUSSION

The acute pain of labor is one of the most severe known types of pain (1,2). Pain in the first stage of labor is mostly characteristic of visceral pain, being felt in the lower region of the abdominal wall, the lumbar region, and the sacrum (22,23). Management of labor pain is one of the primary targets of obstetric nursing care and it may be provided by pharmacological and nonpharmacological methods (24,25). In this study, hot-pack or massage applications, as examples of nonpharmacological methods, were applied to the lumbosacral region, where pain is felt most in the first stage of labor. Throughout those applications, the vital signs (systolic and diastolic blood pressure, pulse rate, body temperature, respiratory rate) of the mothers and fetal heart rates were similar among the three groups (p>0.05). In studies conducted in other countries, it has been determined that hot-pack application and massage have no effects on pregnant women's systolic and diastolic blood pressure, pulse rate, or body temperature or on fetal heart rate (8,13,17,26,27). The fact that the vital signs of the women and the heart rates of the fetuses were not affected by the applications in this study is thus consistent with the findings of previous studies. Furthermore, in this study, the duration of cervical dilatation and effacement in the active and transition phases of labor did not differ among the groups (p>0.05). In other words, massage and hot-pack applications during labor did not affect the time to completion of cervical dilatation or effacement in pregnant women. Gönenç and Terzioğlu (19) and Kaçar and Özcan (28) reported that massage applied to the sacral region in the first stage of labor did not impact the mean duration of cervical dilatation, consistent with the findings of the present study. In this study, no applications were performed in the latent phase of labor and no differences were found in the durations of the latent phase among the groups (p>0.05).

It is recommended in the literature that hot-pack applications be carried out for a period of 20-30 minutes to ensure the therapeutic effect (2,29). In this study, the hot-pack application was continued uninterruptedly for 20 minutes and massage was continued for 30 minutes. The duration of the active phase of labor was found to be significantly shorter in the hot-pack and massage groups than in the control group (p<0.005). The durations of the transition phase of labor were also shorter in the hot-pack and massage groups than in the control group, although the difference was not statistically significant. Thus, in this study, it was found that hot-pack and massage applications both shortened the durations of the active and transition phases of the first stage of labor. This finding confirmed the first and second hypotheses of

the research, that massage and heat applications to the lumbosacral region during labor shorten the durations of the active and transition phases. In previous studies investigating the effects of hot-pack application on the duration of labor, it was reported that the application of heat to the sacral region shortened the latent, active, and transition phases of labor (4,30). The finding of shortened active and transition phases of labor with massage and heat applications in the present study corresponds to the findings of previous research (6,9,20). There are studies in the literature indicating that massaging the lower back region shortens the duration of the phases of the first stage of labor (6,9,20). Similar to the literature reviewed here, it was found in the current study that massage shortened the active and transition phases of labor. However, Akköz Çevik and Karaduman reported that, contrary to the findings of this study, massage applied to the sacral region during labor did not affect the duration of the phases of the first stage of labor (13). The reason for this difference is likely the type of massage performed. In the study conducted by Akköz Çevik and Karaduman, massage was administered with 15 minutes of effleurage and 15 minutes of vibration during labor (13). In the present study, massage was performed with uninterrupted effleurage and friction techniques for 30 minutes. The fact that the active and transition phases of labor were shorter in the hot-pack and massage groups is congruent with the findings of most other previous studies.

In the current study, although the durations of the second and third stages of labor in the hot-pack and massage groups were shorter than those in the control group, the differences were not significant ($p>0.05$). In the literature, there are studies showing that applications of heat and massage both shorten and do not shorten the duration of the stages of labor. Similar to our findings, Bolbol-Haghighi et al. reported that massage applied to the sacral region shortened the duration of the second stage of labor (20). Behmanesh et al., on the other hand, reported that heat application shortened the duration of the third stage of labor but did not shorten the duration of the second stage (4). In the study of Behmanesh et al., the fact that the duration of the second stage of labor was not shortened may have been due to the fact that the heat application was applied to the perineum during this stage. Contrary to the findings of the present study, Kaur et al. concluded that the application of heat to the lumbosacral region did not affect the duration of the three stages of labor (17). Similarly, Karami et al. (2007) reported that massage applied to the sacral region did not affect the duration of the second stage of labor (6).

In parallel with the progression of labor, the severity of labor pain gradually increases (3). As less pain is perceived in the latent phase of labor than in other phases and this early pain can be tolerated by most pregnant women, no application was performed in the latent phase of labor in the current study. In the active and transition phases of labor, hot-pack and massage applications were found to be effective in reducing labor pain. The perceived severity of the pain was found to be lower among the women who underwent hot-pack application compared to the massage and control groups. The perceived pain severity in the massage group was also lower than that in the control group. This study thus revealed that both hot-pack and massage applications in the first stage of labor are effective in relieving labor pain, but hot-pack application is more effective. This finding confirmed the third and fourth hypotheses of the study. Previous studies have also reported that applying heat to the lumbosacral region during the active and transition phases of labor is effective in reducing labor pain. Taavoni et al. stated that applying heat to the sacrum and perineum during the active phase of labor alleviated labor pain (15). Kaur et al. showed that applying heat to the lumbosacral region in the first stage of labor was effective in reducing labor pain (17). Similarly, Ahmad-Shirvani and Ganji (27) and Yazdkhasti et al. (30) found that applying heat in the first stage of labor was effective in reducing labor pain. The literature also contains studies confirming that massage applied to the sacral region in the first stage of labor is effective in reducing labor pain. For example, Akköz and Karaduman examined the effects of sacral massage on labor pain and anxiety and found it to be effective in reducing pain in the active and transition phases of labor (13). Mortazavi et al. (9) and Kaçar et al. (28) similarly demonstrated that massage applied in the first stage of labor was effective in decreasing perceptions of labor pain. Erdogan et al. determined that massage applied to the sacral region during the latent, active, and transition phases of labor reduced the perception of labor pain (14). In another study, Sadat et al. found that manual massage performed during the active phase of labor significantly reduced the severity and duration of labor pain (31). In the present study, it was found that massage applied to the lumbosacral region during the active and transition phases of labor was effective in relieving labor pain, consistent with the findings of previous studies.

5. CONCLUSIONS

The primary findings of this study are that, in the active and transition phases of labor, hot-pack and massage applications shorten labor times and reduce the perceived severity of labor pain. In addition, it has been determined that hot-pack applications are more effective than massage in relieving labor pain. The secondary findings of this study are that hot-pack and massage applications for women in labor do not affect the systolic or diastolic blood pressure, pulse rate, body temperature, respiratory rate, or cervical dilatation and effacement times of the mother or the fetal heart rate. It has been determined that hot-pack applications and massage do not pose any risk in terms of maternal and fetal health and can be safely applied as routine care during labor. Thus, it is concluded that hot-pack and massage applications are effective nursing interventions during labor.

In line with the results obtained in this study, it is suggested that nurses and midwives working in delivery rooms be trained in applications of heat and massage that reduce the perception of pain during labor. Furthermore, pregnant

women should be informed about the safety and advantages of heat applications and massage before birth, and hot-pack applications and massage should be added to routine care during labor.

These methods can be added to routine care during labor, as it has been proven that hot-pack and massage applications to the lumbosacral area in childbirth are effective nursing interventions. Both methods are effective in relieving labor pain, easy to apply, cheap, and harmless.

Limitations

This study has a few limitations. First of all, the pregnant women participating in the study were blinded to the assignment of groups, but due to the nature of the interventions, it was not possible to blind the researchers. In addition, oxytocin, which stimulates uterine contractions and accelerates birth to a certain extent, was administered to all women participating in the study. Therefore, the delivery times calculated in this study may have been slightly affected by oxytocin. Since the findings of this study were specifically obtained from the pregnant women who participated in the study, they cannot be generalized to all pregnant women.

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Clinical and Experimental Health Sciences

An Ethnobotanical Study of Medicinal Plants in Savaştepe (Balıkesir-Turkey)

Ebru Ozdemir Nath[1,2], Sukran Kultur[3]

[1] Altinbas University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Türkiye.
[2] Altinbas University Natural Products Research and Development Center, Istanbul, Türkiye.
[3] Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Türkiye.

Correspondence Author: Ebru Ozdemir Nath
E-mail: pharmebru@gmail.com

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ABSTRACT

Objective: This research planned to record traditional uses and preparations of herbal medicines in Savaştepe, Balıkesir. The study was conducted between the years 2012-2016. The Savaştepe district is located on the borders of Balıkesir province.

Methods: 44 villages connected to Savaştepe were visited during the research. With the help of the local people, the collected plants were diagnosed and converted into herbarium specimens. All herbarium samples are stored in the Herbarium of Istanbul University Faculty of Pharmacy (ISTE). Information about the use of plants were collected by the help of interviews with 205 people (107 men, 98 women).

Results: As a result of this study, there were 163 medicinal plant taxa belonging to 53 families. Mostly used medicinal plant species families are Lamiaceae (22 taxa), Asteraceae (20 taxa), Rosaceae (16 taxa), Fabaceae (6 taxa), Brassicaceae (7 taxa), Apiaceae (4 taxa), Poaceae (4 taxa), Scrophulariaceae (4 taxa) respectively. According to the informations, the most common diseases were categozied into 12 group and their FIC values are calculated. The highest FIC value (0.852) for hemorrhoids. Having high use value represents that this taxa is commonly used in Savaştepe. The highest use value is 0.93 for Urtica dioica

Conclusion: This study documented the medicinal plants used by local people of Savaştepe and also the uses, preparations and vernacular names of medicinal plant species. This study will help to concerve valuable informations of the medicinal plants of Savaştepe.

Keywords: Ethnobotany, Ethnomedicine, Savaştepe, Medicinal plants, Turkey

1. INTRODUCTION

Turkey has a rich flora consisting of more than 12 000 taxa. Approximately 34% of these plants are endemic (1). Turkey has very rich knowledge about the ethnobotany. Ethnobotany is defined as a “human-plant relationship. It can be summarized as the knowledge of utilizing the plants for various purposes in a particular area. With ethnobotanical studies, it is aimed to identify the medicinal plant species in a particular region and as food, in the production of dyes, for cosmetic purposes, in animal diseases and tools. Ethnobotanical studies have been conducted in Turkey and other neighboring countries (2-7). Ethnobotanical studies of Turkey record very important information about the flora of the region, traditional uses of the plants, the cultural values in Turkey. The prospect of ethnobotanical studies have increased more after the migration of local populations to big cities. The villagers who have moved to big cities have forget the uses of plants in their daily life. At the same time, the recording of information about plants used in the region provides a better understanding of the plant’s benefits and leads to the commercial-purpose plant production projects. Savaştepe is located in the southern part of the Marmara region, within the borders of Balıkesir province. According to literature, medicinal plants of Savaştepe have been studied for the first time. This study was carried out to preserve valuable informations about local uses of medicinal plants and helps to treasure up preliminary information for more investigation of chemical studies.

2. METHODS

2.1. Study Area

Savaştepe is in the south part of Balıkesir. It neighbors with İvrindi in the west, Bigadiç in the east and Soma in the South region. It has an area of about 430 km². The climate of the Savaştepe district is similar to the climate of the Marmara and

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Aegean regions. The general vegetation is maquis. The forests of the district are located in the east and south. Although there are a wide variety of plant species in the district, pine and oak trees are the majority. There are 44 villages in Savaştepe; (1) Akpınar, (2) Ardıçlı, (3) Aşağıdanişment, (4) Beyköy, (5) Bozalan, (6) Çaltılı, (7) Çamurlu, (8) Çavlı, (9) Çiftlikdere, (10) Çukurçayır, (11) Deveören, (12) Dikmeler, (13) Eğerci, (14) Esenköy, (15) Güvem, (16) Güvemküçüktarla, (17) Hıdırbalı, (18) İsadere, (19) Kalemköy, (20) Karacalar, (21) Karaçam, (22) Karapınar, (23) Kocabıyıklar, (24) Kocaören, (25) Kongurca, (26) Kovukyurt, (27) Kurudere, (28) Madenmezarı, (29) Mecidiye, (30) Minnetler, (31) Pelitcik, (32) Sarısüleymanlar, (33) Sıtmapınar, (34) Soğucak, (35) Söğütcük, (36) Söğütlügözle, (37) Tavşancık, (38) Türediler, (39) Yazören, (40) Yeşilhisar, (41) Yolcupınarı, (42) Yukarıdanişment, (43) Yukarıkaraçam, (44) Yunakdere (Figure 1). During the War of Independence, the National Army liberated Balıkesir and Savaştepe on 6 September 1922. The people of the district have fought so much in this war. For this reason, the city is called “Savaştepe (Warhill)”. The population of the district was recorded as 20.201. Savaştepe is a district where the nomadic villages are very numerous and the nomadic cultures live intensely. The Karakeçili nomads live in about 20 villages. Apart from this, there are the villages established by the tribes of Mustard, Kılaz, Yüncü nomads. This information is obtained by asking the people of each village during field studies.

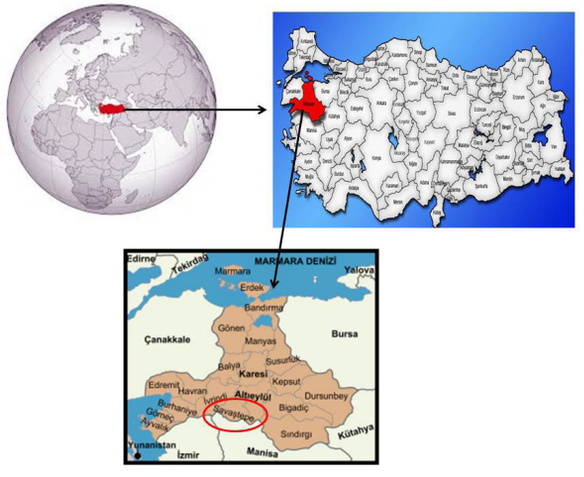

Figure 1. *The geographical location of the study area.*

2.2. Interviews With Local People

During the field studies 205 local people have been interviewed in 44 settlements, between 2012-2016. The ages of the informants were within 30–70 years. The educational status of the local people is usually at the primary, secondary and high school levels. A questionnaire form was used to categorize the use of plants among the public during field studies. In every village, firstly the village reeve was interviewed and information was given about the work done. The names of the people who knew the plants best in the village were asked. The houses of these people and the village shepherds were visited and informations about the medicinal plants was collected from the knowledgeable people (Figure 2-5). During the field studies, the local names of the plants, the demographic characteristics of the participants in the study, the used parts of the plant and preparations written. Whenever possible, audio and video recordings of the interviews were recorded.

Figure 2. *An interview with the local people in Yeşilhisar village tea house*

Figure 3. *Field studies with a vilager in Savaştepe*

Figure 4. *Yörük house in Kovukyurt village*

Figure 5. *A healer woman in Minnetler village*

2.3. Plant Materials

To collect the material of the workshop, 12 times of field trips were organized in the study area between May 2012 and October 2015. In this field trips, 200 medicinal plant specimens were collected, 163 of them to be used for medicinal purpose. The informants who participated in the research were requested to show the plant's sample they used and photographs of the plants were taken in nature. The plant samples were collected, pressed with herbarium press. Some samples were taken from the informant's houses which they use as a medicine, packaged and numbered (E.Ö code). The used plant samples were identified by the help of ''Flora of Turkey and the East Aegean Islands'' (1,8,9) and were compared with the other herbarium samples. The collected plants were deposited at herbarium (ISTE). The plant names were controlled from the plant list (10).

2.4. Calculations

The most common ailments of the study area were categorized into 12 section. Gastrointestinal disease, respiratory and throat disease, dermatological disease, endocrine system disease, cardiovascular disease, kidney disease, nervous system disease, cancer, skeletal muscular system disease, urogenital disease, headache-toothache-ear pain, and hemorrhoids. Informant consensus factors "ICF" (11) were calculated [FIC=(Nur-Nt)/(Nur-1)]. Nur = Number of citations used in each category, Nt= Number of the plant species used. If the FIC value is low (close to 0 value) it shows that plants are not chosen commonly. The use-value "UV" (11) shows the importance of plant species used in the area. It was calculated with following formula. UV=U/N, UV is the use-value of a species; U= the number of citations for every species; N= the number of informants.

3. RESULTS

3.1. Medicinal Plants

Interviews with the informants living in Savaştepe showed that, 163 taxa belonging to 53 families were used in the Savaştepe as a medicine. Among 163 plants, 23 plant species are cultivated. Lamiaceae (22 taxa), Asteraceae (20 taxa), Rosaceae (16 taxa), Leguminosae Fabaceae (6 taxa), Brassicaceae (7 taxa), Apiaceae (4 taxa), Poaceae (4 taxa) and Scrophulariaceae (4 taxa) (Figure 6). The 6 of these taxa are endemic. These are; *Acanthus hirsutus* Boiss., *Alcea pisidica* Hub.-Mor., *Ferulago macrosciadea* Boiss. & Balansa, *Tripleurospermum conoclinium* (Boiss. & Balansa) Hayek, *Stachys cretica* L. subsp. *anatolica* Rech. fil. and *Verbascum simavicum* Hub.-Mor. (1,8,12).

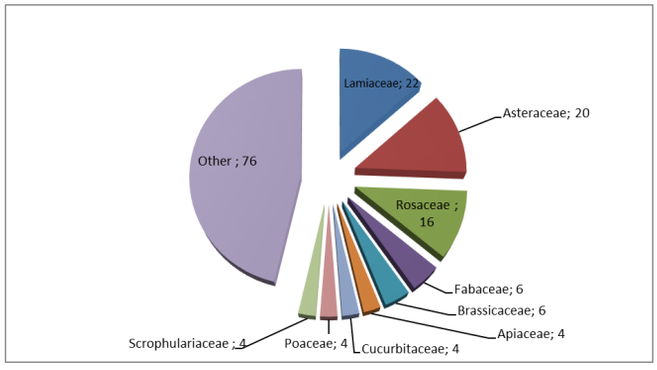

Figure 6. *Most represantative families*

There are different ways of herbal medicine preparations. In this study there were 226 internal uses (%70.9), and 94 external uses (%29.1). The number of methods for the preparation was calculated (Table 3). The most used preparations were infusion (%28.70) and decoction (%25.30). Some plants were used by mixing with honey (*Pinus brutia, Brassica nigra, Nigella sativa, Urtica dioica, Vitis vinifera*). The grated and crushed plant parts (*Allium sativum, Allium cepa, Quercus cerris, Olea europaea, Verbena officinalis*) are mixed with milk, olive oil, snake skin powder, salt, grated soap, yoğurt, tarhana for external application. These applications are the remedies for hair care, joint problems, panaris. The list of wild medicinal plants investigated with their related information and compared with the other ethnomedicinal studies in The West Anatolia. There are 12 taxa have been recorded for the first time as a medicinal plant in region. These taxa are indicated in table 4 in bold. These taxa are; *Onosma aucherana* DC., *Alyssum murale* Waldst. & Kit., *Lepidium spinosum* Ard., *Leontodon tuberosus* L., *Astragalus angustifolius* Lam., *Astragalus hamosus* L., *Melilotus indica* (L.) All., *Trifolium angustifolium* L., *Vicia tetrasperma* (L.) Schreb., *Stachys thirkei* K.Koch, *Veronica pectinata* L., *Scrophularia scopolii* Hoppe ex Pers. When this study is compared with other ethnobotanical studies conducted in the West Anatolia, if the medical use of taxa in Savaştepe is different from the use in other settlements, these uses are shown underlined in table 4. There are 79 taxa with different uses in this way.

Table 1. *FIC values of categories of ailments*

1. Hemorrhoids	0.852
2. Nervous system disease	0.813
3. Headache, tootache, ear pain	0.734
4. Kidney disease	0.702
5. Cancer	0.655
6. Gastrointestinal disease	0.643
7. Dermatological disease	0.637
8. Respiratory and throat disease	0.624
9. Skeletal muscular system diseae	0.594
10. Cardiovascular disease	0.589
11. Endocrine system disease	0.455
12. Urogenital disease	0.428

Table 2. *The most commonly used parts of the plants*

Used part	Number	%
Aerial part	72	24
Leaf	71	23.7
Fruit	57	19
Seed	20	6.7
Flower	18	6
Stem	10	3.3
Cortex	8	2.7
Resin	5	1.7
Latex	2	0.6
Root	35	11.7
Tuber	2	0.6
Total	300	100.0

Table 3. *Preparation methods of the medicinal plants in Savaştepe.*

Preparation methods:		
Directly uses	60	18.52
After processing	264	81.48
Infusion	93	28.70
Decoction	82	25.30
Cooked	26	8.02
Crushed	24	7.40
Grated	7	2.16
Burned	5	1.54
Jam, molasses, marmalade	5	1.54
Mixed with honey	5	1.54
Macerated	5	1.54
Heated	4	1.23
Powdered	4	1.23
Juice	2	0.61
Pickle	1	0.31
Compote	1	0.31
Total	324	100.00

3.2. Data Analysis

Having high use value represents that this taxa is commonly used in Savaştepe/Balıkesir. The use values of *Urtica dioica* L. (0.93), *Salvia fruticosa* Mill (0.80), *Malva sylvestris* L. (0.59), *Matricaria chamomilla* L. (0.58), *Hypericum perforatum* L. (0.56), *Origanum vulgare* L. subsp. *hirtum* (0.54), *Thymus zygioides* Griseb. (0.49), *Pinus brutia* Ten. (0.41), *Platanus orientalis* L. (0.39), *Raphanus raphanistrum* L. (0.39), *Rumex crispus* L. (0.37) and *Rumex acetosella* L. (0.34) were the highest.

The plant species which have high use value can be selected for further pharmacological activity studies and these plant species can be chosen for trading purposes plant farming. In the research area, indications that most people use plants were determined. According to these informations, the most common diseases were categozied into 12 group (Table 1). The highest FIC value (0.852) for hemorrhoids. *Carpinus betulus* L., *Aetheorhiza bulbosa* (L.) Cass., *Carduus pycnocephalus* L. subsp. *albidus* (M.Bieb.) Kazmi, *Centaurea benedicta* (L.) L., *Tripleurospermum conoclinium* (Boiss. & Balansa) Hayek, *Dracunculus vulgaris* Schott, *Arum elongatum* Steven, *Xanthium strumarium* L., *Cornus mas* L., *Ecballium elaterium* (L.) A. Rich., *Dioscorea communis* (L.) Caddick & Wilkin, *Marrubium vulgare* L., *Teucrium chamaedrys* L., *Punica granatum* L., *Malva sylvestris* L. were reported for hemorrhoids. Nervous system disease had the second highest value (0.813). *Juniperus oxycedrus* L., *Pinus brutia* Ten., *Calendula officinalis* L., *Matricaria chamomilla* L., *Hypericum perforatum* L., *Melissa officinalis* L., *Rosmarinus officinalis* L., *Stachys thirkei* K. Koch, *Melilotus indica* (L.) All. were reported for nervous system disease. Headache, toothache, ear pain have 0.734 FIC value, kidney diseases have 0.702 FIC value, cancer have 0.655 FIC value, gastrointestinal diseases have 0.643 FIC value, dermatological diseases have 0.637 FIC value, respiratory and throat diseases have 0.624 FIC value. The last categories were reported to treat skeletal muscular

system diseases have 0.594 FIC value, cardiovascular diseases have 0.589 FIC value, endocrine system diseases have 0.455 FIC value, urogenital diseases have 0.428 FIC value. The aerial part of the plants were used mostly (72 of use-reports), leaf (71 of use reports), fruit (57), radix (35), seed (20), flos (18), stem (10), cortex (8), resin (5), tuber (2), latex (2) (Table 2).

People living in Savaştepe use medicinal plants mostly for Gastrointestinal disease (stomachache, diarrhea, heartburn, flatulence, nausea, reflux, ulcer): 72 taxa; respiratory and throat disease (asthma, bronchitis, cold, cough, dyspnea, hoarseness, sore throat): 57 taxa; dermatological disease (burns, furuncle, cuts, rash, eczema, wounds): 55 taxa; endocrine system disease (thyroid, diabetes): 44 taxa; cardiovascular disease (hypertension, heart diseases, high cholesterol): 17 taxa; kidney disease (kidney gravel, kidney stone, nephritis): 15 taxa; nervous system disease: 12 taxa; cancer: 11 taxa; skeletal muscular system diseases: 29 taxa; urogenital disease (diuretic, dysuria, enuresisnocturna, menstruation problems): 29 taxa; headache, toothache, ear pain: 22 taxa; hemorrhoids: 23 taxa.

Table 4. *The list of wild medicinal plants investigated with their related information*

Family/ Botanical name	Local name in Savaştepe	Used parts	Preparation	Ailments treated, therapeutic effect	Herbarium number (ISTE, E.Ö.)	Use value
PTERIDOPHYTA						
Aspleniaceae						
Ceterach officinarum Willd.	Altın yaldız otu	Aerial part	Infusion, int.	Stomach pain	109566	0.15
Dennstaedtiaceae						
Pteridium aquilinum (L.) Kuhn	Eğrelti	Root	Decoction, int.	Prostate	109726	0.15
Equisetaceae						
Equisetum arvense L.	Eklemeli ot, Kırkkilit, Kilitotu	Aerial part	Decoction, ext.	Allergy	109971	0.18
		Aerial part	Decoction, int.	Kidney pain, prostate		
		Aerial part	Decoction, int.	Constipation		
		Aerial part	Decoction, ext.	Rheumatism		
		Root	Decoction, int.	Prostate		
Equisetum ramosissimum Desf.	Eklemeli otu	Aerial part	Decoction, int.	Rheumatism	109972	0.05
SPERMATOPHYTA GYMNOSPERMAE						
Cupressaceae						
Cupressus sempervirens L.	Selvi	Cone	Decoction, int.	Diabetes	E.Ö.2	0.1
Juniperus oxycedrus L.	Ardıç	Fruit, branch	Decoction, int.	Prostate	109615	0.26
		Fruit, branch	Decoction, ext.	Wound healing		
		Fruit	Decoction, int.	Cough, pain killer, kidney stone, diabetes, urinary system problems, throat pain		
		Stem and branch	Decoction, int.	Abdominal pain		
		Fruit	Eaten, int.	Eczema		
Pinaceae						

Pinus brutia Ten.	Çam	Resin	Eaten, int.	Breathing problems, expectorant	109873	0.41
				pain killer, stomach pain, ulcer, intestine cancer, inflammation		
		Resin	Infusion, int.	Stomach pain		
		Resin	Pastil, int.	Stomach pain		
		Resin	Mixed with honey	Bronchitis, ulcer		
		Resin	Put on skin, ext.	Wound		
		Stem and branch	Decoction, int.	Breathing problems, asthma		
		Stem and branch	Infusion, int.	Circulation problems, cholesterol		
		Young cone	Decoction, int.	Abdominal pain, asthma, bronchitis, expectorant		
		Young Leaf	Decoction, int.	Diabetes		
SPERMATOPHYTA ANGIOSPERMAE						
Acanthaceae						
Acanthus hirsutus Boiss. (Endemic)		Aerial part	Infusion, int.	Fever	109529	0.02
Adoxaceae						
Sambucus nigra L.	Mürver	Cortex	Decoction, int.	Constipation	109530	0.09
Amaranthaceae						
Beta vulgaris L. *	Pancar	Fresh leaf	Freshly put on skin, ext.	Wound healing, panaris	E.Ö.3	0.49
Chenopodium murale L.	Sirken	Leaf	Eaten as a meal, int.	Stomach pain, carminative, constipation	109535	0.42
Amaryllidaceae						
Allium ampeloprasum L.	Pırasa	Aerial part	Eaten, int.	Breathing problems	109757	0.02
Allium cepa L.*	Soğan	Bulb	Grated and mixed with flour and olive oil, ext.	Skin cracks	E.Ö.4	0.11
		Bulb	Grated and mixed with salt, ext.	Ankle joint problems,		
		Bulb	Grated and mixed with grated soap, ext.	Panaris		
		Bulb	Grated and mixed with yogurt and tarhana, ext.	Ankle joint problems		

Allium sativum L. *	Sarımsak	Bulb	Eaten, int.	Bronchitis, blood pressure regulator,	E.Ö.5	0.2
		Bulb	Cooked, ext.	Ear pain,		
		Bulb	Crushed, ext.	Joint problems		
		Bulb	Crushed with salt, ext.	Toothache		
		Bulb	Crushed and mixed with olive oil, ext.	Hair care		
		Bulb	Crushed and mixed with snake skin powder	Hair care		
		Bulb	Grated, ext.	Protection against snake bites		
Anacardiaceae						
Pistacia terebinthus L.	Çetlemik, Çitlembik, Şimşir	Dry fruit	Crushed and infusion, int.	Breathing problems	109537	0.34
		Young leaf	Freshly eaten, int.	asthma		
Rhus coriaria L.	Somak, Sumak	Fruit	Freshly eaten, int.	Diabetes, diarrhea	109540	0.17
		Fruit	Decoction, int.	Stomach pain,		
		Seed		Goitre		
Apiaceae						
Anethum graveolens L.	Kırca	Leaf	Infusion, int.	Throat pain, grip	109550	0.07
Ferulago macrosciadea Boiss. & Balansa (Endemic)	Mide otu	Fruit	Decoction, int.	Stomach sour, carminative	109541	0.06
Foeniculum vulgare Mill.	Arap saçı	Fruit	Infusion, int.	Enurezis	E.Ö.6	0.22
Petroselinum crispum (Mill.) Fuss *	Maydanoz	Leaf	Infusion, int.	Ovarium problems	E.Ö.8	0.33
Araceae						
Arum elongatum Steven	Yılancık, Yılan burçağı, Yılan kavçığı, Yılan yastığı	Root	Crushed, ext.	Wound healing, rheumatism, egzama	109559, 109560	0.37
		Root	Eaten, int.	For animal antihelminthic		
		Root	Cooked as a meal, int.	Hemorrhoids		
		Leaf	Crushed and applied on skin, ext.	Warts, headache		
		Drid leaf	Cooked as a meal, int.	Surgical wound healing		
		Seed	2-3 seed taken with water, int.	Egzama, hand cracks, hemorrhoids, diabetes		
		Seed	Crushed and applied on skin, ext.	Warts		

Dracunculus vulgaris Schott	Yılan burçağı, Yılan kavcığı, Yılan yastığı	Root	Cooked as a meal, int.	Diabetes, breathing problems	109554, 109556, 109557	0.17
		Root	Cut into small pieces taken with water, int.	Hemorrhoids		
		Root	Decoction, int.	Abdominal pain killer		
		Root	Crushed and applied on skin, ext.	Wound healing, pain killer		
		Leaf	Applied on forehead, ext.	Fever		
Asparagaceae						
Asparagus acutifolius L.	Çıtır dikeni	Flower	Infusion, int.	Urinary system problems, kidney inflamation	109763	0.07
Asparagus aphyllus L. subsp. *orientalis* (Baker) P.H.Davis	Ciğer otu	Aerial part	Infusion, int.	Lung problems, heart problems, kidney inflamation	109762	0.08
Ruscus aculeatus L.	Köpek üzümü, Sidikkesen otu, Tavşan memesi, Tavşan topu, Tilki üzümü	Root	Decoction, int.	Prostate	109562	0.19
		Leaf	Infusion, int.	Heart problems		
		Fruit	2-3 fruits eaten, int.	Enuresis, heart problems		
Asteraceae						
Achillea arabica Kotschy	Ayvadana, Populca	Aerial part	Decoction, int.	Gynecological problems, abdominal pain	109618	0.1
		Capitulum	Infusion, ext.	Wound healing		
		Capitulum	Applied on abdomen, ext.	Abdominal pain		
Achillea setacea Waldst. & Kit.	Kurtotu	Aerial part	Decoction, int.	Abdominal pain, ulcer, cancer	109658, 109623	0.16
Aetheorhiza bulbosa (L.) Cass. Monotipik	Mayasıl otu	Tuber	2-3 tuber taken with water, int.	Hemorrhoids, intestine problems, constipation, heel cracked, allergy	109632	0.38
Anthemis cotula L.	Bopatça	Aerial part	Infusion, int.	Abdominal pain, cold,	109647, 109646	0.29
		Aerial part	Infusion, ext.	allergy		
Anthemis cretica L. subsp. *absinthifolia* (Boiss.) Grierson	Papatya	Aerial part	Infusion, int.	Abdominal pain	E.Ö.11	0.25

Anthemis pseudocotula Boiss.	Bopatça	Aerial part	Infusion, int.	Abdominal pain	109648	0.23
			Infusion, ext.	Farengitis		
Artemisia annua L.	Kabe süpürgeliği	Aerial part	Infusion, int.	Menopause, mensturation	109622	0.18
Calendula officinalis L.	Aynısefa otu	Capitulum	Infusion, int.	Sedative	E.Ö.12.	0.08
Carduus nutans L.	Deve dikeni	Seed	Eaten, int.	Immune system tonic	109620	0.07
Carduus pycnocephalus L. subsp. *albidus* (M.Bieb.) Kazmi	Çakır dikeni	Capitulum	Infusion, ext.	Hemorrhoids	109628	0.16
Centaurea benedicta (L.) L.	Sancı dikeni, Şevketi bostan	Aerial part	Decoction, int.	Abdominal pain, hemorrhoids	109621	0.31
			Decoction, ext.	Hemorrhoids		
			Cooked as a meal, int.	Allergy		
Cota tinctoria (L.) J.Gay	Akıllı papatya, Gömeç	Aerial part	Infusion, int.	Cold, abdominal pain	109660, 109662, 109663	0.12
Pilosella hoppeana (Schultes) C.H. &F.W.Schultz subsp. *testimonialis* (Naegli ex Peter) Sell & West	Mercangümüş	Aerial part	Decoction, ext.	Wound healing	109653, 109652	0.08
***Leontodon tuberosus* L.**	Hindibağ	**Root**	Infusion, int.	**Cold, stomach disease**	109636, 109635	0.16
Matricaria chamomilla L.	Bopatça, Papatça, Keloğlan çiçeği	Capitulum	Infusion, int.	Cold, diabetes, abdominal pain, headache, sinusitis, sleep problems, depression, inflamation, urinary system problems	109644	0.58
			Infusion, ext.	Burn, wound healing, rheumatism, leg pain, asthma, allergy		

Silybum marianum (L.) Gaertn.	Sarıkız	Seed	Eaten, int.	Prostate, liver problems	109639	0.17
		Latex	Chewed in the mouth, int.	Toothache		
		Stem	Eaten, int.	Immune system tonic		
Taraxacum hybernum Steven	Karahindibağ	Leaf	Cooked as a meal, int.	Liver problems	109641	0.19
		Root	Decoction, int.	Immune system tonic		
Tripleurospermum conoclinium (Boiss. & Balansa) Hayek (Endemic)	Uzun papatya	Capitulum	Infusion, int.	Abdominal pain	109659, E.Ö.17	0.06
Xanthium strumarium L.	Büyük pıtrak, Domuz pıtrağı	Fruit	Decoction, int.	Hemorrhoids	E.Ö.18	0.07
Xeranthemum cylindraceum Sm.	Duman otu	Aerial part	Infusion, int.	Stomach disease	E.Ö.19	0.11
		Aerial part	Burned and inhalation, ext.	Cold (for animal)		
Betulaceae						
Carpinus betulus L.	Gürgen	Cortex	Crushed, ext.	Hemorrhoids	109574	0.07
Boraginaceae						
Echium plantagineum L.	Engerek otu	Aerial part	Infusion, int.	Expectorant	109580	0.08
***Onosma aucherana* DC.**	Emme otu	**Root**	Infusion, ext.	**Wound healing**	109577	0.14
Brassicaceae						
***Alyssum murale* Waldst. & Kit.**	Sünnetlice	**Aerial part**	Infusion, int.	**Stomach pain**	109604	0.13
Brassica oleracea L.	Kelem	Leaf	Cut into small pieces, cooked with olive oil and applied on the skin, ext.	Pain killer, wound healing,	E.Ö.23	0.19
		Leaf	Heated on the stove, applied on joints with olive oil, ext.	Joint problems, leg pain, joint pain		
		Leaf	Immersed in hot water, applied on joints, ext.	Joint problems		
		Immature flower	Decoction, int.	Stomach pain		
Brassica nigra (L.) K.Koch	Hardala	Seed	Powdered and mixed with honey, int.	Appetizer (for kids)	109608	0.08

***Lepidium spinosum* Ard.**	Muşurat	**Aerial part**	**Freshly eaten, int.**	**Circulation problems**	E.Ö.24	0.21
		Seed	**Crushed and eaten, int.**	**Stomach disease**		
Nasturtium officinale R.Br.	Deli kereviz, Gereviz, Su kazayağı	Leaf	Mashed with tarhana, applied on abdomen, ext.	Abdominal pain	109602, 109603	0.32
		Leaf	Freshly eaten, int.	Kidney stones		
Raphanus sativus L. *	Kara turp	Root	Grated and cooked, applied on skin, ext.	Joint and leg pain	E.Ö.25	0.07
		Root	Carved and put honey, kept one day, and taken, int.	Cough		
Raphanus raphanistrum L.	Eşek turbu, Turp otu	Root	Decoction, int.	Kidney stone	109611	0.39
Cistaceae						
Cistus creticus L.	Pamuklar	Aerial part	Infusion, int.	Diarrhea	109586, 109593	0.11
Cistus salviifolius L.	Pamuklar	Aerial part	Infusion, int.	Diarrhea	109626, 109592	0.09
			Decoction, int.	Urinary system problems, prostate		
Cornaceae						
Cornus mas L.	Kızılcık	Stem	Burned and ash applied on teeth, ext.	Toothache	109595	0.27
		Young shoots	Decoction, int.	Ulcer, hemorrhoids, pain killer		
		Young shoots	Decoction, ext.	Wound healing		
		Fruit	Jam, int.	Bronchitis, snake bites, pain killer		
		Fruit	Decoction, int.	Circulation problems, urinary system problems, kidney stone,		
		Fruit	Compote, int.	Diarrhea		
Cucurbitaceae						
Citrullus lanatus (Thunb.) Matsum. & Nakai *	Karpuz	Exocarp of fruit	Powdered and eaten, int.	Ulcer	E.Ö.26	0.09

Ecballium elaterium (L.) A.Rich.	Acı bostan, Acı dürlek,	Fruit	2 drops juice of fruit, put into the nostrils, int.	Sinusitis	109613, 109612	0.34
		Fruit	2 drops juice of fruit, put into the water, int.	Hepatitis		
		Fruit	Juice of fruit, as a massage, ext.	Cancer pain		
		Root	Grated and mixed with flour, cut into small pieces, int.	Joint pain, itching, lung disease		
		Root	2 drops juice of root, put into the nostrils, int.	Liver inflamation		
		Root	Decoction, int.	Hemorrhoids, itching		
Cucumis melo L. *	Kavun	Seed	Decoction, int.	Carminative	E.Ö.27	0.07
Momordica charantia L. *	Akçakız, Şevketibostan	Fruit	Kept in olive oil for 15 days, ext.	Wound healing	E.Ö.29	0.16
		Fruit	Kept in olive oil for 30 days, eaten int.	Ulcer		
Dioscoreaceae						
Dioscorea communis (L.) Caddick & Wilkin	Acı filiz	Fruit	Crushed, ext.	Wound healing, pain killer	109675, 109677	0.18
		Root	Decoction, int.	Hemorrhoids		
Elaeagnaceae						
Elaeagnus angustifolia L. *	İğde	Fruit	Decoction, int.	Kidney stone	E.Ö.31	0.14
Fabaceae						
***Astragalus angustifolius* Lam.**	Diken otu, Top diken	Root	Decoction, int.	**Prostate**	109729	0.05
***Astragalus hamosus* L.**	Pıtrak	Fruit	Infusion, int.	**Urinary system problems, kidney stone, heel cracked**	109754	0.07
***Melilotus indica* (L.) All.**	Sarı yonca	**Flower, leaf**	Infusion, int.	**Sedative**	109730	0.04
Spartium junceum L.	Katır kuyruğu, Piren	Stem	Decoction, int.	Bronchitis	109753, 109752	0.19
***Trifolium angustifolium* L.**	Trifil	**Aerial part**	Decoction, int.	**Diarrhea**	109738	0.13
***Vicia tetrasperma* (L.) Schreb.**	Mavi kantaron	**Aerial part**	Infusion, int.	**Sedative, pain killer**	109751	0.11
Fagaceae						

Quercus cerris L.	Ak gobak, Gobak, Kara kombalak, Kobak, Kombalak, Kubar	Root	Crushed and mixed with milk, ext.	Abscess	109685, 109690	0.29
		Cortex of stem	Decoction, int.	Diarrhea		
Quercus infectoria G.Olivier	Gobak, Meşe, Palamut	Cortex of stem	Decoction, int.	Organ shape disorders	109688	0.2
		Cortex of stem	Decoction, ext.	Skin disease		
		Fruit	Decoction, int.	Circulation problems		
Geraniaceae						
Geranium asphodeloides Burm.f.	Hıdır otu	Aerial part	Infusion, ext.	Wound healing	109698	0.09
		Leaf, flower	Infusion, int.	Diarrhea		
Hypericaceae						
Hypericum montbretii Spach	Kantaron	Aerial part	Infusion, int.	Stomach pain	109722, 109715	0.13
Hypericum perforatum L.	Boyalık otu, Kantarot, Kantar otu, Sarı kantaron	Aerial part	Infusion, int.	Liver problems, kidney stone, stomach disease, diabetes, sedative, constipation	109724, 109711, 109714	0.56
		Aerial part	Kept in olive oil for 45 days, ext.	Wound healing, pain killer, rheumatism		
Juglandaceae						
Juglans regia L.	Ceviz	Immature fruit	Taken as a pill for 7 days, int.	Antifungal, diabetes	109728	0.24
		Leaf	Decoction, ext.	Egzema, rheumatism		
		Leaf	Decoction as a gargle, ext.	Throat inflamation		
Lamiaceae						
Ballota nigra L.	Çay otu, Kanser otu	Aerial part	Infusion, int.	Cold, stomach pain	109821, 109822	0.09
		Root	Decoction, int.	Cancer		
Clinopodium vulgare L.	Ballıbaba	Aerial part	Infusion, int.	Cold	109853	0.05
Lavandula stoechas L.	Karabaş otu, Lavanta	Aerial part	Infusion, int.	Heart problems	109826	0.12
Marrubium vulgare L.	Bertik otu, Konyalı otu, Mayasıl otu	Aerial part	Infusion, int.	Allergy, breathing problems, hemorrhoids, diabetes	109830, 109848	0.14

Melissa officinalis L.	İliman, Oğulotu	Aerial part	Infusion, int.	Sedative, heart disease, throat pain, stops snoring	109803, 109802, 109851	0.18
		Leaf	Infusion, int.	Improve memory, digestive system problems		
Mentha longifolia (L.) L. subsp. *typhoides* (Briq.) Harley	Dere nanesi	Leaf	Infusion, int.	Gastrointestinal health	109834	0.24
Mentha pulegium L.	Nana, Nane	Leaf	Infusion, int.	Stomach disease, cough, cold	E.Ö.36	0.12
Micromeria myrtifolia Boiss. & Hohen.	Ayaklı kekik	Aerial part	Infusion, int.	Cold	109836, 109837	0.07
Origanum vulgare L. subsp. *hirtum* (Link) Ietsw.	Uzun kekik	Aerial part	Infusion, int.	Abdominal pain, cold, cholesterol, nausea, inflamation, cancer	109829, 109814, 109813	0.54
Prunella laciniata (L.) L.	Horoz ibiği	Aerial part	Infusion, int.	Cough	109844, 109845	0.09
Prunella vulgaris L.	Siğil otu	Aerial part	For prayer, ext.	Warts, headache, eye problems	109842, 109843	0.06
Rosmarinus officinalis L.	Akıl otu	Aerial part	Infusion, int.	Inflamation, breathing problems, sedative, heart problems, constipation, fat burn	109839	0.22
		Leaf	Infusion, int.	Memory enhancer		
Salvia fruticosa Mill.	Adaçayı, Boşotu, Muşapla, Puşapla, Yakıotu	Leaf	Infusion, ext.	Wound healing	109805, 109792	0.8
		Leaf	Kept in vinegar, ext.	Calcification		
		Aerial part	Infusion, int.	Cold, abdominal pain, throat pain, eczema, stomach disease, cough, kidney problems, inflammation, wound healing		
Salvia tomentosa Mill.	Adaçayı	Leaf	Infusion, ext.	Wound healing	109793	0.24
		Aerial part	Infusion, int.	Cold, abdominal pain		
Stachys cretica L. subsp. *anatolica* Rech. fil. (Endemic)	Beyaz şabla	Aerial part	Infusion, int.	Pain killer, stomach disease, cold	109812, 109810, 109789	0.15
Stachys obliqua Waldst. & Kit.	Dağ çayı	Aerial part	Infusion, int.	Cough	109787	0.05
***Stachys thirkei* K.Koch**	Minare otu, Tavşanak otu	**Aerial part**	Infusion, int.	**Cold, digestion problems, carminative, sedative**	109788	0.09

Teucrium chamaedrys L.	Bodur mahmut, Bodur otu, Kebir, Kısa mahmut	Aerial part	Infusion, int.	Immune system tonic, hemorrhoids, tuberculosis	109831, 109786, 109784, 109785, 109783	0.14
Thymbra spicata L.	Bayır kekiği, Karabaş otu, Kaya kekiği, Mercanköşk,	Aerial part	Infusion, int.	Diabetes, rheumatism, stomach disease, cough	109778, 109776	0.11
Thymus longicaulis subsp. *chaubardii* C.Presl subsp. chaubardii (Rchb.f.) Jalas	Nuzlot	Aerial part	Infusion, int.	Abdominal pain	109832	0.09
Thymus zygioides Griseb.	Bayır çayı, Kekik, Nuzla otu, Taş kekiği	Aerial part	Infusion, int.	Cold, diabetes, cancer, abdominal pain, gastrointestinal problems	109819, 109818, 109817, 109816, 109815, 109775	0.49
		Aerial part	Infusion as a gargle, ext.	Gum inflamation		
		Leaf	Chewed in the mouth, int.	Toothache		
Vitex agnus-castus L.	Hayıt	Seed	Decoction, int.	Diarrhea	109828, 109774, 109773	0.08
Lauraceae						
Laurus nobilis L.	Defne	Leaf	Infusion, int.	Cold, constipation	109855	0.06
Lythraceae						
Punica granatum L. *	Nar	Exocarp of fruit	Decoction, ext.	Hemorrhoids, rheumatism, urinary system problems	E.Ö.38	0.14
Malvaceae						
Abelmoschus esculentus(L.) Moench *	Bamya	Fruit	Mashed with milk, applied on joints, ext.	Knee pain, rheumatism,	E.Ö.39	0.07
		Seed	Eaten, int.	Diabetes		
		Flower	2-3 flowers taken with water, int.	Ulcer		
Alcea pisidica Hub.-Mor. (Endemic)	Fatmagül, Hatmi	Flower	Infusion, int.	Cold, cough	109770	0.12
		Seed	Decoction, int.	Breathing problems, enurezis		

Malva sylvestris L.	Evelik	Aerial part	Infusion, int.	Abdominal pain	109767, 109768	0.59
		Leaf, seed, stem	Immersed in hot water, applied on joints, ext.	Edema		
		Leaf	Infusion, int.	Cold, bronchitis		
		Leaf, flower	Freshly eaten, int.	Hemorrhoids		
		Leaf	Cooked as a meal, int.	Stomach pain, intestine cancer		
		Leaf	Kept on fire 30 sec. and applied, ext.	Abscess		
		Leaf	Mashed with milk and cooked, applied, ext.	Abscess		
		Leaf	Mashed with tarhana and cooked, applied, ext.	Wound healing, acarid bites		
		Flower	Infusion, int.	Asthma		
Tilia tomentosa Moench	Ihlamur	Flower	Infusion, int.	Cold, stomach disease, anemia, blood detox	109961	0.28
Tilia rubra DC. subsp. *caucasica* (Rupr.) V.Engl.	Ihlamur	Flower	Infusion, int.	Cold	E.Ö.67	0.05
Moraceae						
Ficus carica L.	İncir	Latex	1,2 drops applied into the ear, int.	Ear pain	109771	0.16
Morus alba L.	Dut	Fruit	Decoction, int.	Eczema, allergy,	109772	0.12
Morus nigra L. *	Kara dut	Fruit	Jam applied into the mouth, int.	Mouth ulcer, diabetes,	E.Ö.40	0.22
		Fruit	Applied, ext.	eczema, allergy		
		Leaf	Applied, ext.	Pain killer, joint health		
Oleaceae						

Olea europaea L. *	Zeytin	Leaf	Decoction, int.	Diabetes, abdominal pain, cholesterol	E.Ö.41	0.31
		Leaf	Infusion, int.	Cholesterol		
		Fruit	Crushed and mixed with salt, ext.	Joint health		
		Fruit oil	Applied in genital organ, int.	Easy delivery for animals		
		Fruit oil	Eaten, int.	Asthma		
		Fruit oil	1-2 drops put into the eyes, int.	Eyes worm		
		Fruit oil soup	Grated soap wiped on hot towel, applied on the chest, ext.	Cold		
Phillyrea latifolia L.	Akçakesme	Leaf	Eaten, int.	Blood pressure problems	109858, 109859, 109860	0.15
Paeoniaceae						
Paeonia peregrina Mill.	Tombak lale	Root	Decoction, ext.	Scabies		0.1
		Root	Crushed and applied on joints, ext.	Rheumatism	109863, 109862	
Papaveraceae						
Papaver somniferum L.	Afyon	Latex	Dried and powdered, mixed with olive oil, used as massage oil, ext.	Sedative, sleep problems	109867	0.06
Plantaginaceae						
Plantago lanceolata L.	Damarlı ot	Leaf	Applied, ext.	Wound healing	109874	0.12
		Leaf	Decoction, int.	Stomach disease, tuberculosis, asthma		
		Aerial part	Infusion, int.	Kidney stone, carminative		
Plantago major L.	Kırkdamar otu	Leaf	Infusion, int.	Asthma, bronchitis, cancer, edema	109876	0.29
		Leaf	Applied, ext.	Warts, wound healing, rheumatism, abscess, edema		

Plantago major L. subsp. *intermedia* (Gilib.) Lange	Beşparmak otu	Leaf	Mashed and applied, ext.	Abscess, wound healing, stomach disease, asthma, tuberculosis	E.Ö.43, 109875	0.07
		Leaf	Decoction, int.	Stomach disease		
		Leaf	Infusion, int.	Asthma, tuberculosis		
***Veronica pectinata* L.**	Bodur mahmut	**Aerial part**	Infusion, int.	**Abdominal pain, wound healing**	109956, 109955	0.07
Platanaceae						
Platanus orientalis L.	Çınar	Leaf	Decoction, int.	Calcification, rheumatism, diarrhea, hepatitis,	109877	0.39
		Leaf	Leaf juice, int.	Hemorrhoids, hepatitis, abdominal pain		
		Leaf	Heated on the stove, applied on joints, ext.	Pain killer		
		Leaf	Applied, ext.	Astringent		
		Leaf	Dried and powdered, eaten with rice, int.	Diabetes		
		Fruit	Decoction, int.	Breathing problems, diarrhea, enuresis		
Poaceae						
Avena sativa L. *	Yulaf	Fruit	Powdered and mixed with water, ext.	Psoriasis	E.Ö.32	0.19
Cynodon dactylon (L.) Pers.	Ayrık otu, Eklem otu	Root	Infusion, int.	Leg pain, calcification,	109707	0.17
		Root	Crushed, eaten, int.	Prostate, diüretic,		
Hordeum vulgare L. *	Arpa	Fruit	Crushed, ext.	Skin disease	E.Ö.33	0.08
		Fruit	Crushed and mixed with soup, int.	Immune system tonic		
Zea mays L. *	Darı	Stilus	Infusion, int.	Urinary system problems, kidney stone	109703	0.22
Polygonaceae						
Rumex acetosella L. *	Kuzukulağı	Seed	Eaten, int.	Immune system tonic	E.Ö.44	0.34
		Leaf	Infusion, int.	Urinary system problems		
		Leaf	Cooked as a meal, int.	Hemorrhoids		

Rumex crispus L.	Alabardağı, Eşek alabadası	Seed	Decoction, int.	Enuresis	109883	0.37
		Leaf	Cooked as a meal, int.	Urinary system problems,		
		Leaf	Decoction, int.	Urinary system problems,		
		Leaf	Cut into the small pieces, cooked with milk, applied on skin, ext.	Wound healing		
Rumex patientia L. *	Pazı	Leaf	Cooked as a meal, int.	Appetizer	E.Ö.46	0.15
Primulaceae						
Primula vulgaris subsp. Huds. *rubra* (Sm.) Arcang.	Dağ marulu, Karga basması, Marul	Leaf	Infusion, ext.	Rheumatism	109887	0.06
Ranunculaceae						
Nigella sativa L. *	Çörek otu	Seed	Eaten, int.	Diabetes	E.Ö.51	0.27
		Seed	Powdered and mixed with hoey, eaten, int.	Breathing problems, cough, carminative		
		Seed	Powdered and mixed with sugar, applied on baby pacifier, int.	Colic		
Ranunculus arvensis L.	Bağdırnağı, Pıtrak	Flower	Applied on skin, ext.	Eczema, abscess, joint pain, allergy	109890	0.15
		Fruit	Decoction, int.	Stomach disease		
		Aerial part	Applied on skin, ext.	Joint pain		
Ranunculus repens L.	Bağdırnağı, Yağlı lale, Yakı otu	Çiçek	Crushed and applied on the legs, ext.	Joint pain	109891	0.13
Rhamnaceae						
Paliurus spina-christi Mill.	Çaltı, Çaltı pulu	Fruit	Decoction, int.	Prostate	109893, 109895	0.14
		Root	Decoction, int.	Allergy, itching		
Rosaceae						
Crataegus monogyna Jacq.	Alıç, yemişen	Leaf	Infusion, int.	Heart problems	109942, 109896	0.12
		Fruit	Jam, int.	Digestive system problems		

Cydonia oblonga Mill. *	Ayva	Leaf	Infusion, int.	Stomach pain, cough, urinary system problems,	E.Ö.52	0.28
		Leaf	Decoction, int.	Diarrhea, breathing problems		
Malus sylvestris (L.) Mill.	Bayır elması	Fruit	Juice of fruit, int.	Bronchitis	109922	0.17
Potentilla recta L.	Aslan pençesi	Leaf	Infusion, int.	Wound healing	109921	0.05
Prunus avium (L.) L.	Kiraz	Fruit stalk	Decoction, int.	Prostate, urinary system problems, edema	109941	0.18
Prunus divaricata A. Sav. subsp. *divaricata*	Dağ eriği	Fruit	Dried fruits eaten, int.	Constipation, diabetes, blood pressure problems	E.Ö.54	0.16
Prunus spinosa L.	Karagüvem, Mamık	Fruit	Eaten, int.	Diabetes	109935, 109909, 109930	0.2
		Fruit	Decoction, int.	Immune system tonic		
		Fruit	Jam, int.	Diabetes		
Pyrus amygdaliformis Vill.	Deli armut	Fruit	Eaten, int.	Diarrhea	109911, 109936	0.11
Pyrus elaeagnifolia Pall.	Ahlat, Alfat, Geyik elması, Yabani armut	Fruit	Pickle, int.	Diabetes	109923	0.18
		Fruit	Eaten, int.	Abdominal pain, diabetes		
Rosa canina L.	Bayırgülü, İtgülü, Kuşburnu, Yabangülü	Fruit	Decoction, int.	Cold, breathing problems, itching, allergy, circulation problems, heart problems, stomach disease	109931	0.34
		Fruit	Jam, int.	Asthma		
		Seed	Decoction, int.	Diabetes		
Rosa sempervirens L.	Kuşburnu, Öküzgötü	Fruit	Decoction, int.	Cold	109901, 109905, 109906	0.1

Rubus canescens DC.	Böğürtlen, Karantı	Root	Immersed in hot water, applied on skin, ext.	Abscess, diabetes, allergy,	109926	0.31
		Root	Decoction, int.	Hemorrhoids, diabetes, allergy, kidney stone		
		Leaf	Applied on skin, ext.	Burn, scabrid		
		Leaf	Burned and ash applied on skin, ext.	Wound healing		
		Fruit	Eaten, int.	Cancer		
Rubus idaeus L.	Karantı	Root	Decoction, int.	Diabetes	109925	0.07
Rubus sanctus Schreb.	Böğürtlen, Kırıntı	Root	Decoction, int.	Rheumatism	109899, 109929, 109939	0.15
		Fruit	Eaten, int.	Headache, abdominal pain		
		Fruit	Decoction, int.	Immune system tonic		
Rubus ulmifolius Schott	Karantı	Fruit	Decoction, int.	Immune system tonic	109927	0.06
Sarcopoterium spinosum (L.) Spach	Çıtır pıtır	Aerial part	Decoction, int.	Urinary system problems, kidney stone	E.Ö.56	0.18
Rubiaceae						
Galium verum L.	Sünnetlice otu, Yoğurt otu	Aerial part	Infusion, int.	Goitre	109947	0.11
		Aerial part	Crushed and applied on the skin, ext.	Wound		
Salicaceae						
Populus nigra L. *	Deli kavak, Telli kavak	Cortex of stem	Decoction, int.	Breathing problems	E.Ö.59	0.09
Salix alba L.	Söğüt	Cortex of Stem	Decoction, int.	Pain killer	109949	0.23
		Cortex of Stem	Burned and mixed with vinegar, applied, ext.	Warts		
		Leaf	Freshly applied, ext.	Warts		
		Leaf	Freshly eaten, int.	Antiagregan		
Santalaceae						

Viscum album L. subsp. *album*	Aflat burcu, Ahlat burcu, Ahlat pürçüğü, Ardıç burcu, Armut burcu, Hurç	Leaf	Decoction, int.	Diabetes, breathing problems	E.Ö.61	0.14
Viscum album L. subsp. *austriacum* (Wiesb.) Vollm.	Burç, Çam burcu, Hurç, Ökse otu	Leaf	Infusion, int.	Blood pressure problems, asthma, demans	E.Ö.62	0.09
			Decoction, int.	Breathing problems		
Scrophulariaceae						
***Scrophularia scopolii* Hoppe ex Pers.**	Basur otu	**Flower**	**Kept in olive oil for 15 days, ext.**	**Wound healing, hemorrhoids**	109953	0.08
Verbascum mucronatum Lam.	Balık otu, Sığır kuyruğu	Aerial part	Infusion, int.	Cold	109951	0.28
		Leaf	Infusion, int.	Hemorrhoids, rheumatism		
		Root	Infusion, int.	Hemorrhoids		
		Flower	Infusion, int.	Hemorrhoids		
		Seed	Decoction, int.	Diarrhea		
Verbascum simavicum Hub.-Mor. (Endemic)	Eşek kulağı, Sığır sidiği	Flower	Infusion, int.	Hemorrhoids	109950, 109952, E.Ö.63	0.13
		Seed	Infusion, int.	Diarrhea		
Solanaceae						

Hyoscyamus niger L.	Diş otu	Seed	The seeds are thrown into the fire, the pot is turned over. The smoke of the seed penetrates into the pot. It is held for 30 minutes on the ground. Then fill the pot with boiling water and hold a cheesecloth on the head of the person with a problem in his eyes holding the face close to the steam of the water. Wait 30 minutes. Insect and fly fall into the water from eyes, ext.	Eye problems	109958, 109957	0.11
Lycopersicon esculentum Mill. (E.Ö.64) *	Domates	Fruit	Cut the fruit in half, apply it on the skin where thorn came in	To remove thorn		0.07
Solanum tuberosum L. *	Patates	Tuber	Remove the skin, cut into the pieces, apply on the eyes or skin, ext.	Eye problems, burn	E.Ö.66	0.14
Urticaceae						

Urtica dioica L.	Kabarcık	Aerial part	Infusion, int.	Cancer, hemorrhoids, blood detox, goitre, allergy, abdominal pain, urinary system problems, diabetes, circulation problems	109962, E.Ö.68	0.93
		Aerial part	Decoction, int.	Cancer, tuberculosis, eczema, pain killer		
		Aerial part	Freshly applied on the skin, ext.	Rheumatism, circulation problems in legs		
		Aerial part	Cooked as a meal, int.	Liver problems, hemorrhoids, eczema, cancer		
		Aerial part	Kept in hot water and applied on skin	Leg sprain		
		Aerial part	Crushed and mixed with olive oil, ext.	Pain killer, circulation problems in legs		
		Leaf	Cooked as a meal, int.	Eczema, hemorrhoids, cancer		
		Root	Decoction, ext.	Leg pain		
		Seed	Mixed with honey eaten, int.	Cancer		
Urtica membranacea Poir. ex Savigny	Isırgan	Aerial part	Infusion, int.	Cancer,	109964	0.121
		Aerial part	Cooked as a meal, int.	Liver problems,		
		Aerial part	Freshly applied on the skin, ext.	Rheumatism		
		Leaf	Cooked as a meal, int.	Hemorrhoids		
Urtica pilulifera L.	Gidişken	Aerial part	Infusion, int.	Cancer	109963	0.08
Verbenaceae						
Verbena officinalis L.	Sultan otu	Aerial part	Crushed and mixed with milk, ext.	Abscess	109965	0.05
Vitaceae						

Vitis vinifera L. *	Üzüm	Fruit	Fruit molasses eaten, int.	Circulation problems, appetizer, milk enhancer for mothers	E.Ö.69	0.18
		Fruit	Dried fruit powdered and mixed with piper nigrum powder and honey eaten one spoon at night, int.	Cough		
		Tendril	Juice of tedril applied on hair, ext.	Hair extender		
Xanthorrhoeaceae						
Asphodelus aestivus Brot.	At otu, Yabani pırasa	Root	Grated and cooked with tarhana (Turkish soup mixture), applied on skin, ext.	Abscess	109969	0.13
		Leaf	Freshly applied on the skin, ext.	Wound healing		
Zygophyllaceae						
Tribulus terrestris L.	Sarı pıtrak	Aerial part	Decoction, int.	Edema, urinary system problems, heel cracked, hemorrhoids, kidney stone, muscle and joint pain, heart problems	E.Ö.71	0.24
		Aerial part	Crushed, ext.	Pain killer		

Bold taxa have been recorded for the first time as a medicinal plant in region. Underlined uses are different uses for the region.

**: Cultivated plants.*

4. DISCUSSION

The results of our research showed that there are some different usages of the plants recorded. It has been compared with other ethnobotanical studies conducted around the study area and different uses of 79 taxa from previous records have been determined. These different uses are indicated in Table 4 as underlined uses (17-47). *Asphodelus aestivus* was commonly used for wound healing and abscess in Savaştepe. It was used against ulcer and wound healing in Edremit Gulf – Balıkesir (29). It was used for hemorrhoids, peptic ulcer, stomach ache in Marmaris (22). *Teucrium chamaedrys* was commonly used for hemorrhoids in Savaştepe. It was used for abdominal pain and kidney stones in Edremit Gulf/ Turkey (29). *Arum elongatum* was commonly used for wound healing, rheumatism, egzema, hemorrhoids in Savaştepe. It was used for hemorrhoids in Gönen-Balıkesir (48). *Ruscus aculeatus* was commonly used for prostate, heart problems, enuresis in Savaştepe. It was used for kidney stones, nephritis in Kırklareli province (26). *Micromeria myrtifolia* was commonly used for cold. It was used for abdominal pain in Manisa province. *Thymbra spicata* was commonly used for diabetes, rheumatism, stomach disease, cough in Savaştepe. It was used for cold and abdominal pain in Manisa (17). *Echium plantagineum* was commonly used as an expectorant in Savaştepe. It was used for stomachache in Marmaris (22).

When compared with previous studies in West Anatolia, the local names are different in other ethnobotanical studies (Manisa, Kırklareli, Marmaris). The local name used for

Micromeria myrtifolia is "Ayaklı kekik" in Savaştepe, but the local names of the same plant are "Boğumlu çay" and "Dağ çayı" in Edremit (29); the local names of the same plant are "Eşek kekiği" and "Kekik" in Manisa (17). The local names used for *Ballota nigra* is "Çay otu" and "Kanser otu" in Savaştepe, it is called as "Grip otu" in Kırklareli (26). It was recorded that 12 taxa have commercial value. The local people collect them from nature to cell medicine companies. These plants are; *Arum elongatum, Ruscus aculeatus, Centaurea benedicta* (L.) L., *Silybum marianum, Quercus cerris, Quercus infectoria, Paeonia peregrina, Primula vulgaris subsp. rubra, Cydonia oblonga, Prunus avium, Populus nigra* and *Urtica dioica.* Identifying the plants that are traded and cultivating them can be an important source for the local people in Savaştepe.

Endemic *Acanthus hirsutus* is very commonly used for the treatment of fever in Savaştepe. *Acanthus hirsutus* is widely used for constipation, for wound healing, as an expectorant, and also used to treat painful disorders in Anatolia. The major components of *Acanthus hirsutus* are; acteoside, hirsutusoide, luteolin-7-O-beta-D-glucuronide. Antinociceptive activity of *A. hirsutus* has been reported (13,14). Endemic *Ferulago macrosciadea* is very commonly used for stomach problems in Savaştepe. The main component of the essential oil of *F. macrosciadea* is carcacrol methyl ether (78,1%) (15). *Onosma aucherana* is very commonly used for the treatment of wound healing in Savaştepe. *Onosma* species contain alkannin, shikonin, flavonoids, vanillic and ferulic acids. It is used for wound healing. It is analgesic, anti-inflammatory, and antibacterial (16).

5. CONCLUSIONS

As a result of this study medicinal uses of the plants in Savaştepe/ Balıkesir have been recorded for the first time. The mean age of the respondents was 55 years (within a range of 30–70 years). In the research area, local people were found to be using 163 medicinal plants from 53 families. Most commonly used plants were *Urtica dioica* , *Salvia fruticosa* , *Malva sylvestris*, *Matricaria chamomilla*, *Hypericum perforatum* , *Origanum vulgare* subsp. *hirtum*, *Thymus zygioides* , *Pinus brutia* , *Platanus orientalis* , *Raphanus raphanistrum* , *Rumex crispus* , *Rumex acetosella* The cultivation of valuable medicinal plants will help to protect nature and environment. It will create a new job opportunity for the local people.

The use value of every taxa and FIC value of important disease groups were calculated. The most common disease were categorized into 12 group. The highest FIC value was 0.852 for hemorrhoids. It represents that the plants which had been used for hemorroids are presumed to be the most effective in Savaştepe/Balıkesir.

163 medicinal plant taxa used for medicinal purposes in Savaştepe district. Among 163 taxa, 12 taxa have been recorded for the first time as a medicinal plant and also the different medicinal uses of 79 taxa have been recorded in Savaştepe.

The vernacular names of the plants can be varied. Recording the vernacular names of medicinal plants in varied geographies is of great importance for us to realize the richness of our culture (47). This study was carried out to preserve informations about the medicinal plants of Savaştepe and their traditional uses. This study constitutes a database for studies on the production of new herbal products.

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Clinical and Experimental Health Sciences

Electron Microscopic Gaba Evaluation in Hippocampal Mossy Terminals of Genetic Absence Epilepsy Rats Receiving Kindling Stimulations

Nuray Nukhet Imdat[1,2], Ozlem Tugce Cilingir Kaya[1], Zehra Nur Turgan Asik[3], Tugba Karamahmutoglu[3], Medine Gülcebi Idrizoglu[3], Dilek Akakin[1], Filiz Onat[3,4], Serap Sirvanci[1]

[1] Marmara University, School of Medicine, Department of Histology and Embryology, Istanbul, Türkiye.
[2] Current address: University of Health Sciences, Tepecik Education and Research Hospital, In Vitro Fertilization Clinics, Izmir, Türkiye.
[3] Marmara University, School of Medicine, Department of Medical Pharmacology, Istanbul, Türkiye.
[4] Current address: Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Medical Pharmacology, Istanbul, Türkiye.

Correspondence Author: Serap Sirvanci
E-mail: ssirvanci@marmara.edu.tr
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ABSTRACT

Objective: The hypotheses related to the fact of epileptic mechanisms are mainly based on excitation-inhibition imbalance in central nervous system. GAERS (Genetic Absence Epilepsy Rats from Strasbourg) is a well-known animal model of absence epilepsy, and frequently used in experimental studies. In the present study, we aimed to examine possible morphological and gamma-aminobutyric acid (GABA) density changes in GAERS hippocampus after electrical kindling stimulations.

Methods: All control and test group rats received 6 kindling stimulations. Rats were decapitated 1 h after the last stimulation. Ultrastructural GABA immunocytochemistry was used to evaluate GABA density quantitatively in mossy terminals of hippocampal CA3 region.

Results: GABA levels were less in kindling groups compared to their controls, and in GAERS groups compared to Wistar groups; mitochondrial and dendritic spine area ratios were greater in GAERS groups compared to Wistar groups, although all these evaluations were statistically nonsignificant. Depletion of synaptic vesicles was evident in the mossy terminals of kindling groups.

Conclusion: The reason of decreased levels of GABA found in the present study might be that GABA has been released from the synaptic pool rapidly at an early time period after the last stimulation, for compansation mechanisms. Depletion of synaptic vesicles observed in kindling groups shows that even 6 kindling stimulations have an impact of changing hippocampal morphology in trisynaptic cycle. The increased mitochondrial area in GAERS might be related to the increased mitochondrial activity. The increased dendritic spine area might be related to the increased performance of learning in GAERS. Our findings indicating that absence epilepsy and temporal lobe epilepsy have different mechanisms of epileptogenesis might be a basis for further experimental studies.

Keywords: Absence epilepsy, GAERS, hippocampus, kindling, GABA, immunocytochemistry

1. INTRODUCTION

Epilepsy is a common neurological disorder with a prevelance of 1% worldwide (1, 2). According to WHO data, there are about 50 million patients with epilepsy. The most common type is temporal lobe epilepsy (TLE) arising from limbic system. Another group is idiopathic generalized epilepsy. In the pathogenesis of absence epilepsy, included in this group, cortico-thalamo-cortical circuit plays a role.

Absence epilepsy and TLE is not commonly seen in the same patient and the reason is not well understood (3, 4). Genetic absence epilepsy rats from Strasbourg (GAERS), a well known animal model of absence epilepsy, is frequently used in epilepsy studies. In the present study, kindling application, which is used for generating TLE model, in GAERS is used to examine possible morphological and gamma-aminobutyric acid (GABA) density changes in hippocampal mossy fiber terminals (MFTs) at the electron microscopical level.

In a previous study, focal seizures in GAERS are investigated by using TLE model and it was demonstrated that Wistar controls had grade 5 seizures, however, GAERS had only grade 2 seizures although they were given 30 kindling stimulations (5). According to the data obtained from Marmara University, School of Medicine, Department of Medical Pharmacology, stimulating Wistar rats 6 times at their after discharge thresholds triggers grade 2 limbic seizures. This number of stimulations provides the same number of stimulations with that of GAERS and staying at the same seizure stage. Thus, in the present study, we tried to standardize possible morphological and GABA density changes in two groups.

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Hippocampal MFTs are giant terminals and contain both GABA and glutamate (6). It was shown that seizures induce a transient increase in glutamic acid decarboxlase 67 (GAD67), GABA synthesizing enzyme, and GABA levels 6 and 24 h after seizures (7).

Cortico-thalamo-cortical circuits in absence epilepsy and limbic structures in TLE were reported to play a role (8, 9). Although seizure circuits of these diseases are thought to be independent from each other, the fact that genetic absence epilepsy rats are resistant to kindling, points that related regions of the brain interact with each other. In the present study, we aimed to investigate possible morphological and GABA density levels at the electron microscopic level and enlighten the neuronal circuits playing role in the mechanisms of these diseases.

In early 1980s, Vergnes and Marescaux discovered that 30% of control Wistar rats showed bilateral synchronized spike and wave discharges on cortical EEG and inbred this strain and named them as GAERS (10). Although the mechanism of seizures in absence epilepsy is excessive GABAergic interactions in thalamo-cortico-thalamic circuit, studies reported the role of neuronal network in the hippocampus. Cellular and molecular mechanisms underlying epilepsy may also present themselves in other brain regions such as the hippocampus. Nehlig et al. found out that energy metabolism and metabolic activity increased in the limbic structures including hippocampus in adult and immature GAERS (11,12). Cerebral glucose utilization was shown to be increased in the primary limbic structures including the hippocampus in immature GAERS, before the appearance of spike and wave discharges (11). Another study demonstrated that basal levels of extracellular glutamate significantly increased in GAERS compared to nonepileptic control rats (13).

Eşkazan et al. reported that although Wistar control rats had stage 5 seizures, GAERS only had stage 2 seizures (5). It was suggested that converse mechanisms may occur in turning of limbic focal seizures in motor seizures, and underlying pathophysiological mechanisms of secondary generalized convulsions might differ from that of absence seizures. They also concluded that mechanisms of generalized absence seizures might be responsible for the resistance of secondary generalization of limbic seizures.

Disturbance underlying spike and wave discharges, especially a possible alteration in GABA function and/or cortical excitability, may change seizure susceptibility to different convulsive agents (14). Previous studies showed that GAERS were more susceptible to focal seizures induced by cortical GABA deprivation and generalized seizures induced by pentylenetetrazole, compared to the controls (15). Therefore, Vergnes et al. investigated susceptibility of GAERS and control rats to seizures by different convulsive agents (16). However, thalamocortical absence seizures are increased by increased GABAergic transmission (17) and they are thought as the seizures of inhibitory GABAergic system (16, 18, 19). It was reported that increase in seizure susceptibility in cortex and/or aberrant susceptibility to the drugs interacting with GABAergic transmission might point to the possible abnormalities which have a role in the generation of spontaneous spike and wave discharges in GAERS. It was also reported that a developmental imbalance between excitation and inhibition might be parallel in the generation of spike and wave discharges.

2. METHODS

Six-month-old male nonepileptic Wistar and GAERS rats were used in the present study. Animals were obtained from Marmara University, The Experimental Animal Implementation and Research Center. All experiments were done according to the national guidelines on animal experimentation and were approved by the Marmara University Local Ethical Committee for Experimental Animals (57.2012.mar). The animals were housed with free access to water and food in a 12-h light/dark cycle controlled room at 20±3 °C. Groups were as follows:

1. Sham-operated Wistar group (n= 4)
2. Sham-operated GAERS group (n= 4)
3. Kindling Wistar group (n= 4)
4. Kindling GAERS group (n= 4)

2.1. Stereotaxic Operation

Animals in the kindling group were anesthetized with ketamine (100 mg/kg) and xylazine hydrochloride (10 mg/kg), and stimulation/recording electrodes were placed into the basolateral amygdala and recording electrodes into the cortex with the aid of a stereotaxic instrument according to Paxinos and Watson Atlas (1998). Then, the animals were allowed for a recovery period of 10 days.

Electrode and cannula coordinates were as follows for EEG recordings: AP 2.0 mm, L 3.5 mm (frontal region), AP – 6.0, L 2.0 mm (parieto-occipital region). Tripolar recording electrodes were placed as referance electrodes on the cerebellum. For kindling procedure, bipolar stimulation/recording electrodes were placed into the basolateral amygdala (AP – 2.6 mm, L 4.8 mm, V – 8.5 mm).

2.2. Kindling

After the recovery period from stereotaxic surgery, 3 h of EEG recordings were obtained for determining the number and duration of basal spike and wave discharges. After that, at the beginning, a stimulation of 50 µA (80 Hz, 1 msn square waves, total duration 2 sn) was given for determining the afterdischarge threshold and continued until the first after discharge by increments of 25 µA. Stimulations were applied two times a day at the intensity of after discharge threshold determined for each animal. Seizures were evaluated according to Racine's seizure scale (20, 21). A total of 6 stimulations were given at the intensity of after discharge threshold for ensuring that all animals had stage 2 seizure.

One hour after the last stimulation, perfusion fixation procedure was applied under deep anesthesia.

2.3. Transmission Electron Microscopy

Deep anesthesia was performed by applying ketamine (100 mg/kg) and xylazine hydrochloride (10 mg/kg) for perfusion fixation and decapitation. Then, perfusion was performed by a perfusion pump at a speed of 50 ml/min by giving a fixative solution containing 0.5% paraformaldehyde, 2.5% glutaraldehyde and 0.1% picric acid in 0.1 M HEPES (pH 7.4). After that, animals were decapitated and brains were obtained and left in the same fixative at 4°C overnight. On the following day, 300-µm-thick sections were obtained by a vibratome (Leica VT1000S) and CA3 region of the hippocampus was dissected under a stereomicroscope. Tissues were post-fixed in 1% osmium tetroxide/1.5% potassium ferricyanide (1:1) for 30 min. Sections were incubated in 0.5% uranyl acetate for 30 min at room temperature in dark and then dehydrated in increasing series of ethanol and cleared in propylene oxide. Sections were incubated in propylene oxide/epon (1:1) mixture overnight. On the following day, sections were incubated in pure epon for 3 h and embedded in Epon 812. Polymerization was achieved by an incubator at 60°C for 24 h. One-µm-thick semi-thin sections were obtained by an ultramicrotome (Leica Ultracut R) and stained with toluidine blue for proper orientation. Thin sections were obtained on grids coated with Coat-Quick 'G' pen.

2.4. Immunogold Method

After drying the sections, they were washed in TBST tampon (0.1% Triton X-100, 0.9% sodium chloride, 0.05 M Tris tampon, pH 7.6). Grids were then incubated in anti-GABA antibody (Sigma A2052, 1:20000) at room temperature. Then the sections were washed in TBST pH 7.6 and pH 8.2 and incubated in 10 nm gold conjugated goat anti-rabbit IgG secondary antibody (Sigma G7402, 1:50) for 90 min. Sections were washed in TBST pH 7.6 and air-dried. After contrasting with uranyl aceate and lead citrate, sections were examined with a SIS Morada CCD camera attached JEOL 1200 EXII transmission electron microscope. MFTs of hippocampal CA3 region were examined for GABA density. For negative controls, primary antibody was omitted.

2.5. Quantitative and Statistical Analysis

MFTs in the stratum lucidum of CA3 region were determined and 10 MFTs were photographed from each animal. Micrographs were evaluated for GABA density with NIH Image Analysis (Image J) program. Terminal areas were calculated and 10 nm gold particles were counted. GABA density was calculated by dividing the number of gold particles by the terminal area. GABA density inside the vessel lumens were calculated for background staining. Final GABA density was determined by subtracting the bacground density from terminal GABA density for each terminal. Mitochondrial, vesicular and cytoplasmic GABA densities were calculated separately. Total mitochondrial area in each terminal was also determined. Similarly, dendritic spine areas synapsing with each terminals were calculated. Data were interpreted as mean±S.E.M. and evaluated with One-Way ANOVA and Tukey multiple comparison tests. Significance level was determined as $p<0.05$.

3. RESULTS

3.1. Light Microscopy

Normal hippocampal structure was observed in sham-operated Wistar (Figure 1a) and GAERS (Figure 1c) groups. Neuronal structure was appeared to be normal in both groups. Dark neurons were observed in kindling Wistar (Figure 1b) and kindling GAERS (Figure 1d) groups and these were interpreted as necrotic neurons.

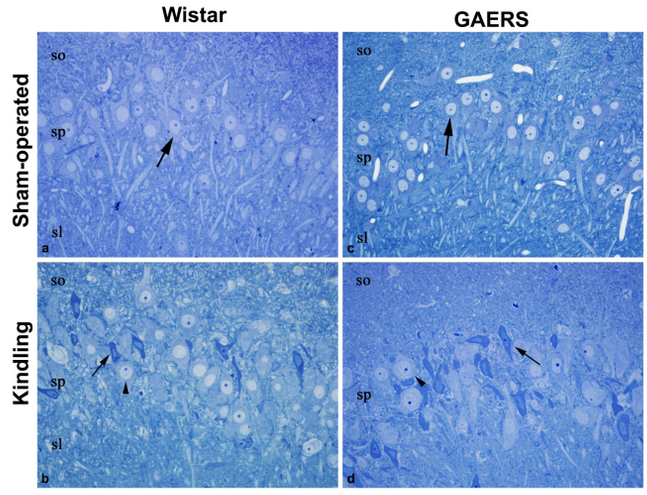

Figure 1. *Semi-thin sections of hippocampal CA3 regions. (a) Sham-operated Wistar group. Normal appearing neuronal cells (arrow). (b) Kindling Wistar group. Arrow: Necrotic dark neuron, arrowhead: normal appearing pyramidal cell. (c) Sham-operated GAERS group. Arrow: Normal appearing pyramidal cell. (d) Kindling GAERS group. Arrow: Necrotic dark neuron, arrowhead: normal appearing pyramidal cell. so: stratum oriens, sp: stratum pyramidale, sl: stratum lucidum. Toluidine blue staining. Original magnification: x400.*

3.2. Transmission Electron Microscopy

MFTs were differentiated as giant terminals including dense core vesicles (Figure 2a-f). They were observed to be GABA (+) in all groups. GABA immunoreactivity was positive on vesicle mebranes, in the vesicles, in the terminal cytoplasm, and on the mitochondria.

In kindling Wistar group, severe vesicle loss in MFTs was observed and the vesicles were seen as clusters (Figure 2b). Some vesicle clusters in or out of the MFTs were surrounded by membranes. Some MFTs were dark and appearead as degenerated (Figure 2c).

Vesicles were seen as clusters in MFTs in kindling GAERS group (Figure 2f) and some terminals appeared as degenerated

(Figure 2e). There were membrane clusters around MFTs, which appeared to be detached from the terminal. Vesicle clusters in or out of the MFTs were surrounded by lamellar membranes in some terminals.

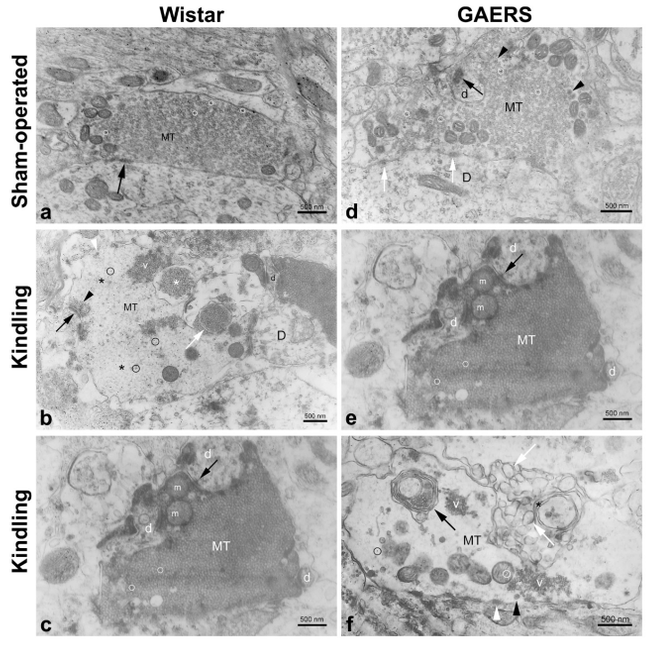

Figure 2. *Transmission electron micrographs. (a) Sham-operated Wistar group. Arrow: Desmosome-like junction between a MFT (MT) and a dendrite, circle: GABA immunoreactivity. (b) Kindling Wistar group. Degenerated MFT (MT). Black arrow: Asymmetric synapse between MFT and a dendritic spine, white arrow: vesicle cluster surrounded by a membrane, arrowhead: synaptic vesicles clustered around a synapse, asterisk: vesicle loss in MFT, v: synaptic vesicles, circle: GABA immunoreactivity, D: dendritic shaft, d: dendritic spine. (c) Kindling Wistar group. Degenerated MFT (MT). Arrow: Asymmetric synapse between MFT and a dendritic spine, m: mitochondria, circle: GABA immunoreactivity, d: dendritic spine. (d) Sham-operated GAERS group. MT: Normal appearing MFT, white arrows: desmosome-like junctions between MFT (MT) and a dendrite, black arrow: asymmetric synapse between MFT and a dendritic spine, circle: GABA immunoreactivity, arrowhead: dense core vesicle, D: dendritic shaft, d: dendritic spine. (e) Kindling GAERS group. MT: Degenerating MFT, black arrow: asymmetric synapse between MFT and a dendritic spine, circle: GABA immunoreactivity, d: dendritic spine, m: mitochondria. (f) Kindling GAERS group. Degenerating MFT (MT), v: vesicle clusters, black arrow: vesicle cluster inside MFT surrounded by lamellar membrane, white arrow: membrane fragments thought to be detached from MFT, asterisk: lamellar membranes out of MFT, black circle: cytoplasmic GABA immunoreactivity, white circle: mitochondrial GABA immunoreactivity, black arrowhead: dense core vesicle, white arrowhead: desmosome-like junction between MFT and a dendrite. Bars: 500 nm.*

3.3. Statistical Analysis

No significance was determined between the groups in parameters. However, the highest terminal area was determined in kindling Wistar group. A tendency to decrease in cytoplasmic, vesicular, and total GABA densities was found in kindling groups compared to their corresponding control groups, and in GAERS groups compared to Wistar groups; and a tendency to increase in mean ratio of mitochondrial area/MFT area and in ratio of spine area/MFT area in GAERS groups compared to Wistar groups.

Mean MFT area was 8.14±1.16 μm^2, mean vesicular GABA density 4.42±1.66, mean cytoplasmic GABA density 3.41±1.75, mean mitochondrial GABA density 1.25±0.43 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.59±0.005, and mean spine area/MFT area 0.07±0.01 in sham-operated Wistar group (Figure 3).

Mean MFT area was 8.08±0.78 μm^2, mean vesicular GABA density 2.50±0.68, mean cytoplasmic GABA density 2.42±0.76, mean mitochondrial GABA density 0.81±0.28 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.07±0.01, and mean spine area/MFT area 0.17±0.17 in sham-operated GAERS group (Figure 3).

Mean MFT area was 10.30±1.09 μm^2, mean vesicular GABA density 2.99±1.64, mean cytoplasmic GABA density 2.75±1.46, mean mitochondrial GABA density 0.58±0.18 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.06±0.001, and mean spine area/MFT area 0.08±0.02 in kindling Wistar group (Figure 3).

Mean MFT area was 7.89±0.76 μm^2, mean vesicular GABA density 1.60±0.31, mean cytoplasmic GABA density 2.11±0.70, mean mitochondrial GABA density 0.52±0.14 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.07±0.006, and mean spine area/MFT area 0.11±0.02 in kindling GAERS group (Figure 3).

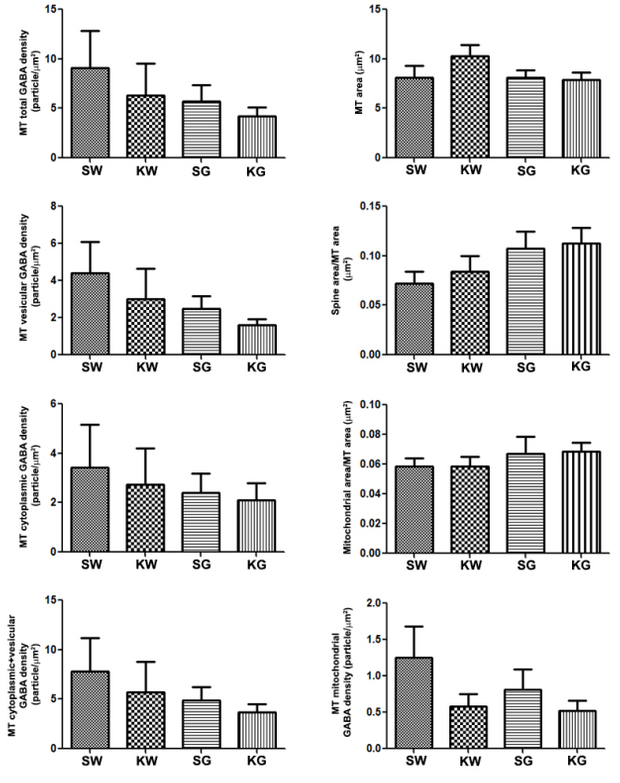

Figure 3. *Statistical analysis. SW: Sham-operated Wistar group, KW: kindling Wistar group, SG: sham-operated GAERS group, KG: kindling GAERS group.*

4. DISCUSSION

MFTs are giant terminals with a idameter of 4-10 µm. In rats, mean MFT area was found to be about 8.2 µm^2 (22). In another study, CA3 mean MFT area was found as 4.75±0.26 µm^2 in naive Wistar control rats and 4.45±0.30 µm^2 in naive GAERS and there was no statistical difference between two groups (23). Akakın et al. found that mean MFT area in CA3 region of sham-operated Wistar rats was 4.48±0.44 µm^2, 4.07±0.50 µm^2 in sham-operated GAERS, 4.29±0.18 µm^2 in kindling Wistar (stage 5 seizure), and 4.07±0.32 µm^2 in kindling GAERS (stage 2 seizure) groups (24). In the present study, we found that mean MFT area in CA3 region was 8.14±1.16 µm^2 in sham-operated Wistar, 8.08±0.78µm^2 in sham-operated GAERS, 10.30±1.09 µm^2 in kindling Wistar groups (6 stimulations); and 7.89±0.76 µm^2 in kindling GAERS group. Although there was no statistical significance, the highest terminal area was found in kindling Wistar group. The highest terminal area in this group was 30.6 µm^2 . This is a very high number for the area of MFTs. The reason for nonsignifacence in areas between the groups may be that this high number of area was not seen in all terminals. Pierce et al. examined MFTs in dorsal and ventral hippocampal dentate area in rats given pentylenetetrazole and had grade 5 seizures, and found no significance between the groups in MFT areas in dorsal hippocampus (25). However, they showed significant increase in MFT areas in ventral hippocampus in seizure group. Other studies demonstrated increased terminal areas after cerebellar parallel fiber lesion and septohippocampal deafferentation (26, 27). This increase was reported as a mechanism of compensation. We also concluded that there may be a similar compensation mechanism in the terminals with an increased areas in kindling Wistar group. Small, membrane-coated cytoplasmic fragments around MFTs in kindling groups may have detached from neighboring degenerating MFTs. Increased areas in some MFTs may be a result of a compensation mechanism for the function of these terminals. Another possibility is that degeneration might have caused swelling in the terminal. The vesicles were observed to occupy a small space in these terminals.

Excitatory amino acids play a role in cellular abnormalities in pathological conditions, as well as in normal neuronal functions because of their high concentration and wide distribution in the central nervous system. It was shown in a previous study on the toxic effect of glutamate that there was a delayed neuronal death in neurons exposed to glutamate for a short period (9). The most vulnerable region to glutamate toxicity in epilepsy is CA3 region of the hippocampus (28, 29). Degeneration in CA3 pyramidal neurons in kindling groups in the present study might be a result of excessive glutamate release from MFTs after the stimulations.

It was shown in previous studies that MFTs contain GABA, an inhibitory neurotransmitter, as well as glutamate, an excitatory neurotransmitter. Sandler and Smith reported for the first time the presence of GABA in MFTs in 1991 (6). The presence of GABA in CA3 MFTs in GAERS rats was demonstrated in the studies of Şirvancı et al. (23, 30) and Akakın et al. (24). Similarly, we also demonstrated the presence of GABA in MFTs of sham-operated and kindling Wistar and GAERS rats.

Akakın et al. found that GABA density was higher in kindling Wistar group compared to sham-operated Wistar and kindling GAERS groups (24). In that study, kindling Wistar group animals were decapitated 24 h after the last grade 5 seizure and kindling GAERS group animals after the last grade 2 seizure. In the present study, GABA density in MFTs were determined 1 h after the last grade 2 seizure. This might be the reason for no increase in GABA density in Wistar rats having grade 2 seizures. There was reported a rapid but transient GAD67 mRNA increase in granule cells of rats 6 and 24 h after kainic acid injection (7). In the present study, we observed a tendency to decrease in GABA density in kindling groups compared to the control groups, although no statistical significance was determined. This finding suggests that in the first hours after kindling stimulations, GABA might be decreased in MFTs. The reason for this might be that only 6 stimulations were given and GABA density was examined at very early hours after the last stimulation. GABA might be rapidly released from the terminal for compensation and this might decrease the GABA pool. Another reason for no difference in GABA density between the groups in the present study might be that kindling Wistar group had only grade 2 seizures like kindling GAERS group and they did not reach stage 5 seizure state. In the study of Akakın et al., it was found that Wistar rats had grade 5 seizures and GAERS rats had grade 2 seizures, althoug they were given 30 kindling stimulations. There was no significant difference in GABA density between kindling GAERS group having grade 2 seizures and sham-operated GAERS group. Similarly, there was no significant difference between kindling groups having grade 2 seizures and their controls. We thought that GABA might be released rapidly from the terminal in the first hours after kindling stimulations and then increased. Akakın et al. found that GABA density in MFTs of Wistar rats having grade 5 seizures increased 24 hours after the last grade 5 seizure compared to kindling GAERS group having grade 2 seizures. In the present study, we observed that GABA levels were increased in kindling Wistar group compared to kindling GAERS group but no statistical significance was found. The reason for this insignificance might be that animals had only grade 2 seizures and they were sacrificed 1 h after the last seizure.

In the present study, we found decreased GABA density in GAERS groups compared to Wistar groups but no statistical significance was found. MFTs synapse mostly with inhibitory neurons (31, 32-34). Şirvancı et al. examined the presence of GAD65/67, GABA synthesizing enzyme, in MFTs, and found no significant difference between the groups (35). However, there was reported to be a tendency to decrease in GAD65/67 in GAERS group. This finding is in line with the findings of the present study. Decreased GABA in MFTs of GAERS might decrease the disinhibition effect on inhibitory neurons, resulting in more effective function of these neurons. This

might be a reason for the resistance of GAERS to kindling stimulations.

GABA density in MFTs in the present study was evaluated as vesicular, cytoplasmic, and mitochondrial separately. Gold particles in the vesicles or vesicular membrane were evaluated as vesicular GABA pool, and the particles out of the vesicles as cytoplasmic pool. Gold particles on mitochondria represent mitochondrial GABA pool. Meshul et al. examined GABA pool in 3 regions, and reported that cytoplasmic pool might be evaluated together with the vesicular pool (36). Similarly, we evaluated these pools both separately and together.

In most regions of the brain, neurons are surrounded by dendritic spines. These spines are the main places of excitatory synaptic transmission and play a key role in the transmission in the brain. Distribution and structure of the spines are destroyed in many diseases and they play a role in plasticity (37-39). Şirvancı et al. found no significant difference in the area and number of dendritic spines in hippocampal CA3 and dentate regions between GAERS and nonepileptic controls (40). The reason for lack of difference in absence epilepsy was suggested to be due to different pathogenetic mechanisms in absence epilepsy and TLE. Drakew et al. generated an epileptic state with bicuculine and picrotoxin in hippocampal culture, and showed decrease in the number of dendritic spines of CA3 pyramidal neurons in the experimental group, but no difference was found in the areas between the groups (41). In line with the previous studies, in the present study, no significant difference was observed in the ratio of spine area/MFT area. However, there was a tendency to increase in ratio of spine area/MFT area in GAERS groups compared to Wistar groups. Hippocampus is involved in learning and memory (42). GAERS were reported to show better performance in learning compared to controls (43, 44). In the present study, we suggest that the tendency to increase in the ratio of spine area/MFT area in GAERS group might be related to the increased performance of this strain in learning abilities.

In the present study, we found increased ratio of mitochondrial ara/MFT area in GAERS groups compared to Wistar groups, however, no statistical difference was observed. In line with this finding, increased mitochondrial activity was reported in neurons and astrocytes in GAERS cortex (45), and increased glucose metabolism in GAERS hippocampus (46) was reported. Although the absence seizures were reported to be limited to thalamocortical circuit, this mutation in GAERS strain includes all brain cells (45). Therefore, increased ratio of mitochondrial area/MFT area in GAERS in the present study is in line with the increased mitochondrial activity and cerebral glucose utilization in this strain (11, 47).

Synaptic vesicle clustering in MFTs was previously shown in genetically epilepsy prone gerbil (48). Akakın et al. also reported vesicle loss and clustering in kindling groups (24). Similarly, in the present study, we observed severe vesicle loss in MFTs of kindling groups, and that other vesicles were clustered in some regions of the terminal. These findings show that even 6 kindling stimulations might result in structural changes in hippocampal trisynaptic circuit.

5. CONCLUSION

In conclusion, differences in the GABA density, mitochondrial area/MFT area, and spine area/MFT area between GAERS and control Wistar groups suggest that there are different epileptogenetic mechanisms between absence epilepsy and TLE. Our study might be a basis for future experimental studies.

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Clinical and Experimental Health Sciences

The Effect of Individual Education on the Participation of Relatives of Cancer Patients in General Health and Cancer Screenings: A One Group Pretest-Post Test Study

Habibe Ozcelik[1], Sebahat Gozum[1], Hasan Senol Coskun[2]

[1] Akdeniz University, Faculty of Nursing, Department of Public Health Nursing, Antalya, Türkiye.
[2] Akdeniz University, School of Medicine, Department of Medical Oncology, Antalya, Türkiye.

Correspondence Author: Habibe Ozcelik
E-mail: hozcelik@akdeniz.edu.tr
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ABSTRACT

Objective: The aim of this study was to evaluate whether or not there were changes in the behaviors of relatives of cancer patients such as learning the warning signs of cancer, engaging in cancer prevention strategies, and participating in screenings after education sessions in the clinic.

Methods: This one-group pretest-posttest study was conducted with 238 relatives in a medical oncology clinic in Antalya, Turkey. Individual education sessions were conducted as an intervention, banners were hung about cancer, and general health information on checkups in the form of leaflets were distributed to each relative regarding cancer screenings. Reminder messages were sent to individuals to participate in screenings one and two months after the first interview. The posttest data were collected by phone in the third month. Four questionnaires were prepared based on the literature and national cancer screening standards. The face validity of the tools was evaluated by three experts and 15 relatives who not included as participants.

Results: The rate of having a mammography increased from 19.8% to 33.9%, rates of having the fecal occult blood test increased from 16.9% to 23.8%, and rates of having the HPV test increased from 43.5% to 49.6%. The rate of having blood pressure checks within the last 12 months increased from 75.8% to 83.1%, rates of blood cholesterol measurement increased from 68.5% to 79%, and rates of blood glucose measurement increased from 70.2% to 79%.

Conclusion: Education provided to the relatives increased participation in screenings.

Keywords: Relatives, individual education, cancer screening, health behaviors, cancer prevention

1. INTRODUCTION

A wide range of studies have revealed the impact of family history in terms of the etiology of cancer in addition to environmental factors. People with a family history of cancer have a higher risk of cancer themselves (1-5). The National Comprehensive Cancer Network (NCCN) suggests different screening protocols for first and second degree relatives (FSDR's) other than community screenings (3). Taking family history regarding cancer from relatives up to the fourth degree can provide a more comprehensive view of the individual's health history, assist in evaluating potential risks, and help in providing preventive care (6). But, there is no screening standard for FSDRs in Turkey (7). According to the Turkey Health Survey data for 2016, the mammography rate during the previous two years was 16.1%, the Human Papillomavirus (HPV)/pap test rate during the previous five years was 25.6%, the Fecal Occult Blood Test (FOBT) rate was 11.4%, and the colonoscopy rate was 2.5% (7, 8). These rates show that participation in cancer screenings is relatively low.

The presence of individuals diagnosed with cancer in their immediate family, relatives, and acquaintances may make people more aware of cancer and more willing to think about cancer, to participate in cancer screening, and to learn about cancer. In the active treatment period of a diagnosed patient, educating the relatives about cancer in the clinic and directing them to a screening can be much more effective than the referrals made to asymptomatic people in the community. It has been emphasized that early diagnosis and treatment can be provided by determining the risk levels of individuals and participating in screening programs required for the appropriate age ranges (9). In a study conducted in Turkey in which the first-degree relatives of the patients diagnosed with breast cancer were specified as the sample group, the rate of participation in screenings

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was determined to be 18%, not different from the tendencies of the general population (10); whereas, in another study, it was revealed that relatives of patients diagnosed with lung cancer changed their attitudes positively because 10.2% of them began exercising, 13.8% of them paid more attention to nutrition, 21.1% of them quit smoking, and 2.8% of them showed interest in and participated in cancer screening (11). With respect to the other study, it was concluded that the rate of colonoscopy of the first degree relatives of patients with colorectal cancer (CRC) was low (22.2%) and that they were more likely to participate if the health motivation was increased. It is recommended that strategies should be developed to increase knowledge, awareness, and participation in CRC screening tests of first-degree relatives for patients diagnosed with CRC (12). In another study, first and second-degree relatives of breast cancer patients that knowledge of regarding inheritance characteristics of breast cancer and risk reduction strategies was moderate. But still majority of women have moderate or higher level of risk perception and are worried about getting breast cancer (13).

In a case-control study, while the participation rates of caregiver spouses in the colorectal, cervical, gastric, and breast cancer screenings were higher than the control group, no significant difference was found between controls for risky health behaviors and controls for chronic diseases (hypertension, diabetes, and hypercholesterolemia) (14). In another study, individuals with a family history of cancer showed no differences in terms of their preventive health behaviors such as routine screening, smoking, and physical activity behavior compared to the general population (15). But, in a systematic review, a clear link between breast cancer risk perception and some cancer preventive behaviour was determined (16). Whether it is biologically driven or not, a diagnosis of cancer in the family history may cause a person to think about cancer, participate in cancer and general health checkups, and being aware of the need for cancer screening. For this reason, oncology clinics can be used as an opportunity to inform and direct relatives of patients. Nurses can provide individual education to patient of relatives in here. Since the relatives of patients who are hospitalized in oncology clinics are both sensitive and a high-risk group due to their family history, they may benefit more from the training given about cancer prevention, participation in screenings and knowing the cancer signs.

The aim of this study was to evaluate the effect of individual education on the participation of relatives of cancer patients in general health and cancer screenings. The study was investigated the rates of awareness of the warning signs of cancer and rates of behaviors that support cancer prevention including cancer screening behaviors, general health checkups behaviors, and participation in post-referral scans in the relatives of cancer patients in Oncology Clinic. The answers to the following questions will be sought in this study;

- Is there a change in post-tests with respect to pre-tests in terms of knowledge of protection from cancer behaviours for the relatives of patients diagnosed with cancer?
- Is there a change in post-tests with respect to pre-tests in terms of actual practice of protection from cancer behaviours for the relatives of patients diagnosed with cancer?
- Is there a change in post-tests with respect to pre-tests in terms of the rate of knowledge of warning signs of cancer for the relatives of patients diagnosed with cancer?
- What is the rate of warning signs of cancer' for the relatives of the patients diagnosed with cancer?
- Is there a change in post-tests with respect to pre-tests in terms of the rate of participation in cancer screenings for the relatives of patients diagnosed with cancer?
- Is there a change in post-tests with respect to pre-tests in terms of the rate of participation in general health screenings for the relatives of patients diagnosed with cancer?

2. METHODS

This study, which evaluates the effect of an intervention in design of one-group pretest-posttest was conducted in the Medical Oncology Clinic in a university hospital. Chemotherapy, radiotherapy and palliative treatment are carried out in the 46-bed medical oncology clinic where the study was conducted. Relatives accompanies the patients in the clinic. There are informative educational materials for patients, and two nurses providing training. Relatives are only informed about the patient. The data collection was initiated on 01.06.2016 and continued for 15 months until adequate sample numbers were reached.

2.1. Ethical Approval and Informed Consent

Informed consent from the relatives of the patients and approvals from the clinic (dated 04.08.2016 with no. 60590709/ONK-2502) and from the ethics committee (dated 24.02.2016 with decision no. 161) were obtained in order for the study to be conducted. Ethical approval was obtained from the Clinical Research Ethics Committee of Akdeniz University, Antalya, Turkey.

2.2. Sample

The population of the study was comprised of the relatives of the patients diagnosed with all forms of cancer and hospitalized at the Medical Oncology Clinic. Relatives of all patients who were hospitalized with a diagnosis of cancer, whether biological or non-biological, who were over 30 years of age, who not to have a sort of mental disability, and who volunteered were included in the study. Since cervix cancer screening in Cancer Early Diagnosis, Screening and Training Centers (CEDSTC) starts at the age of 30, relatives aged 30

and over were included. Both biological and non-biological relatives were included in this study. One relative of each cancer patient was included the study. The biological relatives of the cancer patients were included in this study because of they were more at risk than the general population and the non-biological relatives would have more awareness than the general population. The sample size was calculated by using a formula appropriate for studies in which the actual number of the population is unknown, but the ratio of a variable is examined, and, for this particular study, the power was assumed to be 80%, and the error margin was assumed to be five % (17). For the related calculation, the reference value of a prior study (20%) was taken into consideration (18) and, with respect to this, 250 relatives of patients were included in the sample group. The data collection process continued until the specified number of a minimum of 250 individuals had been reached, and 270 individuals could for be reached the pre-test. Thirty two relatives could not be reached for the post-test level. For this reason, the comparison analyses of the pre-test and the post-test were conducted on 238 matched individuals.

2.3. Measurement

Four measurement tools were used to collect data. Questionnaires were prepared based on the literature and national cancer screening standards (7, 11, 12, 14, 15, 19-21) The face validity of the tools was evaluated by three experts, an oncologist, a physician from CEDSTC, and a public health nurse with a convenience sample of 15 relatives. Minor comments were then evaluated in the research team, and the final instruments were formulated. At baseline, we obtained sociodemographic information (Table 1) from a self-reported questionnaire and medical information from electronic records. After that, a questionnaire on the knowledge and application of behavior that support cancer prevention with 13 statements revealing the cancer prevention behavior of the relatives of cancer patients was prepared (Table 4).

A third questionnaire was used for the participants to evaluate whether they were aware of the warning signs of cancer and the status of having these symptoms (Table 5).

The fourth questionnaire was used for participants to determine whether they participated in general health checkups and cancer screenings (Table 2, 3).

The dependent variables of the study were knowing and doing cancer prevention behaviors of cancer patients' relatives, knowing the early danger signs of cancer, having of signs, participation in cancer screening and general health checkups. The independent variable of this study was the information and guidance to be made in the clinic.

Table 1. *Sociodemographic Attributes of the Patient's and Patient's Relative(s).*

Patient's Sociodemographic Variables (n=270)		**n**	**%**
Gender	Woman	103	38.1
	Man	167	61.9
Education	Illiterate	29	10.7
	Literate	9	3.3
	Elementary and Secondary School	148	54.8
	(Senior) High School	47	17.4
	College/University and above	37	13.7
Diagnosis of Patients'	Lung Cancer	69	25.6
	Gastric Cancer	39	14.4
	Colon Cancer	23	8.5
	Breast Cancer	17	6.3
	Pancreatic Cancer	17	6.3
	Brain Tumor	11	4.1
	Pharyngeal Cancer	11	4.1
	Others (malignant melanoma, over cancer, cervical cancer, renal cell carcinoma...)	83	30.7
Current treatment of the patient	Chemotherapy	161	59.6
	Radiotherapy	34	12.6
	Surgical	4	1.5
	Palliative care only	71	26.3
Patient's Relative(s) Sociodemographic Variables (n=270)			
Gender	Woman	237	87.8
	Man	33	12.2
Education	Illiterate	16	5.9
	Literate	17	6.3
	Elementary School	128	47.4
	Secondary School	28	104
	(Senior) High School	52	19.3
	College/University and above	29	10.7

Table 1. *(Continued)*

Marital Status	Married	244	90.4
	Single	26	9.6
Health Insurance	Available	252	93.3
	N/A	18	6.7
Income Status	My income is less than my expenditures	143	53
	My income is equal to my expenditures	118	43.7
	My income is more than my expenditures	9	3.3
Affinity degree to the patient	Spouse	137	50.7
	First Degree Relative (Daughter, Sister, Mother, Son, Brother)	98	36.3
	Others (Daughter-in-law, wife's sister-in-law, nephew/niece, maternal aunt, paternal aunt , son-in-law, cousine etc.)	39	15.4
Chronic illness	Available	113	41.9
	N/A	157	58.1
Frequent chronic diseases	Hypertension	63	23.3
	Diabetes Mellitus	35	13.0
	Thyrocele	23	8.5
	Respiratory system diseases such as asthma	9	3.3
Smoking habits	I have never smoked	172	63.7
	I smoke	59	21.9
	I have quitted smoking	39	14.4
Do you consider quitting if you are a smoker?	No	34	57.6
	I consider quitting smoking in a month	6	10.2
	I consider quitting smoking in 6 months	19	32.2
Did the family health center guide provide guidance to you for screening programs?	Yes	96	35.6
	No	174	64.4
Have you had a discussion with the physician who treated your relatives who had cancer that family history increased the risk of cancer?	Yes	36	13.3
	No	234	86.7
Did the physician who treated your relative who had cancer provide guidance to you for screening programs?	Yes	27	10
	No	243	90
Do you know that screening services are free of charge in CEDSTC?	Yes	130	48.1
	No	140	51.9
Have you been to cancer screenings sutiable for your age and gender prior to cancer diagnosis of your relative?	Yes	104	38.5
	No	166	61.5

Table 2. *Participation Status to Cancer Screenings and General Health Checkups.*

Participation status to cancer screenings*		**Pre-test n (%)**	**Post-test n (%)**	**P****
CBE***		55 (25.7)	77 (31.0)	**0.000**
Mammography (within the last 2 years)		49 (19.8)	84 (33.9)	**0.000**
Breast Ultrasonography		49 (19.8)	59 (23.8)	**0.013**
BSE**** (regular in monthly basis)		155 (62.5)	168 (67.7)	**0.007**
FOBT***** (within the last 2 years)		42 (16.9)	59 (23.8)	**0.007**
Colonoscopy (within the last 10 years)		27 (10.9)	30 (12.1)	0.219
Pap smear test (within the last 5 years)		108 (43.5)	123 (49.6)	**0.000**
Spiloma control		4 (1.6)	10 (4.0)	0.109
General Health Checkups*				
Blood pressure control	I have done it during the last 12 months	188 (75.8)	206 (83.1)	**0.000 %7.3 ↑**
Blood cholesterol measurement	I have done it during the last 12 months	170 (68.5)	196 (79.0)	**0.000 %9.5↑**
Blood glucose measurement	I have done it during the last 12 months	174 (70.2)	196 (79.0)	**0.000 %8.8↑**

**Only the number of participants and the percentage are given in the table. **McNemar analysis had been conducted. ***CBE: Clinical Breast Examination, ****BSE: Breast Self-Examination, *****FOBT: Fecal Occult Blood Test.*

Table 3. *The Reasons for not Having Screening for Breast Cancer, Colorectal Cancer and Cervical Cancer.*

The reasons for not having screening for breast cancer (n=238)	**n (%)**
Not within the suitable age range for screenings	35 (14.1)
The relative of the patient is a man	24 (9.7)
I was not aware that I should have had a screening	**50 (20.2)**
I did not know where to go and how to have a screening	**49 (19.8)**
I have procrastinated	**48 (19.4)**
I did not know that this service was complimentary of charge	32 (12.9)
I did not have time	25 (10.1)
I was afraid that a malady would come up	22 (8.9)
I did not have the opportunity since I am giving caretaking for my patient	17 (6.9)
I was ashamed	5 (2.0)
The procedure is painful	4 (1.6)
The reasons for not having screening for cervical cancer (n=238)	**n (%)**
Not within the suitable age range for screenings	7 (2.8)
The relative of the patient is a man	24 (9.7)
I am not sexually active	5 (2.0)
I did not know where to go and how to have a screening	**34 (13.7)**
I was not aware that I should have had a screening	**33 (13.3)**
I have procrastinated	**28 (11.3)**
I was afraid that a malady would come up	20 (8.1)
I did not know that this service was complimentary of charge	17 (6.9)
I did not have time	12 (4.8)
I did not have the opportunity since I am giving caretaking for my patient	12 (4.8)
I had histerectomy before	11 (4.4)
I was ashamed	6 (2.4)
The procedure is painful	1 (0.4)
The reasons for not having screening for CRC* (n=238)	**n (%)**
Not within the suitable age range for screenings	102 (41.1)
I was not aware that I should have had a screening	**77 (31.0)**
I did not know where to go and how to have a screening	**52 (21.0)**
I have procrastinated	**29 (11.7)**
I did not know that this service was complimentary of charge	28 (11.3)
I was afraid that a malady would come up	18 (7.3)
I did not have the opportunity since I am giving caretaking for my patient	14 (5.6)
I did not have time	10 (4.0)
I was ashamed	5 (2.0)
The procedure is painful	2 (0.8)

**CRC: Colorectal Cancer.*

Table 4. *Knowledge and Practice of Cancer Prevention Behaviours (n=238)*

Knowledge and practice of cancer prevention behaviours*		**Pre Test n (%)**	**Post Test n (%)**	**P****
Increase consumption of fresh vegetables and fruits	Knows	177 (74.4)	232 (97.5)	**0.000**
	Practices	218 (91.6)	233 (97.9)	**0.001**
Increase consumption of fiber food	Knows	168 (78.6)	231 (97.1)	**0.000**
	Practices	204 (85.7)	235 (98.7)	**0.000**
Increase consumption of vitamin A and C	Knows	167 (70.2)	230 (96.6)	**0.000**
	Practices	207 (87.0)	237 (99.6)	**0.000**
Decrease consumption of fats	Knows	159 (66.8)	224 (94.1)	**0.000**
	Practices	175 (73.5)	206 (86.6)	**0.000**
Decrease consumption of salty, additives and nitrite including foods and smoked food	Knows	205 (86.1)	234 (98.3)	**0.000**
	Practices	200 (84)	224 (94.1)	**0.000**
Maintenance of ideal body weight	Knows	182 (76.5)	222 (93.3)	**0.000**
	Practices	77 (32.4)	75 (31.5)	0.727
Not smoking or quit smoking	Knows	231 (97.1)	236 (99.2)	0.125
	Practices	188 (79.0)	196 (82.4)	**0.021**
Keeping away from smoking free environments in order not to breathe in cigarette smoke	Knows	233 (97.9)	237 (99.6)	0.125
	Practices	197 (82.8)	209 (87.8)	**0.000**
Not consuming alcohol or quitting	Knows	212 (89.1)	232 (97.5)	**0.000**
	Practices	221 (92.9)	228 (95.8)	**0.039**
At least 30 minutes of exercise at least three days a week	Knows	176 (73.9)	225 (94.5)	**0.000**
	Practices	112 (47.1)	132 (55.5)	**0.001**
Not being exposed to harmful solar rays – protection from sun	Knows	201 (84.5)	226 (95.0)	**0.000**
	Practices	208 (87.4)	232 (97.5)	**0.000**
Having protective sexual intercourse (monogamy, using condoms with multiple partners)	Knows	174 (73.1)	215 (90.3)	**0.000**
	Practices	232 (97.5)	236 (99.2)	0.125
Sleeping for 6-8 hours a day	Knows	164 (68.8)	217 (91.2)	**0.000**
	Practices	199 (83.6)	234 (98.3)	**0.000**

**Only the number and rate individuals who know and practice are given in the table. **McNemar analysis is applied.*

Table 5. *Warning Signs of Cancer (n=238)*

Warning Signs of Cancer*		Pre Test n (%)	Post Test n (%)	P**
Weight loss (losing more than 5% of body weight within a month)	Knows	171 (71.8)	224 (94.1)	**0.000**
	Available	4 (1.7)		
Fever (recurrent fever and infection)	Knows	103 (43.3)	195 (81.9)	**0.000**
	Available	3 (1.3)		
Malaise and prostration other than the reason of being obliged to caring for the patient in the hospital (decrease of performance, lack of energy, excessive sleepiness, insomnia)	Knows	139 (58.4)	204 (85.7)	**0.000**
	Available	39 (16.4)		
Pain (unknown origin, intractable pain nonresponsive to treatment)	Knows	112 (47.1)	204 (85.7)	**0.000**
	Available	6 (2.5)		
Mass felt in breasts or in body	Knows	218 (91.6)	235 (98.7)	**0.000**
	Available	23 (9.7)		
Variations on skin (jaundice, livering and rubescence on skin)	Knows	134 (56.3)	215 (90.3)	**0.000**
	Available	5 (2.1)		
Bleeding/ haemorrhage (blood in pituitary, urine, faeces or abnormal vaginal bleeding)	Knows	177 (74.4)	234 (98.3)	**0.000**
	Available	7 (2.9)		
Change of defecation habits (diarrhea lasting longer than 2-3 weeks, defecation twice or less than twice per week, transition from diarrhea to intestinal obstruction)	Knows	124 (52.1)	220 (92.4)	**0.000**
	Available	22 (9.2)		
Persistent cough nonresponsive to treatment	Knows	135 (56.7)	202 (84.9)	**0.000**
	Available	5 (2.1)		
Alteration of body marks and papillomas	Knows	176 (73.9)	231 (97.1)	**0.000**
	Available	20 (8.4)		

**Only the number individuals who know about the risks are given in the table. The number and rate of individuals who have the risks are given. **McNemar analysis is applied.*

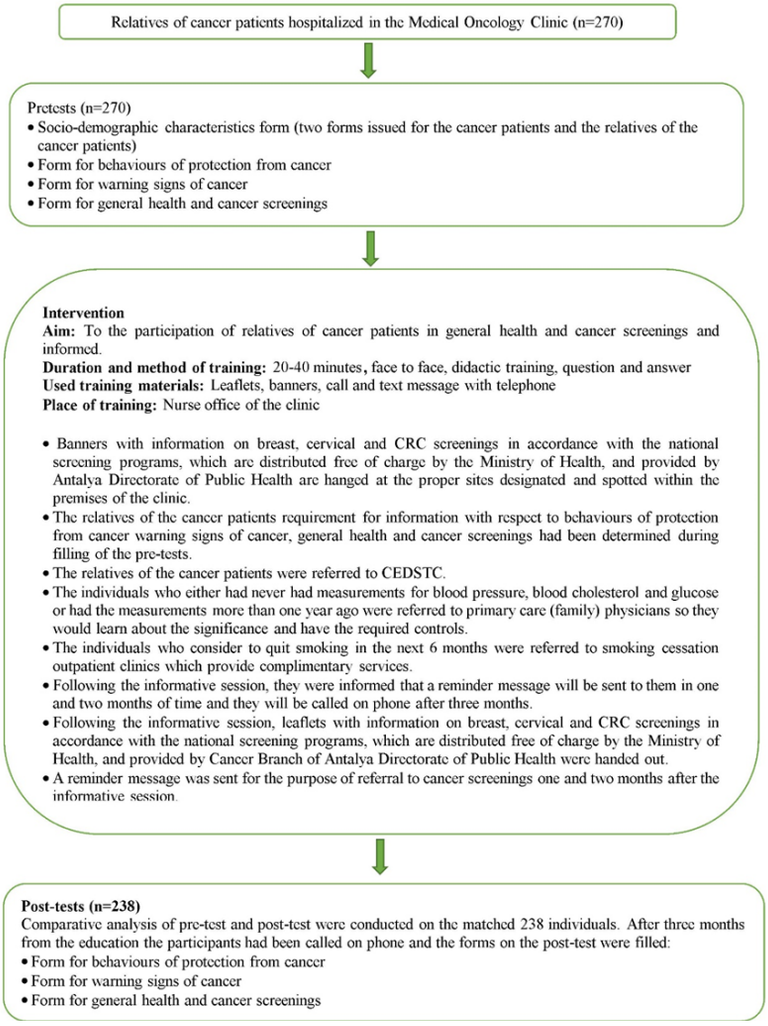

Figure 1. *Flow chart of the study*

2.4. Intervention Procedures

Banners with information on breast, cervical, and colorectal cancer screenings in accordance with the national screening programs, which are offered free of charge by the Ministry of Health and provided by Antalya Directorate of Public Health, were hung at the proper sites designated and visible within the premises of the clinic. At least one of the participants was chosen, and she/he was educated with respect to behaviors that support cancer prevention including knowledge of the warning signs of cancer, general health checkups, and cancer screenings was specified during the completion of the pre-tests. Indispensable, detailed was given to the individuals who lacked information during the data collection process. The data was collected by the first researcher, an oncology nurse, by the face-to-face interview method. Each individual education interview lasted between 20 minutes and 40 minutes depending on the level of education and the perception skills of the participant. The interventions made are explained with the flow chart of the study (Figure 1). Each subject was enriched by examples in accordance with the needs of the participant; thus, clarification and understanding of the matter was realized. Each relative of the patients was referred to CEDSTC that offer cancer screening free of charge according to age, gender, and risk factors. In this individual education, reference was made to the national screening program guide.

The individuals who either had never had measurements for blood pressure, blood cholesterol, and blood glucose or had the measurements more than one year ago were referred to primary care (family) physicians so they would learn about the significance of these tests and have them performed. The individuals who was considering quitting smoking in the next six months were referred to smoking cessation outpatient clinics which provide complimentary services. Following the education session, they were informed that a reminder message would be sent to them after one month and again after two months, and they would be called on phone after three months. Following the education session, leaflets with information on breast, cervical, and colorectal cancer screenings in accordance with the national screening programs were handed out; these are distributed free of charge by the Ministry of Health and provided by the cancer branch of the Antalya Directorate of Public Health. A reminder message was sent for the purpose of a referral to cancer screenings after one month and again after two months after the education session. The post tests were completed by telephone three months after the individual education intervention in the clinic.

2.5. Statistical Analysis

Comparative analysis of pre-test and post-test were conducted on the matched 238 individuals. Data were analyzed using IBM SPSS Statistics 20. Frequency distributions were determined for categorical variables. The relationship between two independent categorical variables was examined with c^2 tests. The McNemar test was used to evaluate and analyze the relationship between the pre-test and post-test for the categorical data. Statistical significance level was accepted as $p<0.05$.

3. RESULTS

The average age of 270 cancer patients hospitalized in the Oncology Clinic who had participated in this particular study was 59.4±12.0 (min=21, max=89); 61.9% of them were men, 54.8% of them were elementary school graduates, 25.6% of them were diagnosed with lung cancer, the elapsed time after the diagnosis was 19.4±26.3 months (min=1, max=132), and 59.6% of them were receiving chemotherapy (Table 1).

The average age of the 270 relatives of the cancer patients who were involved in the study was 51.16±9.85 (min=30, max=70). 87.8% of them were women, 47.4% of them were elementary school graduates, 90.4% of them were married, 93.3% of them had health insurance, 43.7% had income equal to expenditures, 50.7% of them were wives of the cancer patients. 21.9% of the smoked cigarettes. 35.6% of them were referred to cancer screening programs by the primary care (family) physician/nurse whereas 10% of them were referred to cancer screening programs by the physician in charge of treatment of the cancer patient, and 13.3% of them discussed the increase in cancer risk when there is cancer in their family history with the physician in charge of treatment of the cancer patient. 48.1% of them knew that cancer screening services are provided on a complimentary basis from CEDSTC, and 38.5% had their cancer screening done prior to the cancer diagnosis of the relative (Table 1).

The participants' rates of clinical breast examination, mammography, breast ultrasonography, breast self-examination, screening for HPV, and FOBT doing behaviors frequency increased as significant ($p<0.05$). According to the pre-test mammography, HPV screening and FOBT rates were 19.8%, 43.5%, and 16.9%, respectively, whereas the rates increased up to 33.9%, 49.6%, and 23.8%. According the evaluation and analysis of general health checkups, the rate of individuals who had their blood pressure taken during the last 12 months increased by 7.3%, those who had their blood cholesterol measurement increased by 9.5%, and those who had their blood glucose measured increased by 8.8%. (Table 2). There was not a significant change in terms of body-mass index (BMI) measurement [for pre-test and post-test respectively 28.4±5.2 (min=18.2, max=49.1), 28.3±5.0 (min=18.2, max=49.1)]

The participants stated the three most prominent reasons for not having breast cancer, CRC, and cervical cancer screenings as not being aware of the necessity to have a screening, not knowing where to apply for a screening or how to get the screening done, and being inclined to procrastination (Table 3).

An increase was observed in knowledge of behaviors that support cancer prevention other than not smoking, quitting smoking, and keeping away from heavy smoking environments**,** and the rates in the range of 66.8% and 97.96% before the intervention increased to the range of

90.3% and 99.6% after the intervention. An increase was observed in knowledge of behaviors that support cancer prevention other than maintaining ideal body weight and having protective sexual intercourse, and the rates in the range of 32.4% and 97.5% before the intervention increased to 31.5% and 99.6% after the intervention (Table 4).

The knowledge level of the participants who participated in the study on the warning signs of cancer showed a significant change in terms of all subjects during the post-test. The rates of knowledge of the warning signs of cancer were between 43.3% and 98.7% before the intervention whereas the range increased to between 84.9% and 98.7% after the intervention. The presence of the warning signs of cancer varied between 1.3% and 16.4% (Table 5).

4. DISCUSSION

4.1. Participation in Breast Cancer, Cervical Cancer, and CRC Screenings

In the study, a significant increase in the rates of breast, cervical, and CRC screening of the relatives of cancer patients who were hospitalized in the Oncology Clinic was determined. More than half of the participants stated that they had done breast self-examination during the pre-test and post-test. However, the mammography rate, which is deemed the gold standard for breast cancer screening, was lower than expectations according to the pre-tests. According to 2016 Turkey Health Survey data, the mammography rate for women in the last two years was 16.1%, the HPV test rate in the last five years was 25.6%, and the rate of FOBT during the last two years was 11.4% (7, 8). Within the scope of this study, participation in mammography screening and FOBT was found to be slightly above the average in Turkey for individuals who have a cancer diagnosis in their family history whereas the rate for HPV screenings were almost twice as much. This situation may be explained by the fact that cervical cancer screenings are initiated at before more than breast and colorectal cancer screening in Turkey, and cervical cancer screenings are easily accessible in numerous family health centers. With respect to a prior study conducted in the same hospital, the mammography rate for the participants was 18% (10) and, although the rate increased up to 19.8%, the participation was still insufficient for the group prone to a high risk of breast cancer.

With respect to the findings of this particular study's pre-test, the participation rates in screenings was slightly higher than the screening rates of the general population in Turkey who have a normal (middle) risk (7, 8) and the rates stated in other studies (10, 19). Participants in the study were better at participating in cervical cancer screening, but four out of five did not participate in breast and CRC screenings. In a study conducted in Australia, approximately 30% of first-degree relatives had undergone CRC screening in the last five years (22). In a comparative study in South Korea, it was determined that the rates of participation of the spouses of cancer patients in breast, cervical, and CRC screenings were higher than the general population (14). These results suggest that initiatives are needed to provide opportunities for high risk groups to participate in such screenings, particularly breast cancer and CRC screenings.

In this study, participation in breast, cervical, and CRC screenings increased with the help of education sessions in the clinic and referrals (Table 2). Similarly, initiatives in clinic areas such as training and education during the other studies (23) consultancy (23-25), and referral (23-26) increased participation in breast, cervical, and colorectal cancer screenings. Based on the studies, it can be asserted that individuals with a family history have similar rates of participation in cancer screening as other individuals, which was lower than expected, but interventions are effective in increasing participation in screenings. The fact that perceived sensitivity in this group, is high may have had a positive effect on the result. Nurses are the ideal professionals for mobilizing an aware group in clinical settings.

4.2. Participation in General Health Checkups

The participation rates of the participants involved in the study for the previous 12 months in terms of measurements based on blood pressure, blood cholesterol, and blood glucose were much better compared to cancer screenings and had been increased through the medium of interventions (Table 2). The rate of individuals who had blood pressure checks, blood cholesterol measurement and blood glucose measurement increased. In a study conducted in South Korea, it was determined in general health checkups that the participation of spouses who cared for cancer patients was not different from the comparison group (14). As a result, the interventions in our study group, who had high awareness due to family history, increased their participation in general health checkups and cancer screening.

4.3. Reasons for Not Having Done Cancer Screenings

When the participants' level of knowledge about cancer screening was examined, it was concluded that about half of them knew that cancer screening services are given free of charge in CEDSTC, which implements the National Cancer Control Program, and 35% of them were directed to cancer screening by the family health center employees. The participants stated that the three most prominent reasons for not having cancer screenings as not being aware of the necessity to have a screening, not knowing where to apply and how to get the screening done, and being inclined to procrastination. Although according to the results of research investigating the lack of information about cancer screenings in Turkey varied with respect to the regions where the data was collected, knowledge of breast, cervical, and CRC was in the range of 17% and 51% (27, 28). Similarly, in a study conducted in the USA, it was concluded that the most important obstacle to participating in screening for colorectal cancer was not their provider had not recommended it (29).

The reason that the lack of awareness in terms of breast and CRC was higher in comparison to cervical cancer is thought to be related to the fact that cervical cancer is started the earliest the national cancer screening program (21). Cervical screening starts at an earlier age than others and is also applied in family health centers. We should aim to eliminate barriers by means of initiatives by determining the participation barriers to screenings.

4.4. Behaviors That Support Cancer Prevention

When the behaviors of the participants involved in our study regarding behaviors that support cancer prevention were evaluated, we observed that not smoking or quitting smoking and keeping away from heavy smoking environments were the best-known subjects, so a significant increase could not be determined. The high initial knowledge of the importance of not smoking and avoidance of heavy smoking environments suggested that public health programs (30) for tobacco control promoted by the Ministry of Health had been effective. The fact that there is a significant increase in the rates of knowing the anti-cancer behaviors such as being aware of the need for good nutrition, limiting alcohol consumption, doing exercise, avoiding excessive sun exposure, having safe sexual intercourse, and sleeping for at least 6-8 hours a day indicated that the education provided within the scope of this study was effective.

Studies show that the behaviors of the individuals with a family history of cancer were based on anti-cancer elements such as physical activity, good nutrition, not smoking, and weight control are not different from those in the larger society (15, 20), yet they revealed that interventions with this group were effective (20, 26, 31). Similarly, in our study, a significant increase was determined in terms of anti-cancer behaviors in terms of subjects other than maintaining ideal body weight and having protective sexual intercourse. Lack of change in these two areas could be explained by the fact that weight loss is a time-consuming action, and the participants were monogamous. On the other hand, there was a significant increase in quitting smoking. This shows that the education given about smoking cessation in outpatient clinics is effective and that individuals who are ready to quit smoking will have an increase in the rate of using smoking cessation outpatient clinics. In this study, the higher rates of anti-cancer behaviors may be explained by the fact that the everyone in the study group was related to a cancer patient. When family members observed the symptoms of the patient in the clinic, their awareness increased, and their positive health behaviors were developed; thus, the education sessions were effective for the aware group.

4.5. Knowledge of the Warning Signs of Cancer

While the rate of the participants' knowledge of the warning signs of cancer was in the range of 43.3% to 91.6% before the intervention, it ranged between 84.9% and 98.7% after the intervention. This result indicated that the individual education initiated in the clinic had been effective. The most well-known signs include a lump in the breast, bleeding, changes in body marks and warts, and weight loss while lesser known signs include fatigue, persistent cough, skin changes, change in defecation habits, and pain and fever. The studies conducted in Turkey, it was determined that the rate of knowledge of the signs of cancer was lower than in our study (19, 27). In our study, the fact that the rate of knowledge of the signs of cancer was higher than in the other studies suggested that the participants may have learned by observing the signs experienced by the patient. Our education interventions may have affected the learning process, and the experiences of other patients and their relatives may have affected their learning about the signs of cancer.

Limitations

The biggest limitation of this study was that the study was done with all biological and non-biological relatives of cancer patients (spouse, uncle, in laws, etc.). In this study, we worked with relatives whom we thought would be aware due to the diagnosis of cancer. In future studies, we recommend that biological relatives and non-biological relatives should be included in the study separately. In addition, the pre-test-post-test design is the only group in which the effect of an initiative was evaluated. When evaluating the results of this study, it should be noted that only face validity was used for the questionnaires used. In future studies, it is recommended more validity and reliability evidence for surveys be provided.

5. CONCLUSIONS

With the help of education sessions and referral lasting for nearly half an hour to relatives of hospitalized cancer patients and reminder messages to them with respect to cancer screenings in the following one or two months, the relatives of cancer patients were observed to have increased knowledge, increased inclination toward anti-cancer action and prevention behaviors, increased recognition of cancer signs, and increased participation in general health checkups and cancer screenings suitable for their age and gender. The individual education and guidance given by the nurses to the relatives while the cancer patient is lying in the clinic is effective. In order to increase the participation of individuals with a family history of cancer in general health and cancer screening, relatives of patients in oncology clinics can be considered as the target population. Educational programs can be created for this population.

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Competing Interests

The authors declare that they have no competing interests

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Authors' Contributions

OH, GS and CHS conceived and designed of study. OH acquired, analysed and interpreted the data. OH and GS analysed and interpreted the data. OH, GS and CHS drafted the manuscript. All authors read and approved the final manuscript.

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Clinical and Experimental Health Sciences

Rosacea-Specific Quality of Life Scale (RosaQoL): The Study of Adaptation and Validation for Turkish Rosacea Patients

Mustafa Tosun[1], Yalcin Karagoz[2]

[1] Sivas Cumhuriyet University, Faculty of Medicine, Department of Dermatology, Sivas, Türkiye.
[2] Duzce University, Faculty of Business, Department of Health Management, Duzce, Türkiye.

Correspondence Author: Mustafa Tosun
E-mail: mustafatosun@cumhuriyet.edu.tr
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ABSTRACT

Objective: Rosacea-Specific Quality of Life Scale (RosaQoL) developed specifically for rosacea. The aim of the study was to adapt the RosaQoL Scale that is specific to Rosacea used in evaluating the quality of life of patients with Rosacea into Turkish, and evaluate its validity and reliability.

Methods: The RosaQoL Scale is a 21-item index that is specific to Rosacea, and was developed originally in English. The Turkish Scale, which was created after the clinical examinations of the patients, was applied to the patients. A total of 285 people, 240 females (84.2%) and 45 males (15.8%), who were diagnosed with Rosacea, admitting to the dermatology clinic between May 2019 and August 2019 were included in the study.

Results: The mean age of the patients was found to be 44.8 ± 12.5 years in the study. The internal consistency of the scale (Cronbach's Alpha Coefficient) was found to be 0.952. The correlation coefficient was calculated as r=0.988 in the test-retest reliability. The total RosaQoL score was 62.4 ± 11.5 (Mean ± SD), and the total DLQI score was 7.85 ± 5.04 (Mean ± SD). For validity analysis, the correlation coefficient between RosaQoL and DLQI was calculated as r=0.411 ($p<0.05$).

Conclusion: The Turkish version of the RosaQoL was valid and reliable for evaluating the quality of life of Turkish Rosacea patients.

Keywords: Quality of Life, Turkish version, Validation, Reliability, Rosacea

1. INTRODUCTION

Rosacea is a common and chronic inflammatory skin disease that involves the middle part of the face. Although its prevalence varies among countries, it ranges between 1% and 20%, is more common in women between the ages of 30-50 and in people who have skin phenotype as 1-3. It can be seen in the central region of the face, which is always visible, in various clinical forms with erythema, telangiectasia, rhinophyma, papules, and pustules (1, 2). There are 4 subtypes of Rosacea identified as Erythematotelengiectatic, Papulopustular, Phymatous, and Ocular (3).

Since Rosacea is a chronic disease progressing with attacks through various triggering environmental factors, and is in the visible area of the face, it brings serious limitations to the daily lives of patients. It affects the quality of life of patients and causes psychosocial problems (4).

Studies conducted on Rosacea showed that patients complain about embarrassment, disappointment, and low self-confidence scores. Patients face rude comments, jokes, and misunderstandings from their circles (5).

If merely the physical symptoms and findings are considered when the severity of Rosacea is evaluated, the perception of the patient regarding his/her disease is not evaluated. For this reason, quality of life scales was developed to measure the quality of life to enable patients to express their own perceptions. In this way, it is ensured that the psychosocial condition and quality of life are evaluated along with the dermatological findings in determining the severity of the disease (6, 7).

The Rosacea-specific Quality of Life (RosaQoL) Scale is a disease-specific scale that is employed for evaluating the quality of life of patients with Rosacea, and was developed by Nicholson et al. in 2007. The scale consists of 21 questions and 3 sub-dimensions (8). The validity and reliability of the scale were shown for many languages, and was used in studies conducted in many countries worldwide (9, 10).

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The DLQI Scale was developed in 1994 by Finlay and Khan. Its reliability and validity study for Turkish was conducted by Öztürkcan et al. It is a practical questionnaire form that consists of 10 short and easily understandable questions on emotions, symptoms, daily activities, leisure time activities, school and work life, personal relations, and treatment parameters, and is prepared to understand the effects of the existing dermatological disorders on the life of the patient. The answers consist of "not relevant / none, little, more, and too many" options with a Likert-type scale. In the evaluation of the scores, 0, 1, 2 and 3 points are given for these answers, respectively; and the resulting scores are added up. In this way, the minimum score is obtained as 0, and the maximum score is 30 (11, 12).

There is no specific scale that is used for Rosacea in our country. For this reason, the purpose of our study was to adapt the RosaQoL Scale, which is used commonly in other countries, into Turkish to evaluate the quality of life of patients with Rosacea.

2. METHODS

2.1. Ethics

For the study Sivas Cumhuriyet University ethics committee permission was received (2019-04/53). The purpose and contents of the study were explained to patients, and informed consent forms were received from volunteering ones. The study was conducted in line with the World Medical Association Declaration of Helsinki, Regulation of Patient Rights, and Ethical Rules.

2.2. Subjects

A total of 285 patients (240 females, 45 males) diagnosed with Rosacea admitting to dermatology clinic between May and August 2019 were included in this study.

Patients who were illiterate, who had a history of psychological diseases, or conditions which could affect the ability to understand the conditions of the study, and patients who were under the age of 18 were excluded from the study.

2.3. Rosacea-specific Quality of Life Scale (RosaQoL)

It was developed by Nicholson et al. in 2007. The scale consists of 21 questions and 3 sub-dimensions as the Emotions Dimension (7 items), Functions Dimension (3 items), and Symptoms Dimension (11 items). The answer options are structured in 5-Point Likert style as "never, rarely, sometimes, often, and always". The source language of the scale is English. The scale determines the specific problems and expectations of patients with Rosacea about their disease, enabling the doctor to record the viewpoints of patients (8).

2.4. Translation Process

– Communication was established with Dr. Chen (the responsible author), who is one of the authors of the scale, and the necessary permission was obtained for the scale to be adapted into the Turkish Language and to conduct its validity and reliability study.

– Firstly, the scale was translated into Turkish by 3 experts, two of whom were in the field of dermatology and one in the field of linguistics. The 3 texts obtained in this way were converted into a common text by 2 different dermatologists. This text was then translated into English by a bilingual person.

– The English form was then sent to Dr. Chen (the responsible author), and his opinions were received.

– Then, the English of the text was reviewed by the linguists, clinicians, and academicians board, and the language validity of the scale was approved. A pilot scheme was administered to 20 people with this scale, whose language validity was approved, and the scale was evaluated in terms of understandability to give its final form.

2.5. Field Testing

The final Turkish version of the RosaQoL scale was applied to 285 patients diagnosed with Rosacea treated in the dermatology clinic. Dermatology Quality of Life Index (DLQI) generic tool was also applied consequently to the same patients for the purpose of demonstrating convergent validity. The data of this pilot application were used in the reliability and validity analyses. The Turkish Language version of the RosaQoL is presented in Table I.

2.6. Statistical Analysis

The data were analyzed by the SPSS version 22.0 Statistical Package. In the evaluation of the data, in addition to the descriptive statistics (mean, standard deviation), One-Way ANOVA was used in the comparison of quantitative data means of more than two groups.

Internal consistency, item-total score correlations test-retest reliability were used for reliability analyses. Internal consistency was tested using the Cronbach α value, whereas item–score and total-score relationships were explored by using the Pearson Correlation Analysis. For Test-Retest Reliability, the Scale was applied twice, initially and 2 weeks later, to 30 patients. The Test-Retest Reliability was evaluated with Pearson Correlation Test in statistical terms.

Validity Analysis was carried out using convergent and construct validity. The DLQI, a well-documented and widely used generic health-related QoL scale, was used in parallel to the RosaQoL in order to test convergent validity. Construct Validity was tested by using the principal components factor analysis, and convergent validity by Pearson correlations. The significance level of the ***p*** value was taken as $p<0.05$.

Table 1. *Turkish-language Rosacea-Specific Quality of Life Scale (RosaQol)*

RosaQoL Items	Domains
1. Rosacea hastalığımın ciddi olabileceğinden endişelenirim (kaygı duyarım).	Emotion
2. Rosacea hastalığım batma veya yanma hissettirir.	Symptom
3. Rosacea hastalığımdan kaynaklanan izlerden dolayı endişelenirim (kaygı duyarım).	Emotion
4. Rosacea hastalığımın daha kötü olabileceğinden endişelenirim (kaygı duyarım).	Emotion
5. Rosacea tedavisinden kaynaklanan yan etkilerden endişelenirim (kaygı duyarım).	Emotion
6. Rozase lezyonlarım beni tedirgin (rahatsız) eder.	Symptom
7. Rosacea hastalığımdan dolayı utanırım.	Emotion
8. Rosacea hastalığımdan dolayı hayal kırıklığına uğrarım.	Emotion
9. Rosacea hastalığım cildimi hassas yapar.	Symptom
10. Rosacea hastalığımdan dolayı sinirlenirim.	Emotion
11. Cildimin görünüşünden (kızarıklık, leke) dolayı sıkılırım.	Emotion
12. Rosacea hastalığım beni utangaç yapar.	Emotion
13. Rosacea lezyonlarımı makyaj ile örtmeye (gizlemeye) çalışırım.	Function
14. Rosacea hastalığımın sürekli olmasından veya tekrarlamasından dolayı sıkıldım.	Emotion
15. Rosacea hastalığıma neden olan bazı yiyecek veya içeceklerden uzak dururum.	Function
16. Cildimi pürüzlü hissederim (düzensiz, eşitsiz, pürüzlü).	Symptom
17. Cildim kızarır.	Symptom
18. Cildim kolayca tahriş olur (kozmetikler, temizleyiciler, tıraş losyonu).	Symptom
19. Gözlerim beni rahatsız eder (kuru veya pütürlü).	Symptom
20. Rosacea hastalığım ile ilgili düşünürüm.	Emotion
21. Rosacea hastalığıma neden olan belirli çevresel etkenlerden (nem, sıcak, soğuk) kaçınırım.	Function

3. RESULTS

Demographic characteristics of the Rosacea patients are presented in Table II.

The Study Group consisted of a total of 285 people, 240 women (84.2%) and 45 men (15.8%). The ages of the patients who were included in the study ranged between 21 and 72, and the mean age was 44.86 ± 12.50. The mean disease duration was found to be 28 ± 31.2 (months), and the mean age of onset of disease was found as 42 ± 12.2 (years).

When the educational status of the patients was evaluated, it was found that 227 (79.6%) were primary school graduates, 55 (19.3%) were high school, and 3 (1.1%) were university graduates. When the subtypes of Rosacea were evaluated, it was found that 75% (214) of the patients had Erythematotelengiectatic, 17.8% (51) had Papulopustular, 4.2% (12) had Phymatous, and 3% (8) had Ocular type. The severity of Rosacea disease was found to be moderate in 58.2% (166), mild in 26.7% (76), and severe in 15.1% of patients (43). In evaluating the reliability of RosaQoL, an internal consistency analysis was conducted, and the Cronbach's Alpha Coefficient score was found to be 0.952. When the corrected item-total correlation values were evaluated, no items were excluded from the scale because all of the items of the scale were higher than 0.30.

Test-Retest was administered to 30 patients for reliability analysis. A total of 30 patients, who were selected with the Simple Random Sampling Method, and to whom the questionnaire was administered, were called again 2 weeks later, and the questionnaire was administered again. The level (degree) of the Pearson Correlation Coefficient between the first and the second test administrations was found as 0.988 (98.8%). A very strong (very high) positive correlation was detected between the first and the second administration.

Data from the 285 Rosacea patients were analyzed with Factor Analysis with a rotational method of Varimax, and three factors were extracted: Symptoms and Feelings (questions 1, 2, 4, 6-12, 14, 16, 17-20); Anxiety (questions 3, 5, 13); and Functions (questions 15 and 21).

The total RosaQoL score was found as Mean ± SD of 62.4 ± 11.5 points (range 38-82). The values for individual domains were (Mean ± SD) 3.16 ± 0.57 for the symptoms and feelings domain, 2.42± 0.68 for the anxiety domain, 2.56±0.81 for the domain of the function. No significant differences were detected between gender, age, and total RosaQoL score ($p>0.05$). Statistically significant differences were detected between disease duration, disease severity, and total RosaQoL score ($p < 0.05$).

Table 2. *Demographic characteristics of rosacea patients (n = 285).*

Items	Results
Age (years), mean±SD	44.86±12.5
Sex, n (%)	
Female	240 (84.2)
Male	45 (15.8)
Education, n (%)	
Primary education	227 (79.6)
High school	55 (19.3)
University	3 (1.1)
Age at onset (years), mean±SD	42±12.2
Disease duration (months), mean±SD	28±14.3
Rosacea disease severity, n (%)	
Mild	76 (26.7)
Moderate	166 (58.2)
Severe	43 (15.1)
Subtype of rosacea, n (%)	
Erythematotelangiectatic	214 (75)
Papulopustular	51 (17.8)
Phymatous	12 (4.2)
Ocular	8 (3)

The total DLQI score was found as Mean ± SD of 7.85 ± 5.04 points. The values for individual domains were (Mean ± SD) 3.28 ± 1.2 for the symptoms and feelings (items 1 and 2) domain, 1.58± 1.21 for the daily activities (items 3 and 4) domain, 1.31± 1.40 for the leisure (items 5 and 6) domain,

0.50± 0.81 for the work / school (item 7) domain, 0.75± 1.0 for the personal relationships (items 8 and 9) domain, and 0.38± 0.68 for the treatment (item 10) domain.

The relation between total RosaQoL and total DLQI scores was calculated with the Pearson Correlation Coefficient, and a high-level correlation was detected between the scales (r = 0.411) (Table III).

The correlation coefficients between the total RosaQoL scale and its subscales varied between 0.374 and 0.979. A high level correlation was detected between the total RosaQoL scale and its subscales (p <0.05) (Table IV).

Table 3. *Pearson correlation analysis between RosaQoL and DLQ.*

DLQI							
RosaQoL	Symptoms and feelings	Daily activities	Leisure	Work/school	Personal relationships	Treatment	Total DLQI
	r	r	r	r	r	r	r
Symptoms and feelings	0.247***	0.288***	0.278***	0.253***	0.395***	0.324***	0.367***
Anxiety	0.310***	0.337***	0.371***	0.262***	0.317***	0.317***	0.403***
Function	0.172***	0.220***	0.258***	0.129***	0.247***	0.099	0.257***
Total RosaQoL	0.291***	0.316***	0.316***	0.294***	0.413***	0.356***	0.411***

****p<0.05*

Table 4. *Pearson correlation analysis between RosaQoL domains*

RosaQoL	Symptoms and feelings	Anxiety	Function
	r	r	r
Symptoms and feelings			
Anxiety	0.614***		
Function	0.516***	0.374***	
Total RosaQoL	0.979***	0.678***	0.607***

****p<0.05*

4. DISCUSSION

Rosacea is a very common inflammatory disease that is characterized by papule, pustule, erythema and skin tenderness in the middle area of the face (13). It is a chronic disease with several clinical manifestations in the visible face area, and might be accompanied by eye involvement and rhinophyma. It usually appears after the age of thirties, and might cause important limitations in the daily lives of patients with the effects of various triggering environmental factors (14).

Because rosacea clinical severity scores are based on physical symptoms, they are insufficient to reflect the true burden of disease experienced by patients and thus the negative impact on their quality of life. Not all rosacea patients are affected to the same degree. It is important to determine how much the patient is affected and to give individualized treatment. Emotional distress occurs both as a triggering factor for rosacea and as a result of the disease. In monitoring the effectiveness of therapeutic approaches, quality of life should be evaluated on standard scales. To achieve ideal medical outcomes, the doctor-patient relationship must be developed and the patient must be provided with the correct response. The use of scales that evaluate the quality of life is an inexpensive and practical method that contributes to the documentation and storage of this situation (4).

The evaluation of the patient's quality of life with subjective criteria often reveals different results from the physician's prediction and clinical evaluation. Studies of the severity of the disease indicated by the patient show that it is higher than determined by the physician. Measuring quality of life is of primary importance (11).

In Rosacea, the patient, who constantly faces his/her skin findings in the mirror, becomes unhappy and remains under the follow-up of people around him/her. Also, the chronic progression of Rosacea causes psychosocial problems to increase gradually. The clinical severity scores used by doctors for Rosacea are insufficient in determining the negative effects on the quality of life and the actual disease burden. For this reason, quality of life scales was developed to evaluate the opinions of patients. It is necessary that clinical severity scores and quality of life scales are evaluated together before and after the treatment by the doctor (6).

In Rosacea, 3 types of quality of life scales can be used, which are the general quality of life scales, dermatology-specific quality of life scales, and Rosacea-specific quality of life scales. The general quality of life scales and dermatology-specific

quality of life scales might overlook some disease-related challenges, remain time-consuming and complex; and when they are used to measure changes after an intervention related to the disease, they might not be as sensitive to change as the disease-specific scales (15).

The general quality of life scales and dermatology-specific quality of life scales provide clinicians with the opportunity to compare different diseases. However, Rosacea-specific scales focus on the dimensions related to the disease more. For this reason, the general quality of life scales is less sensitive to the changes in terms of the severity of Rosacea than Rosacea-specific scales. Hence, using Rosacea-specific scales can be more specific guidance in the follow-up and treatment of the disease and in studies conducted on Rosacea. The only quality of life scale that is specific to Rosacea, RosaQoL, was developed by Nicholson et al. in 2007. It evaluates 21 characteristics of 3 subclasses in terms of emotions, symptoms, and functions, and it is a quality of life scale that was created by considering the symptoms that are specific to Rosacea and the negative factors expected by patients (8).

In order to make the most accurate evaluation and impression of the results, it is imperative that these scales are adapted to religion, language, and socio-cultural according to the societies in which they are used, and that their validity and reliability are shown. These scales should be standardized at the national and international levels in order to compare the results of different treatment programs and different patient groups in the same disease group in the society and to make comparisons between societies.

In the present study, the purpose was to adapt the RosaQoL Scale, which is a quality of life scale acknowledged globally for Rosacea, into Turkish; and to test its validity and reliability in the follow-up and treatment of Rosacea patients in our country, and also to present it for use in studies related to Rosacea. The RosaQoL Scale is used in studies in many countries all around the world, and its validity and reliability were proven for different languages (9, 10).

Internal Structure Consistency and Test-Retest Methods were applied to measure the reliability of the scale. The Cronbach Alpha Coefficient was found to be 0.952 for the entire scale, and the Correlation Coefficient of the Test-Retest Method was found to be r = 0.988 in internal structure consistency ($p < 0.001$). The results obtained in this way show that the Turkish reliability of our scale is proven. None of the items were removed from the scale.

Explanatory Factor Analysis and External Tests Method were used for the validity study of the scale; the structural validity of the scale was evaluated with the DLQI Scale, which measures similar conceptual structures and which is frequently used in dermatological diseases with correlation analysis.

According to the Factor Matrix, 1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, and 20 were grouped under the first factor structure; questions 3, 5, and 13 under the second factor; questions 15 and 21 were grouped under the third factor structure. Considering the meanings of the items in the factors, and by using the alternating factor loads, since the questions in the first factor were related with mood and symptoms, it was called the “Symptoms and Emotions” dimension; since the questions in the second factor were about anxiety, it was called the “Anxiety” dimension; and since the questions in the third factor were related with the functions of the organs, it was called “Functions” dimension.

In the present study, a high-level correlation was detected between the total and subscale scores of RosaQoL and DLQI. Also, a high correlation was detected between the RosaQoL total score and the RosaQoL subscale scores, and it was demonstrated that the whole scale was in an important relation with subscales. This result supports the validity of the scale.

There was a statistically significant difference between the RosaQoL total score and all subgroups of the scale and the severity of the disease. According to the results obtained in our study, as the severity of the disease increases, the RosaQoL score also increases.

No statistically significant differences were detected between gender, age, and total RosaQoL Score ($p > 0.05$); however, statistically significant differences were detected between the duration and severity of the disease and total RosaQoL score ($p < 0.05$), which supports that patients are negatively affected in social and psychological terms due to the presence of the disease in a visible area and due to the prolonged and severe course of the disease.

5. CONCLUSION

In conclusion, the results of this validation study have clearly demonstrated that the Turkish version of the RosaQoL is an appropriate, clinically firm, and valid instrument with strong psychometric properties to be used with Turkish-speaking patients who have Rosacea disease.

We recommend that the scale is applied before and after the Rosacea treatment in future studies to examine the extent to which the scale is beneficial in evaluating the treatment response and patient follow-ups.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

The Role of p-Coumaric Acid on Reproductive and Remote Organ Damages Created by Adnexal Torsion/Detorsion: Biochemical and Immunohistochemical A Study

Ayhan Tanyeli[1], Fazile Nur Ekinci Akdemir[2]*, Ersen Eraslan[3], Mustafa Can Guler[1], Tuncer Nacar[1], Selim Comakli[4], Ilhami Gulcin[5]

[1] Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye.
[2] Department of Nutrition and Dietetics, Faculty of Health Science, Agri İbrahim Cecen University, Agri, Türkiye.
[3] Department of Physiology, Faculty of Medicine, Bozok University, Yozgat, Türkiye.
[4] Department of Pathology, Faculty of Veterinary, Atatürk University, Erzurum, Türkiye.
[5] Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Türkiye.

Correspondence Author: Fazile Nur Ekinci Akdemir
E-mail: fazilenur85@gmail.com
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ABSTRACT

Objective: We planned to search the effects of p-coumaric acid on ovary and lung injuries formed via bilateral adnexal torsion detorsion (T/D) in experimental rat model.

Methods: 24 female, Sprague-Dawley rats were sorted out as 3 groups. Design of the groups was performed as sham (group I) and T/D (group II), p-coumaric acid+T/D (group III) groups. Sham group; abdomen area was applied incision and repaired with no T/D model application. T/D group; 3 h of torsion phase completed and then 3 h of detorsion stage was established. P-coumaric acid+T/D group; p-coumaric acid was administered at the dose of 100 mg/kg for 15 days by oral gavage and then, T/D model was performed. Following detorsion phase, rats were sacrificed, lung and ovarian tissues were excised for biochemical and immunohistochemical evaluations.

Results: When it is compared to group I, oxidant parameters elevated significantly in group II ($p<0.05$) while the activity of antioxidant enzymes and TAS level decreased. On the other side, antioxidant enzyme activity raised and oxidant parameter levels diminished in group III compared to group II ($p<0.05$). Moreover, NF-kB, caspase 3 and LC3B protein expression levels increased in ovary and lung tissues of the group II. But NF-kB, caspase 3 and LC3B protein expression levels decreased in group III.

Conclusion: As a consequence, p-coumaric acid acted a protective performance against ovary and lung injuries arising from adnexal T/D model in rats.

Keywords: p-Coumaric Acid, Adnexal Torsion/Detorsion, Ovary, Remote organ, Rat.

1. INTRODUCTION

Adnexal/ovarian torsion (OT), which is more prevalent during reproductive age, is an emergency clinical condition rarely diagnosed preoperatively. In OT, the ovaries and fallopian tube portions rotate on the vascular pedicle. Ovarian/adnexal torsion causes to ischemia. In case of the OT, ischemia is defined as deterioration of organ blooding as a result of clogging due to clot or mechanical reasons of the blood vessels feeding ovaries. The regulation of this incident, re-oxygenation of the tissue and correction of blood flow is called as reperfusion. Pathologies which are caused by ischemia-reperfusion (I/R) are observed in many clinic cases such as burn, sepsis, shock, and torsion (1). There will be no permanent damage when it is noticed and operated in the early period, in ovarian tissue. However, in neglected or delayed cases, the necrosis in the ovaries occurs because the blood flow is cut off for a long time. In other words, irreversible damage is emerged in ovarian tissue. This case leads to infertility in woman. Some local and systemic consequences occur as a result of reperfusion of ovaries owing to detorsion of the torsional adnexa. Ovarian reperfusion results in activation, infiltration, and adhesion of leukocytes, because free radicals and proinflammatory substance levels increase (2) In addition to hypoperfusion in ischemia and I/R injury occurs inflammatory responses and many organ dysfunctions (3).

Numerous experimental studies have been conducted to alleviate ovarian tissue damage due to adnexal torsion/detorsion (T/D) (4, 5). Also, it is known that I/R directly leads to primary organ injury. Besides, I/R results in remote organ damage via starting an array of oxidative and inflammatory reactions in the secondary organs' tissues (6, 7). To the best

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of our knowledge, no studies were found on remote organ damage induced by adnexal T/D in the literature. Upon this reason, this search will make a significant contribution to the literature.

p-Coumaric acid is a phenolic compound commonly present in various plants (8, 9). The chemical structure of p-comaric acid is given in Figure 1. The main dietary phenolic acid sources are beverages and fruits (10). Several researches have reported the association between the consumption of rich diets from phenolic acid derivatives and the protection of various diseases (11). The studies on food phenolics have enhanced due to their effects as antioxidants and their implication in the prevention of some diseases.

Here, we investigated the effects of p-coumaric acid on adnexal T/D-induced ovarian and lung tissue injury.

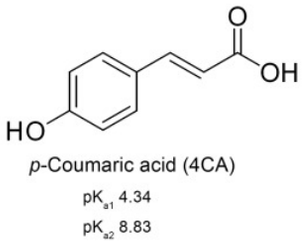

Figure 1. *The chemical structure of p-coumaric acid (42).*

2. MATERIALS AND METHODS

We established the experimental procedure at Atatürk University Experimental Animal Research and Application Center (ATADEM). The rats were provided by ATADEM. They were housed in standard cages with laboratory medium including appropriate humidity, light/dark cycle and temperature. Rats were fed with standard pellet feed and water. In order to avoid anesthesia complications, rats were famished before 12 hours of the experiment. This study was carried out via confirmation of Atatürk University Experimental Animals Local Ethics Committee (30.03.2018/94).

2.1. Groups and Torsion/Detorsion Model

24 female, Sprague-Dawley rats were weighted (220±10 g) and randomly divided into 3 groups. Group I; following the anesthesia, the rats were immobilized in the dorsal position. The abdominal region was prepared by shaving and cleaning. Povidone iodine was preferred for disinfection. Median laparotomy incision was performed in the size of 1-2 cm, and it was repaired with silk 3/0 suture without any T/D model or medical process. Group II; all steps were carried out as in the group I but the ovaries and their structures were spun in clockwise for 360 degrees and fixed by an atraumatic microvascular clamp for 3h. Thus, bilateral ovarian torsion was applied (12). It was allowed blood stream for 3 hours by releasing the clamps in the detorsion phase and the incision was closed. In the group III, 100 mg/kg of p-Coumaric acid (purchased from Sigma Aldrich Co. USA) was administered to rats orally (by gavage) for 15 days (13, 14) and T/D model was performed. All steps were established using anesthesia (100 mg/kg intraperitoneal (i.p.) Ketalar®, Pfizer, Istanbul, and 15 mg/kg i.p. xylazine hydrochloride, Rompun®, Bayer, Istanbul). When the detorsion ended, the rats were euthanized under overdose anesthesia. The ovarian and lung tissues were excised, washed, and preserved frozen for the biochemical analysis.

2.2. Biochemical Measurements

Malondialdehyde (MDA) levels were measured to find out the lipid peroxidation status (15). The results were demonstrated as µmol/g protein. The protocol defined by Sun et al. (16) was chosen to determine the superoxide dismutase (SOD) activity (U/mg protein). Myeloperoxidase (MPO) activity (U/g protein) was evaluated with a method defined by Bradley et al. (17). The total antioxidant status (TAS) was evaluated with a commercial kit (Rel Assay Diagnostics, Product Code: RL0017). Total oxidant status (TOS) was determined with an available kit (Rel Assay Diagnostics, Product Code: RL024). TAS and TOS levels were given as nmol/L. The ratio of TOS to TAS was accepted as the oxidative stress index (OSI).

2.3. Immunohistochemical Staining

Ovarian and lung tissues were taken into 10% buffered formalin solution. They were detected in neutral formaldehyde solution and washed with tap water. Tissues were blocked in the paraffin through the following-up of alcohol-xylol process. They were taken on the polylysine slide, incubated in 3% H_2O_2 to inactivate peroxidase, and washed in phosphate-buffered saline (PBS). Thereafter, incubation was executed with the antigen retrieval solution for 10 min at 500W. Nonspecific binding prevented by adding protein block solution. Cleaved Caspase-3 (Novus Biological, Cat. No. NB600-1235, Dilution: 1/100), microtubule-associated protein light chain 3 (LC3B) (Abcam, Cat. No. ab48394 Dilution: 1/200) and Nuclear Factor kappa-B (NF-kB) (Abcam, Cat. No: ab7971, Dilution:1/150) were applied as the primer antibody. The exposure according to the mouse and rabbit specific procedure HRP/DAB detection IHC kit (Abcam: ab80436) was applied. 3,3'-diaminobenzidine chromogen was used and contrasted with hematoxylin. Positive cells were evaluated at 20x magnification in a light microscope.

2.4. Statistical Analysis

One-way ANOVA with the Tukey test was used for multiple comparisons. Descriptive statistics are given as the Mean±SD. In immunohistochemical examination, it was graded as intense immunopositivity (+++), moderate immunopositivity (++), mild immunopositivity (+), and negativity (-) in the ovary and lung tissues. Immunohistochemical findings were analyzed with Kruskal-Wallis test and Mann-Whitney U test was used for the binary group comparison. The statistically significant results were accepted as $p<0.05$.

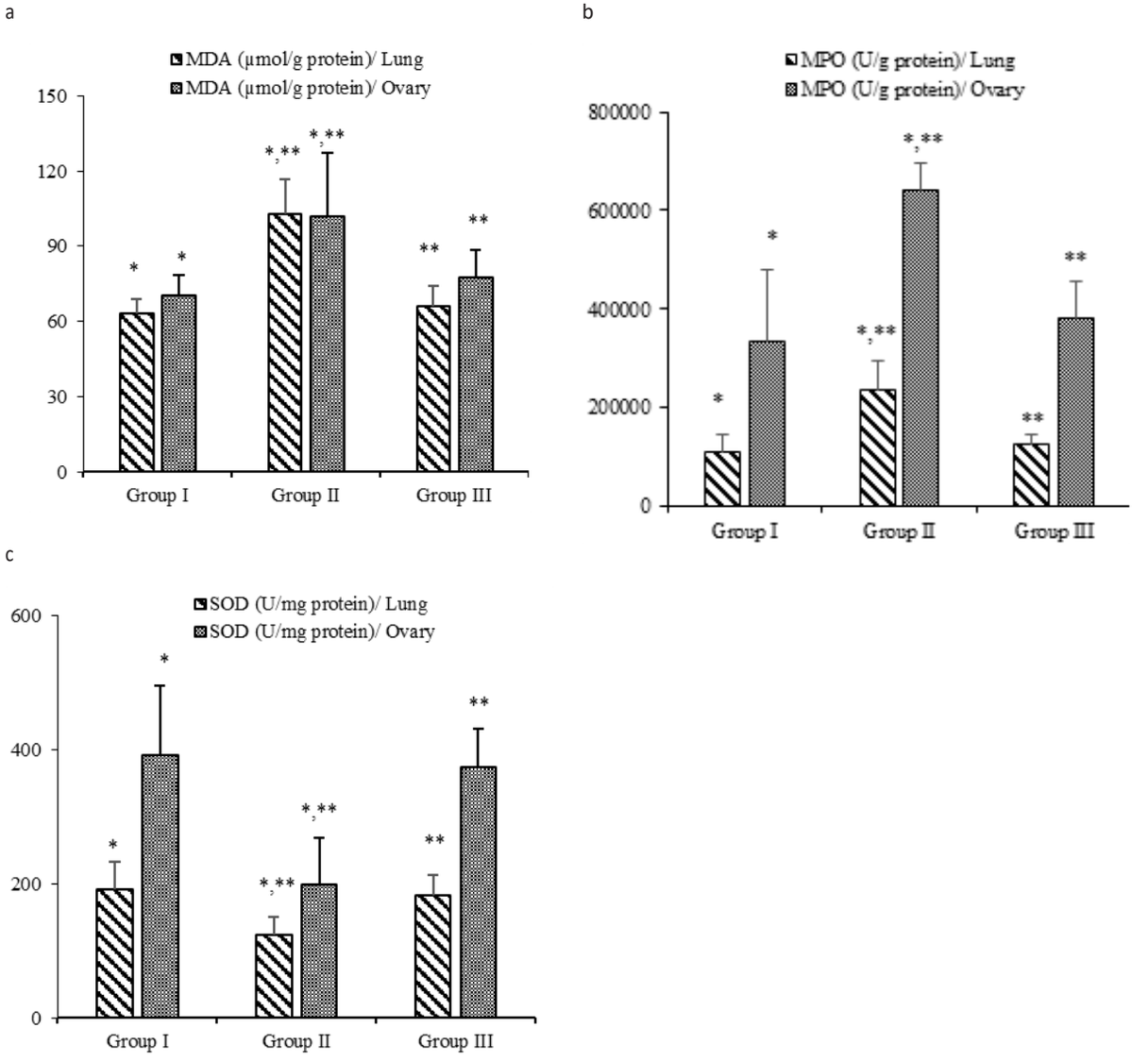

Figure 2.a. *There is statistical significance between the groups that denoted by the same symbols(p<0.05).* ***b.*** *There is statistical significance between the groups that denoted by the same symbols(p<0.05).* ***c.*** *There is statistical significance between the groups that denoted by the same symbol (p<0.05).*

3. RESULTS

3.1. Biochemical Results of Ovary and Lung Tissues

Biochemical analyses were performed on ovarian and lung tissues. MDA level was presented in Figure 2a. In group II, MDA value raised significantly compared to group I. Besides, MDA measurement declined in group III compared to group II ($p<0.05$). In group II, MPO activity was significantly higher than group I (Figure 2b). In group III, MPO activity diminished significantly compared to group II ($p<0.05$). Post hoc analysis of SOD revealed that it was low in group II compared to group. In addition, SOD activity elevated significantly in group III compared to group II (Figure 2c $p<0.05$). TAS value decreased in group II compared to group I. Also, TAS value increased in group III compared to group II ($p<0.05$). TOS level elevated in group II compared to group I. OSI level raised in group II compared to group III ($p<0.05$, see Table 1).

3.2. Immunohistochemical Results of Ovary and Lung Tissues

In the immunohistochemical staining for inflammatory reaction of NF-kB immunopositivity, it did not show immunopositivity in ovarian tissues of the group I (Figure 3a and Table 2). There was intense immunopositivity in the group II (Figure 3b). In group III, there was a significant decrease in the severity of immunopositivity (Figure 3c). But in inflammatory cells in the interstitial area and in lutein cells was found immunopositivity. In immunohistochemical staining for apoptotic cell death and autophagic cell

death; caspase-3 (Figure 4c) and LC3B protein (Figure 5c) immunopositivity was negative in group I, but the most intense caspase-3 and LC3B protein immunopositivity was found in group II (Figure 4b and Figure 5b). Whereas group III showed a decrease in caspase 3 and LC3B protein immunopositivity (Figures 4c and 5c). immunopositivity was also found in luteal cells and interstitial areas.

Immunohistochemical staining revealed no NF-kB immunopositivity in the group I in terms of inflammatory reaction (Fig. 6a and Table 2). The most intense immunopositivity was observed in the lung tissues of the group II (Fig. 6b). In the group III, the severity of immunopositivity decreased (Fig. 6c). Apoptotic cell death and autophagic cell death did not reveal caspase-3 and LC3B protein immunopositivity in the lung tissues of the group I (Fig 7a and Fig 8a). The most intense caspase-3 and LC3B protein immunopositivity was observed in the group II (Fig 7b and Fig 8b). In the lung tissues of the group III, there was a decrease in caspase-3 and LC3B protein immunopositivity (Fig 7c and Fig 8c).

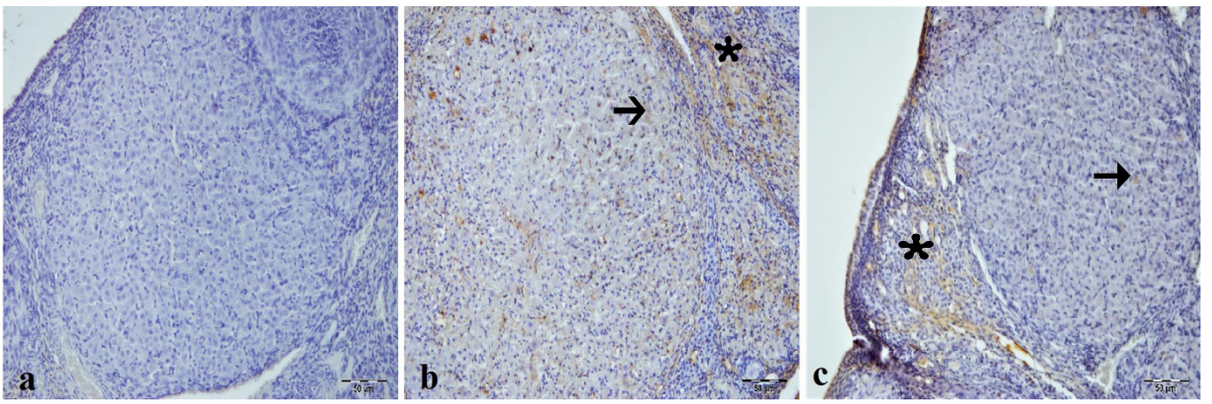

Figure 3. a. *Group I* ***b.*** *group II, intense NF-kB immunopositivity in interstitial region (star) and luteal cells (arrow)* ***c.*** *group III, mild NF-kB immunopositivity in luteal cells (arrow), moderate-intensity NF-kB immunopositivity in interstitial cells.*

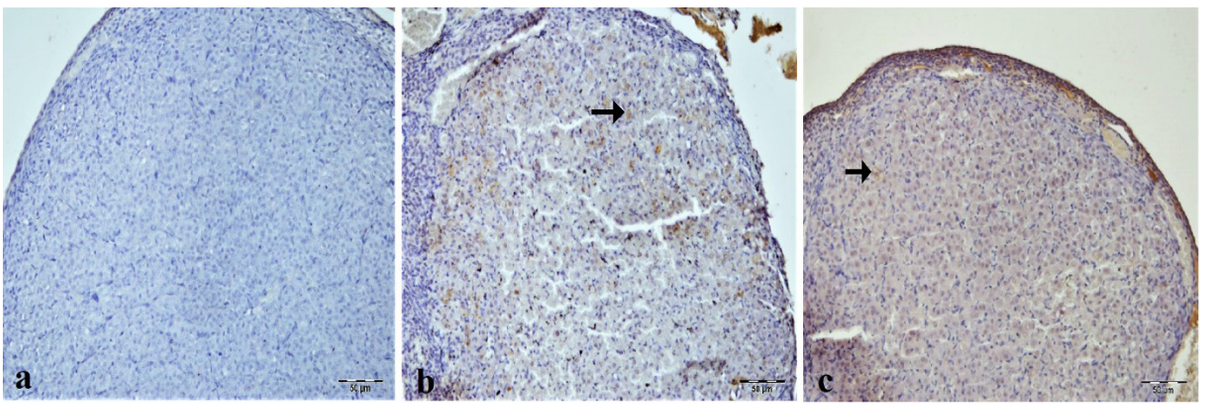

Figure 4. a. *Group I* ***b.*** *group II, intense caspase-3 immunopositivity in luteal cells (arrow)* ***c.*** *in group III, mild caspase-3 immunopositivity in luteal cells (arrow).*

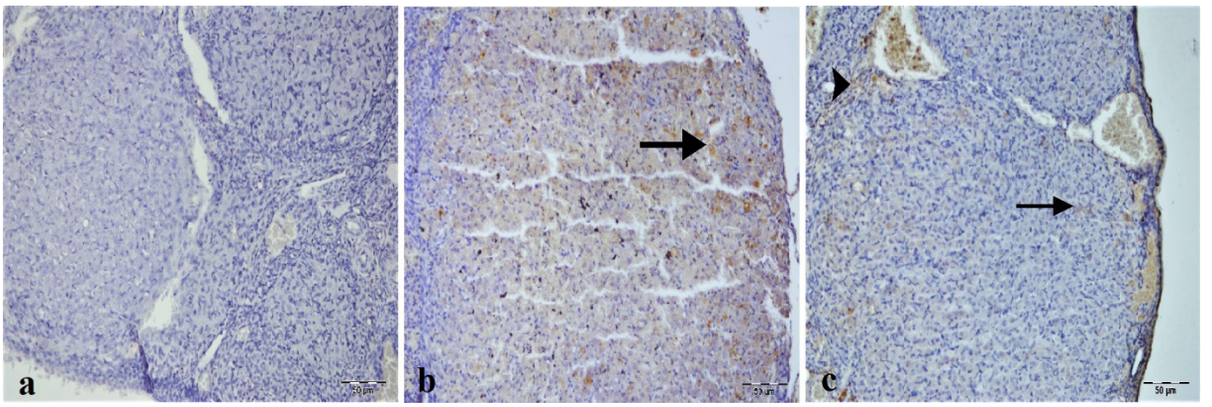

Figure 5. *a. Group I* ***b.*** *Group II, dense LC3B protein immunopositivity in luteal cells (arrow)* ***c.*** *Group III, mild LC3B protein (arrow) in luteal cells, moderate LC3B protein immunopositivity in interstitial cells (arrowhead).*

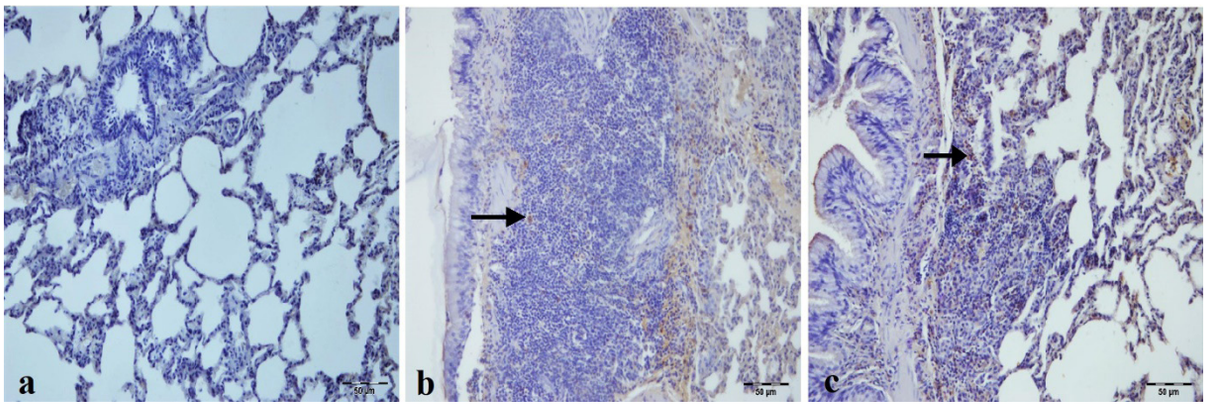

Figure 6. a. *Group I* ***b.*** *group II, dense NF-kB immunopositivity-arrow in inflammatory cells around the bronchiole (arrow)* ***c.*** *group III, decreased NF-kB immunopositivity-arrow in the inflammatory cell group around the bronchiole.*

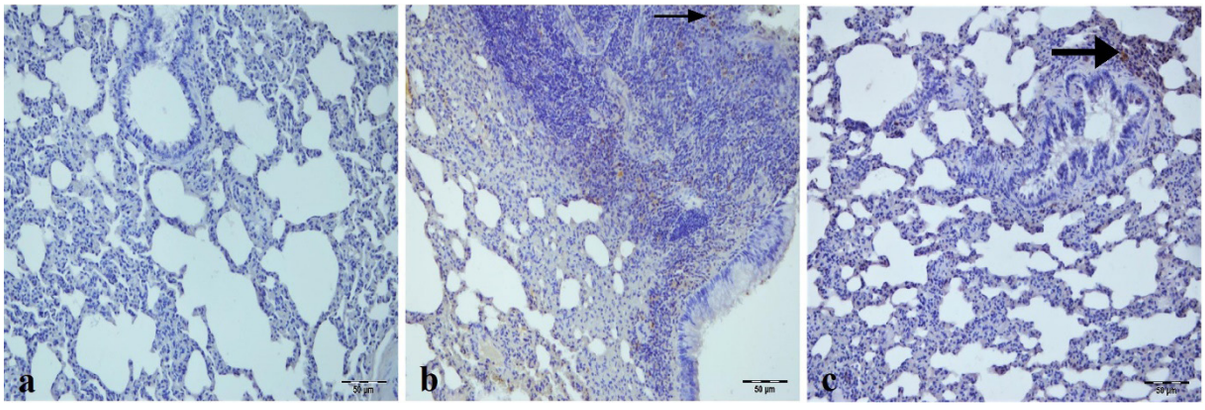

Figure 7. a. *Group I* ***b.*** *group II, intense caspase 3 immunopositivity-arrow in inflammatory cells in the peribronchiolar region (arrow)* ***c.*** *group III, very light caspase 3 immunopositivity in inflammatory cell group around the bronchiole (arrow)*

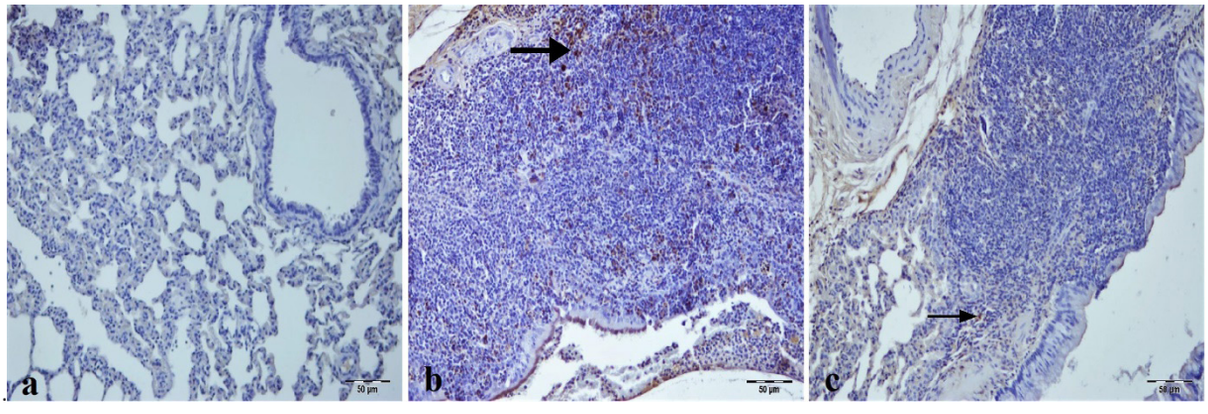

Figure 8. a. *Group I* ***b.*** *group II, very dense LC3 protein immunopositivity in inflammatory cells in the peribronchiolar region (arrow)* ***c.*** *group III, mild LC3B protein immunopositivity-arrow in inflammatory cell group around the bronchioles (arrow).*

Table 1. *Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values in lung and ovarian tissues were presented as a Mean±SD.*

	Groups	TAS[a]	TOS[b]	OSI
Lung	I	1.094±0.215*	8.931±1.538*	0.845±0.226*
	II	0.795±0.172*,**	12.476±0.753*,**	1.633±0.355*,**
	III	1.031±0.140**	8.259±1.062**	0.815±0.149**
Ovary	I	0.453±0.152*	5.027±0.455*	1.199±0.330*
	II	0.257±0.118*,**	8.384±1.265*,**	3.976±1.970*,**
	III	0.448±0.104**	5.733±1.037**	1.352±0.450**

a: mmol Trolox equivalent/L; b: µmol H_2O_2 equivalent/L; is statistical significance between the groups that denoted by the same symbols (p<0.05).*

Table 2. *Nuclear Factor kappa-B (NF-kB), caspase-3 and LC3B protein immunopositivity as intense immunopositivity (+++), moderate immunopositivity (++), mild immunopositivity (+), and negativity (-) in lung and ovarian tissues.*

Ovarian tissue			
	Group I	Group II	Group III
NF-$_K$B	-	+++	+
Caspase 3	-	+++	+
LC3B protein	-	+++	+
Lung tissue			
	Group I	Group II	Group III
NF-$_K$B	-	+++	+
Caspase 3	-	+++	+
LC3B protein	-	+++	+

4. DISCUSSION

The basic functions of the ovaries are the production of hormones and the development of the ovum. These functions can be interrupted by many factors. Torsion may occur and vascular insufficiency may cause necrosis. For this reason, OT must be recognized and treated quickly as surgically to avoid potential necrosis causing to infertility. Adnexal torsion is a gynecological condition. It decreases ovarian blood flow leading to tissue injury. The main goal in the treatment of ischemia is to renew blood circulation and ameliorate the tissue oxygenation (3, 18-20). Besides, ischemic organ reperfusion may cause much more critical injury than ischemia. This case is accepted as reperfusion damage (21). Neutrophil infiltration, reactive oxygen species (ROS) generation, release of cytokines, and inflammation are enounced to have important act in the etiology of this injury (22). After reperfusion, free radicals and ROS generate in severe quantity. Free radicals can cause damage by interacting with the entire biomolecules due to their extreme activities. Lipids, carbohydrates, proteins, amino acids, DNA, and complex molecules are damaged by free radicals. This is called as peroxidation of biomolecules (23-25). The main antioxidant defense in cell against radical damage is maintained by antioxidant enzymes including SOD and catalase (CAT) (25, 26). MDA is an important lipid peroxidation product and a toxic substance indicating oxidative stress (23, 24). SOD enzyme catalyzes the ROS neutralization (23, 24). MPO acts as a catalysor in hydroxyl radical formation (27). Until today, it has been made numerous experimental adnexal-T/D models, as well as remote organ damage studies induced by I/R in different organs (4, 12, 28-30). In a previous study, MPO activity and MDA levels increased in I/R groups (31). SOD activity decreased due to I/R in another adnexal T/D model [28, 32]. MDA levels significantly increased in an I/R experimental study (33). MDA level raised and antioxidant enzyme level diminished in another I/R research (7). In current study, MDA level and MPO activity elevated, and SOD activity diminished in both lungs and ovaries.

NF-kB plays role in different physiologic processes such as neurodegeneration, cell growth, tumorigenesis, immunity, inflammation, and apoptosis (34). During an inflammatory response, NF-kB plays as role in gene expression (35). Oxidative stress induces NF-kB transcription factor activation (36). Further, there is a powerful relationship between autophagic response and NF-kB (37). Hunger, hypoxia and various stress conditions stimulate cellular autophagic activity. ROS have been shown to be effective in autophagic vesicle and regulation of autophagia on cell death or survival (11, 26). The autophagy regulation of ROS takes place via Atg4, which is important in the formation of autophagosomes. The Atg4 gene is involved in the ripening of LC3B proteins and their degradation from fat molecules (38-40). Caspase 3 is vital for apoptosis and overexpressed during apoptosis events. Apoptosis was shown by increased caspase-3 levels in experimental animals (41). In this study caspase 3, NF-kB, and LC3B immunopositivity was intense in group II. Autophagy and apoptotic markers have been shown to be triggered by oxidative stress induced by ROS production. Whereas NF-kB, caspase 3 and LC3B protein showed less immunopositivity in ovarian and lung tissues owing to p-coumaric acid treatment.

In addition to this knowledge, p-coumaric acid was effective against lung and ovarian damage in adnexal T/D. Based on current biochemical data, p-coumaric acid has provided effective protection against ovarian injury induced by adnexal T/D.

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Data availability statement

Research data not shared

Declaration of conflict of interest

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Clinical and Experimental Health Sciences

Proteomic Analysis in Nifedipine Induced Gingival Overgrowth: A Pilot Study

Ece Yetis[1], Aysen Yarat[2], Onur Eroglu[1], Hafize Ozturk Ozener[3], Leyla Kuru[3]

[1] Marmara University, Institute of Health Sciences, Department of Periodontology, Istanbul, Türkiye.
[2] Marmara University, Faculty of Dentistry, Department of Basic Medical Sciences, Istanbul, Turkiye.
[3] Marmara University, Faculty of Dentistry, Department of Periodontology, Istanbul, Türkiye.

Correspondence Author: Leyla Kuru
E-mail: lkuru@marmara.edu.tr
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ABSTRACT

Objective: The aims of the present study were to investigate the proteomic profile of nifedipine induced overgrown gingiva and compare with non-overgrown gingival tissues obtained from the same patients.

Methods: Seven subjects under nifedipine medication for at least 6 months and diagnosed as nifedipine induced gingival overgrowth (NIGO) participated in the study. Periodontal clinical parameters were recorded. Gingival tissue samples were harvested from overgrown (GO+ Group, n=7) and non-overgrown regions (GO- Group, n=7) of the same patients. Proteomics was performed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) technique. The identified proteins were further classified according to their molecular functions, biological processes and cellular component distribution for functional gene ontology analysis using a web-based bioinformatics tool. Mann Whitney-U and ANOVA tests were performed to compare clinical parameters and identified proteins with proteomics, respectively.

Results: Bleeding on probing and gingival overgrowth index of the GO+ group were statistically significantly higher than the GO- group ($p<0.05$, $p<0.01$, respectively). A total of 143 proteins were identified in 14 gingival tissue samples using proteomics. Among the proteins identified, 79 of them were detected in higher quantities in the GO+ group ($p<0.05$) whereas remaining 64 were found higher in the GO- group ($p<0.05$). The analysis of functional gene ontology demonstrated that certain proteins exhibit roles in either stimulatory or inhibitory processes including cell proliferation, growth and apoptosis.

Conclusion: The proteomic profiles of overgrown and non-overgrown gingiva suggest that the identified proteins expressed at different levels in both groups may contribute to the pathogenesis and progression of NIGO.

Keywords: gingival overgrowth, nifedipine, proteomics

1. INTRODUCTION

Gingival overgrowth (GO), a pathological clinical situation, is defined as the increase in size and the change in contour of gingiva observed as a common symptom in various gingival diseases and systemic conditions. It can be temporary and reversible or chronic and irreversible, associated with many factors such as inflammation, side effects of certain medications, and neoplastic pathologies (1). When it occurs as a side effect and an undesirable consequence of systemically used drugs, it is then called drug-induced GO (DIGO) which is limited to the gingival tissues, but not a periodontium-induced pathology. The inducing drugs are anticonvulsants, immunosuppressants and calcium channel blockers (CCBs). Worldwide used CCBs have proven to be extremely effective to take the hypertension under control. According to their chemical composition, CCBs can be classified under three main classes as benzothiazepines, phenylalkylamines and dihydropyridines (2).

The gingival changes due to the use of CCBs present similar clinical features. CCB induced GO is localized on attached gingiva and gingival margin, and visible first on interdental papillae with a lobular or nodular morphology extending through occlusal surfaces coronally and mucogingival junction apically, covering the clinical crown (3). Lesions are located mostly on the anterior vestibular surfaces whereas some regions in the same subject may not show any sign of GO. With the reach of overgrown gingiva coronally, aesthetic, functional and phonational difficulties may occur (4).

Among CCBs, nifedipine has been the most frequently used drug inducing GO with a wide prevalence ranging from 15%

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to 83% (5), followed by diltiazem (6) and verapamil (7) with ratios of 21% and 4%, respectively.

It is proposed that the incidence of nifedipine induced GO (NIGO) appears to increase in the next period of time, since the use of antihypertensive drugs, especially nifedipine and the indication of hypertension as a symptom of various systemic diseases such as metabolic syndrome in the society, increase. Thereby, it is of utmost importance to clarify the cellular and molecular mechanisms of NIGO.

The upregulatory process or causative molecule is still under the concern of researchers. Numerous theories and hypotheses have been speculated in the literature. Nifedipine may act indirectly by stimulating the production of interleukin-2 by T cells (8) or testosterone metabolites (9). Some changes in calcium ion metabolism may also account for GO (5). The upregulation in proliferative and synthetic activity of fibroblasts are followed by the increase in extracellular matrix (ECM) and tissue volume. In addition, transforming growth factor-ß (TGF-ß), fibroblast growth factor and heparan sulphate glycosaminoglycan play a role in NIGO (10). Angiotensin-II, which is involved in fibrosis mechanism by increasing the TGF-ß and connective tissue growth factor levels, was found to be higher in gingival samples with NIGO compared to healthy controls (11).

With the development of genomic, proteomic, or metabolomic techniques, it is now possible to define the key factors of disease pathogenesis more efficiently. Proteomics provides a great advantage on identifying potential disease markers since very low levels of proteins can be detected, and also compares the expression profile of proteins from a cell, tissue or organism with state of health and non-standard condition such as a disease or presence of a toxin in a spontaneous reference state (12). In dental field, enamel, dentin, pulp, microorganisms, stem cells and various dental materials have been successfully examined using this novel technique (13, 14). Proteomics has been also applied in periodontology to investigate dental plaque, oral mucosa, gingiva, periodontal ligament, cementum, alveolar bone, gingival crevicular fluid (GCF) and saliva (15, 13).

Until now, there is only one experimental study examining the upregulated and downregulated proteins expressed by cultured gingival fibroblasts treated with 10 µM of immunosuppressant cyclosporin-A for 48 hours using proteomics technique (16). Jung et al. reported that 17 proteins are related with cell proliferation. However so far, the exact pathogenic mechanism of NIGO has still not been clarified. Therefore, the aim of the present study was to investigate the proteomic profile of nifedipine induced overgrown gingiva, and compare with non-overgrown gingival tissues obtained from the same patients.

2. METHODS

2.1. Study Population

This cross-sectional study was approved by the Ethical Committee of Marmara Faculty of Dentistry with a protocol number of 2018-175. Written informed consent, detailing the study protocol was obtained from all participants. Seven subjects (Male/ Female (M/F), 3/4; mean age of 43.42± 15.29 years) under nifedipine medication of 30 mg per day, for at least 6 months and diagnosed with NIGO were enrolled in the study.

Inclusion criteria were: 1) having at least 12 teeth, 2) non-smoker, 3) not being pregnant, 4) not using antibiotics or anti-inflammatory drugs in the past 6 months, 5) not having periodontal treatment in the past 6 months.

2.2. Clinical Procedures

Clinical parameters including plaque index (PI) (17), gingival index (GI) (18), probing depth (PD) and bleeding on probing (BOP) were recorded by a calibrated single researcher (E.Y.) with using a 0.5 mm diameter and 15 mm periodontal probe (University of North Carolina, PCPUNC15, Hu-Friedy Ins Co, USA). In addition, GO index developed by Ellis et al. (19) was evaluated on 10 anterior papilla (13 to 23, and 33 to 43) using intraoral photographs taken from patients and projected on a screen. The calibration of the researcher was calculated by Cohen's Kappa analysis on GO index which was measured twice with 24 hours interval (κ= 0.84, $p<0.001$).

Criteria for assessing GO on adjacent tooth surfaces for a gingival unit are as follows:

0= No encroachment of interdental papilla onto tooth surface

1= Mild encroachment of interdental papilla, producing a blunted appearance to papilla tip

2= Moderate encroachment, involving lateral spread of papilla across buccal tooth surface of less than 1/3 tooth width

3= Marked encroachment of papilla, more than 1/4 tooth width. Loss of normal papilla form

Using this index, the maximum score obtainable was 30 which was then converted to a percentage GO score and recorded.

2.3. Sample Collection

Gingival tissue samples were harvested from overgrown (GO+ Group, n=7) and non-overgrown regions (GO- Group, n=7) of the same patients diagnosed with NIGO. While the GO+ group tissues were obtained by the excision for therapeutic purposes during flap operation or gingivectomy procedure, the GO- group samples were obtained during crown lengthening operation. The excised tissue samples were placed in low-binding tubes (Eppendorf, USA) containing sterile saline and stored at – 80°C prior to the experiment.

2.4. Proteomic Analysis

2.4.1. Protein Extraction from Gingival Samples

Gingival tissue samples were homogenized and pooled for each group. For protein extraction, the tissue homogenate was mixed with Universal Protein Extraction (UPX) Kit (Abcam, UK) and protease inhibitor cocktail (Abcam, UK) at a ratio of 1:100. The tissue homogenates were centrifuged at 18.000 rpm for 10 minutes. The supernatant containing soluble proteins was obtained and transferred into low-binding tubes (Eppendorf, USA). Protein concentrations in the samples were determined by the method of Biorad Bradford protocol (Bio-Rad, Hercules, USA) (20). Peptide concentration was measured by Pierce's quantitative colorimetric peptide determination method (Pierce ™ Quantitative Colorimetric Peptide Assay, Thermo Fisher Scientific, USA) (21). The samples were diluted with 0.1% formic acid so that the final concentration was 200 ng/µL.

2.4.2. Identification and Quantitative Analysis of Proteins

Following the chromatographic separation by liquid chromatography (LC), molecules were ionized. The interface used in this study was the LC-Tandem Mass Spectrometry (LC-MS/MS) method was based on electrospray ionization. Further specificity was obtained with multiple ionization events. The analyzed data were collected for peptides that could be identified in the m/z 50-2000 range. MS was performed following the electrospray ionization. MS analysis was performed for 0.5 seconds and information about the entire peptide was collected. Then, MS/MS analysis was performed for 0.5 seconds, and the peptide was fragmented and sequence information was obtained.

Protein identification was carried out using human protein sequence information in the UniProt protein database (22). Primary accession number, entry name of UniProt database and molecular weight for each identified protein were reported.

2.5. Classification of Identified Proteins

These identified proteins were further classified according to their molecular functions, biological processes and cell localization for functional gene ontology analysis using a web-based bioinformatics tool (Panther Analysis Gene Ontology Classification System, version 16.0, Panther, USA) (23).

2.6. Statistical Analysis

The analysis for clinical data was performed using the IBM SPSS, version 22.0 for Windows. Mann Whitney-U test was performed to compare clinical parameters between the groups. The analysis for proteomic data was performed by the Progenesis QIP software (Waters, USA). The ANOVA test was applied to compare the identified protein levels between the groups. A P value of <0.05 was considered statistically significant.

3. RESULTS

Clinical periodontal parameters for whole mouth and selected regions for GO+ and GO- groups are shown in Table 1. While PI, GI and PD values of both groups were similar (p>0.05), BOP and GO index of the GO+ group were statistically significantly higher than the GO- group (p<0.05, p<0.01, respectively). All of the subjects were diagnosed as periodontitis and the details were as follows: 1 patient Grade A Stage III, 2 patients Grade B Stage III, 2 patients Grade B Stage IV and 2 patients Grade C Stage IV.

Table 1. *Clinical periodontal parameters*

	Whole Mouth n=7	GO+ Group n=7	GO- Group n=7	P*
PI	2.31±0.48	2.41±0.44	2.15±0.55	0.318
GI	1.70±0.26	1.89±0.47	1.51±0.27	0.097
PD (mm)	4.29±1.36	4.61±1.58	3.68±1.61	0.209
BOP (%)	59.42±22.81	70.17±32.98	37.90±20.60	0.038
GOI (%)	66.67±18.95	66.67±18.95	0	0.001

** Mann Whitney-U Test, p<0.05, PI: Plaque Index, GI: Gingival Index, PD: Probing Depth, BOP: Bleeding on Probing, GOI: Gingival Overgrowth Index*

A total of 143 proteins were identified in 14 gingival tissue samples using LC-MS/MS method, as shown in Table 2. The collective data revealed that among the total number of human proteins identified, 79 of them were detected in higher quantities in the GO+ group (p<0.05) whereas remaining 64 were found higher in the GO- group (p<0.05).

As shown in Figure 1, the molecular functions of the identified proteins in the GO+ group were associated with catalytic activity (53.8%), binding (38.5%), molecular function regulator (3.8%), structural molecule activity (1.9%) and transporter activity (1.9%), while the distribution of the identified proteins in the GO- group were related with, catalytic activity (33.3%), binding (48.9%), molecular function regulator (4.4%), structural molecule activity (4.4%), molecular adaptor activity (4.4%), and transporter activity (4.4%). The most up-regulated proteins in the GO+ group were found to be related with catalytic activity, whereas the proteins having a role in binding to cell surface receptors were noticed at the highest rate in the GO- group.

Figure 2 shows the distribution of identified proteins involved in biological processes for the GO+ group were as follows, cellular process (30.2%), metabolic process (22.2%), biological regulation (12.7%), response to stimulus (10.3%), localization (9.5%), signaling (4.8%), immune system process (4%), interspecies interaction between organisms (3.2%), developmental process (1.6%), multicellular organismal process (0.8%) and biological adhesion (0.8%); while the distribution for the GO- group were cellular process (30.9%), metabolic process (14.4%), localization (11.3%), biological regulation (11.3%), response to stimulus (9.3%), signaling (6.2%), developmental process (4.1%), biological adhesion (4.1%), multicellular organismal process (3.1%), immune system process (2.1%), interspecies interaction between

organisms (1%), locomotion (1%) and growth (1%). Regarding the biological processes, proteins taking role in cellular process were found to be the most markedly up-regulated proteins in both groups.

Further examination of functional and biological characteristics revealed that some of the identified proteins having roles in stimulatory or inhibitory processes may be associated with NIGO. These are; pyruvate dehydrogenase kinase isozyme 1, macrophage migration inhibitory factor, striatin, myeloid-derived growth factor, derlin, thioredoxin domain containing protein 17, annexin, peroxiredoxin-4, dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2, guanine nucleotide-binding protein subunit beta-1, integrin-linked protein kinase, CDKN2A-interacting protein and programmed cell death protein.

As shown in Figure 3, the cellular component distribution of proteins were classified as; cellular anatomical entity, protein containing complexes and intracellular proteins in both groups which were rated as 45.7%, 19% and 35.2% for the GO+ group, respectively; and 48.6%, 17.1% and 34.3% for the GO- group, respectively. The proteins which have cellular anatomical entity demonstrated the most elevated levels in both groups.

Table 2. *The list of identified proteins*

Protein Name	Accession Number	P Value	Q Value	Fold Change	Power
Small nuclear ribonucleoprotein	P62314	0.0494506	0.3444506	2.74500693	0.68099941
Zinc finger protein 700	Q9H0M5	0.0478779	0.3125406	4.72471546	0.795083946
Macrophage migration inhibitory factor	P34884	0.0469577	0.3905696	1.76736934	0.587594274
Coiled-coil-helix-coiled-coil-helix domain-containing protein 2	Q9Y6H1;Q5T1J5	0.0465955	0.3943175	1.77652293	0.57250119
Transcobalamin-2	Q9R0D6	0.0463088	0.2726492	4.7581034	0.861144554
S-adenosylmethionine synthase	Q4L7C7	0.0460559	0.3964464	1.7423305	0.555635061
Ankyrin repeat domain-containing protein 22	Q5VYY1	0.0460435	0.2270366	16.0487573	0.934527505
Platelet-activating factor acetylhydrolase IB subunit beta	P68402	0.045364	0.3284671	1.743945	0.700942142
Protein S100-A2	P29034	0.0437645	0.2000892	29.0562674	0.986536066
OCIA domain-containing protein 1	Q6NYD7	0.0436608	0.2479149	3.15127175	0.906039036
Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1	Q15118;Q63065	0.0429072	0.1410083	12.639472	0.999123168
39S Ribosomal rotein L41_ mitochondrial	Q8IXM3	0.0418746	0.3255255	9.76112542	0.711532306
Myristoylated alanine-rich C-kinase substrate	P29966	0.0418546	0.3223091	1.73490353	0.76171896
Atypical kinase COQ8A	Q60936	0.0413078	0.3943175	3.04513551	0.573975836
CDKN2A-interacting protein	Q9NXV6	0.040622	0.2135866	2.13707375	0.976937958
Dynein light chain roadblock-type 1	Q9NP97;Q8TF09	0.0401211	0.2261721	2.24604037	0.960450498
Sterol-4-alpha-carboxylate 3-dehydrogenase decarboxylating	Q15738	0.0382684	0.3943175	1.61296849	0.573906578
Minor histocompatibility antigen H13	Q8TCT9	0.0373349	0.2323764	1.85436527	0.922115453
Orotidine 5'-phosphate decarboxylase	A8Z6D0	0.0360385	0.2543735	2.44554601	0.89727168
Retroviral-like aspartic protease 1	Q53RT3	0.0354336	0.2261721	11.1586553	0.961766064
Inhibitor of Bruton tyrosine kinase	Q9P2D0	0.0345971	0.3123982	2.75312185	0.802580257
Striatin	O43815	0.0345886	0.3223091	1.67677781	0.750769371
Phosphomethylpyrimidine synthase	Q4JVZ0	0.0345613	0.3964464	1.53558654	0.563943207
Myotubularin	Q13496;Q9Z2C5	0.0344052	0.3887715	10.6141569	0.592047309
Surfeit locus protein 4	O15260	0.0337239	0.1230189	1.72952404	0.999940605
Myeloid-derived growth factor	Q969H8;Q9CPT4;P62248	0.0333449	0.277251	1.93441014	0.844173717
Grancalcin	P28676	0.033282	0.2543735	1.86803851	0.897186983
Signal recognition particle 54 kDa protein	P61011	0.0330232	0.3905696	1.52457041	0.586986556
Peptidyl-prolyl cis-trans isomerase FKBP11	Q9NYL4	0.032833	0.3223091	1.93834411	0.739459546
Programmed cell death protein 4	Q53EL6	0.0279365	0.1811616	1.70692707	0.994490283
Leucine-rich repeat-containing protein 59	Q96AG4	0.027685	0.3255255	2.37681336	0.728506738

Table 2. *The list of identified proteins (continued)*

Protein Name	Accession Number	P Value	Q Value	Fold Change	Power
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P10515	0.0271859	0.3943175	1.72991847	0.577789621
Gag-Pro-Pol polyprotein	P03361	0.0266269	0.3223091	5.55732404	0.740907027
Signal peptidase complex catalytic subunit SEC11C	Q9BY50	0.0265996	0.3223091	2.03108407	0.745842721
Signal peptidase complex subunit 3	P61009	0.0265591	0.2726492	1.5691254	0.853652628
Thioredoxin domain-containing protein 17	Q9BRA2	0.0264845	0.2261721	1.60326151	0.958475264
Proliferating cell nuclear antigen	Q9DEA3	0.0264741	0.3656748	18.4921742	0.621154602
S-adenosylmethionine synthase	Q9ZMN5	0.0263273	0.3444506	2.39962297	0.647887343
Annexin A3	P12429	0.0261257	0.3255255	1.79484398	0.711440398
Succinate-CoA ligase [ADP-forming] subunit beta	Q2GEF1	0.025605	0.2046882	3.70168551	0.983097635
DnaJ homolog subfamily B member 11	Q9UBS4	0.025593	0.2270366	1.53692691	0.92866995
Mesencephalic astrocyte-derived neurotrophic factor	P55145	0.0247768	0.2135866	1.77713195	0.975413303
Extracellular superoxide dismutase [Cu-Zn]	P08294	0.0247699	0.3284671	1.73638144	0.697792648
Spondin-1	Q9HCB6;Q9GLX9	0.0246131	0.3284671	1.69274015	0.70526491
Very-long-chain enoyl-CoA reductase	Q9NZ01	0.0243144	0.3255255	2.07954226	0.727043325
Dolichol-phosphate mannosyltransferase subunit 1	O60762	0.0234614	0.3444506	1.67444642	0.646561629
Aldo-keto reductase family 1 member B15	C9JRZ8	0.0233771	0.3284671	6.12802928	0.698785084
Translocon-associated protein subunit alpha	P43307	0.0233119	0.1811616	1.59502778	0.992895743
Mitochondrial 2-oxoglutarate/malate carrier protein	Q02978	0.0232578	0.1811616	1.86519549	0.993412988
Lysozyme C	P61626	0.0230058	0.3223091	2.66994427	0.740274942
Ras-related protein Rab-3C	Q96E17	0.022807	0.3444506	7.97927353	0.667355132
Glycogen phosphorylase muscle form	P11217	0.0223101	0.1410083	2.1824605	0.999180605
Putative HLA class I histocompatibility antigen alpha chain H	P01893	0.0221405	0.3444506	2.10721218	0.658071653
ATP synthase subunit alpha	Q2GER5	0.0216382	0.3223091	1.51863186	0.74143263
Marginal zone B – and B1-cell-specific protein	Q8WU39	0.0212303	0.1261953	2.41340595	0.999857756
Fumarate hydratase_ mitochondrial	P97807	0.0211739	0.3125406	3.70671211	0.787282188
Endoplasmic reticulum resident protein 29	P30040;P81623	0.0202626	0.1261953	1.64950507	0.999833658
ADP-ribosylation factor-like protein 8A	Q96BM9	0.0201756	0.3483712	2.05341452	0.63628478
Phosphoglycerate kinase 2	P09041	0.0197738	0.3588169	1.78912135	0.627392388
N-acetyl-D-glucosamine kinase	Q9QZ08	0.0191735	0.2885533	3.0009926	0.82026268
Signal peptidase complex subunit 2	Q15005	0.0188479	0.0930501	2.12728597	0.999999925
Nucleobindin-1	Q02818	0.0188328	0.3255255	1.9367718	0.719351807
Olfactomedin-like protein 1	Q6UWY5	0.0186546	0.2881296	1.86819209	0.828938891
Erythrocyte band 7 integral membrane protein	P27105	0.0181331	0.2316996	1.60684163	0.924409994
Nidogen-1	P14543	0.0174738	0.2261721	2.72995478	0.953511525
Methylmalonate-semialdehyde dehydrogenase [acylating]	Q02252	0.0159665	0.3223091	2.64178933	0.752466404
Guanine nucleotide-binding protein G(i) subunit alpha	P08754;P08753	0.0158337	0.3444506	1.98163028	0.667244381
Protein-glutamine gamma-glutamyltransferase K	P22735	0.0158215	0.3255255	2.53768358	0.710419009
Galectin-3	P17931	0.0155269	0.3444506	1.75854584	0.659501018
Eukaryotic translation initiation factor 6	O55135;P56537	0.0152499	0.3444506	2.32651092	0.655670144
Olfactomedin-like protein 3	Q9NRN5	0.0143952	0.2270366	1.71008181	0.940712533
26S proteasome regulatory subunit 4	Q90732;P46466	0.0141739	0.2000892	1.95399027	0.98548403
Leukocyte elastase inhibitor	P30740	0.014025	0.2783066	1.62640482	0.839515115
Peroxiredoxin-4	Q13162	0.0135143	0.0880851	3.13111297	0.999999996
ATP synthase subunit beta	B9E8E6	0.0132827	0.3125406	1.94261605	0.787116367
Septin-6	Q14141	0.0131362	0.1811616	2.43375325	0.993686597
Small proline-rich protein 3	Q9UBC9	0.0130156	0.1410083	4.90568906	0.99947845
Septin-14	Q6ZU15	0.0127058	0.27402	2.7950391	0.850675595
Replicase polyprotein 1ab	K9N7C7	0.0121312	0.3255255	2.07353472	0.714644895
Peripherin	P41219	0.0119551	0.2261721	2.34766417	0.953857353
Tubulin beta chain	Q7KQL5	0.0111684	0.3255255	1.71872222	0.710777958
Src substrate cortactin	Q14247	0.0109764	0.2885533	8.06699095	0.822004131
Chaperone protein DnaK	A7GXU4	0.0109734	0.3847723	3.45751798	0.601744719
Cystatin-B	P04080	0.0108682	0.3171161	1.76846961	0.772746792

Table 2. *The list of identified proteins (continued)*

Protein Name	Accession Number	P Value	Q Value	Fold Change	Power
Cartilage oligomeric matrix protein	P49747	0.0101003	0.0880851	2.47772586	0.999999996
Transmembrane protein 43	Q9BTV4	0.0100313	0.2543735	1.53673907	0.885697972
Aldehyde dehydrogenase family 3 member A2	P51648	0.0100252	0.2543735	3.32059814	0.881620602
Immunoglobulin heavy constant alpha 1	P01876	0.0093938	0.3444506	1.82173435	0.663923212
Desmin	P31001;P17661	0.0093368	0.3255255	9.10387636	0.710177479
Protein S100-A8	P05109	0.0090665	0.3255255	1.68831625	0.731313778
Biglycan	P21810	0.0087172	0.2270366	2.70289177	0.931861137
Inter-alpha-trypsin inhibitor heavy chain H1	P19827	0.0082459	0.3223091	2.53867226	0.757542475
Mimecan	P20774	0.0080826	0.113302	2.729652	0.999983623
Aldehyde dehydrogenase_ mitochondrial	P05091	0.0077833	0.241562	1.5090911	0.915501195
ATP-dependent RNA helicase eIF4A	P0CQ70;P0CQ71	0.0077791	0.3444506	2.31695327	0.66741294
Galectin-7	P47929	0.0075516	0.3125406	2.34418073	0.783540276
Catalase	P04040	0.0073611	0.277251	1.61494597	0.842294719
Heterogeneous nuclear ribonucleoprotein L	P14866	0.0069176	0.3444506	1.88765147	0.646758634
Myeloperoxidase	P05164	0.0067079	0.3444506	2.82568957	0.672256008
Hypoxia up-regulated protein 1	Q63617	0.0064996	0.3444506	1.80619151	0.677177949
Collagen alpha-2(VI) chain	Q02788	0.0059853	0.3444506	Infinity	0.671439997
Epoxide hydrolase 1	P07099	0.0059597	0.3943175	1.57980836	0.570916727
Transforming growth factor-beta-induced protein ig-h3	Q15582	0.0056148	0.3815853	1.85984727	0.606008932
Heterogeneous nuclear ribonucleoprotein R	O43390	0.0054654	0.2726492	1.53695077	0.857107301
Desmoglein-1	Q02413	0.0053651	0.3444506	1.66593836	0.65613829
Nucleoprotein TPR	P12270	0.0042724	0.3125406	3.18188131	0.789254833
Glycogen phosphorylase-brain form	P11216	0.0042699	0.3478382	1.6140184	0.64052864
Asporin	Q9BXN1	0.0041418	0.3444506	1.55111128	0.652864531
Thioredoxin domain-containing protein 5	Q8NBS9	0.0035843	0.2885533	1.59440314	0.825564784
Prolargin	P51888	0.0033696	0.2270366	2.96971508	0.943620631
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2	P04844	0.0033509	0.0116242	1.53964014	1
Plastin-2	Q61233	0.0033047	0.3847723	1.99018513	0.598390619
Decorin	P07585	0.0032437	0.3171161	1.83686709	0.771824683
Protein disulfide-isomerase A4	P13667	0.0025061	0.2686129	1.69255519	0.871053227
Immunoglobulin heavy constant gamma 3	P01860	0.0024355	0.2726492	2.45801404	0.855543903
Heat shock-related 70 kDa protein 2	P54652	0.0023972	0.113302	1.71989491	0.999993783
Probable ATP-dependent RNA helicase DDX5	A5A6J2;P17844	0.00228	0.3255255	1.97935445	0.712840555
Coatomer subunit beta	O55029	0.0021076	0.2270366	3.10673893	0.946494099
Protein disulfide-isomerase A3	P30101	0.0017214	0.2000892	1.62917084	0.9859406
Immunoglobulin heavy constant gamma 4	P01861	0.0014507	0.3444506	2.15666663	0.672341363
Transitional endoplasmic reticulum ATPase	Q7ZU99	0.0012721	0.2270366	4.0405704	0.92861101
Periostin	Q62009	0.0012484	0.1683598	2.11026082	0.99758383
Keratin type II cytoskeletal 4	P19013	0.0011481	0.3943175	1.8453487	0.568928235
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1	P04843	0.0011056	0.2135866	1.63474264	0.975442197
Actin aortic smooth muscle	P62736;P63267	0.0008349	0.3255255	7.85237412	0.726979068
Keratin type II cytoskeletal 1	P04264;A5A6M6	0.0008055	0.1811616	2.45738148	0.995578171
Keratin type II cytoskeletal 6B	Q9Z331	0.0006659	0.1410083	6.63077012	0.999398198

Table 2. *The list of identified proteins (continued)*

Protein Name	Accession Number	P Value	Q Value	Fold Change	Power
Periostin	Q15063	0.0005452	0.2543735	1.57656593	0.884225394
Keratin type I cytoskeletal 19	P08727	0.0005163	0.1507498	2.40782814	0.998614781
Protein-glutamine gamma-glutamyltransferase E	Q08188	0.0004337	0.2479149	1.95355944	0.906833537
Endoplasmin	P14625	0.0002239	0.2543735	1.64650041	0.884267123
Keratin type I cytoskeletal 13	P13646;A5A6P3;O76009;O76011;Q9C075	0.0001589	0.113302	3.93747234	0.999978162
Keratin type II cytoskeletal 6A	P02538	0.000158	0.2543735	2.64170703	0.896233369
Apolipoprotein B-100	P04114	0.000000699	0.2689355	1.50061659	0.868712906
Derlin-2	Q9GZP9	0.0322501	0.3790223	1.59148063	0.609164362
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	P62873	0.0318179	0.3223091	1.65624766	0.743890989
Integrin-linked protein kinase	O55222;Q13418	0.0318036	0.3444506	19.6263433	0.646500435
Alpha-mannosidase 2	Q16706	0.0317961	0.2000892	2.24080842	0.985297612
ATP synthase subunit beta	P85446	0.0312786	0.2885533	5.80844931	0.821857339
High mobility group protein B1	P09429;P10103;P63159;B2RPK0	0.0311746	0.2471222	2.48853462	0.910609495
Immunoglobulin heavy variable 3-7	P01780;A0A0B4J1V1;P01762;P01763;A0A0B4J1X8;A0A0C4DH32;P01766	0.0311637	0.3171161	1.95019881	0.777037742
Torsin-1A-interacting protein 1	Q5JTV8	0.0305529	0.3847723	1.73495521	0.59826925
Ras-related protein Rab-3A	P20336	0.0300769	0.3255255	5.28473034	0.719461125
GTP-binding protein SAR1a	Q9NR31;Q9Y6B6	0.0280528	0.3223091	1.81775252	0.762300497

Figure 1. *Molecular function distribution of identified proteins by Panther Analysis*

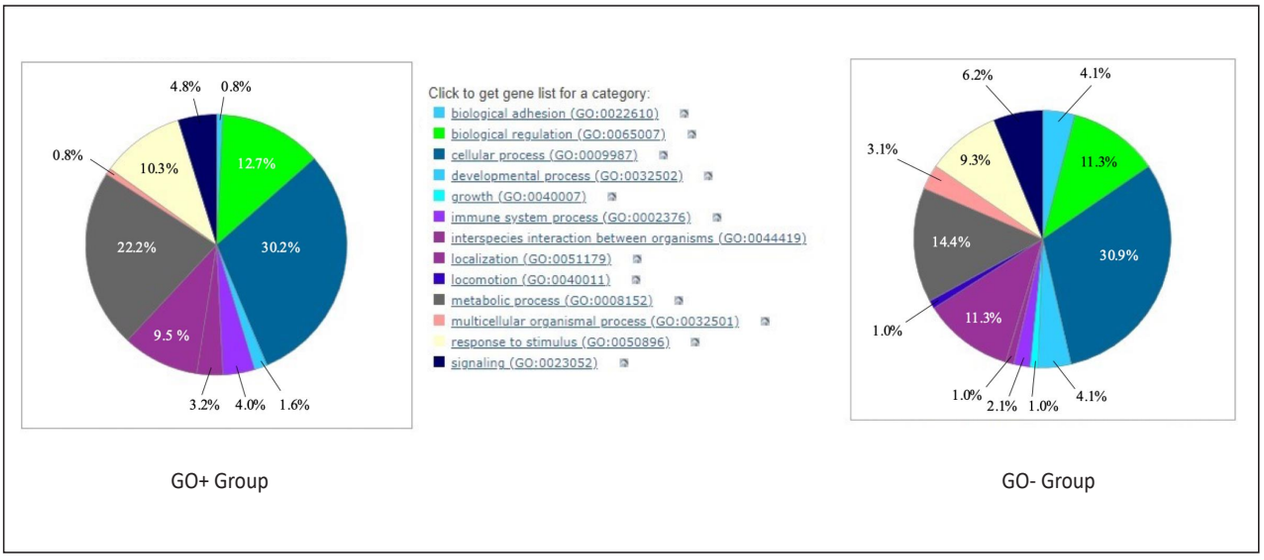

Figure 2. *Biological process distribution of proteins by Panther Analysis*

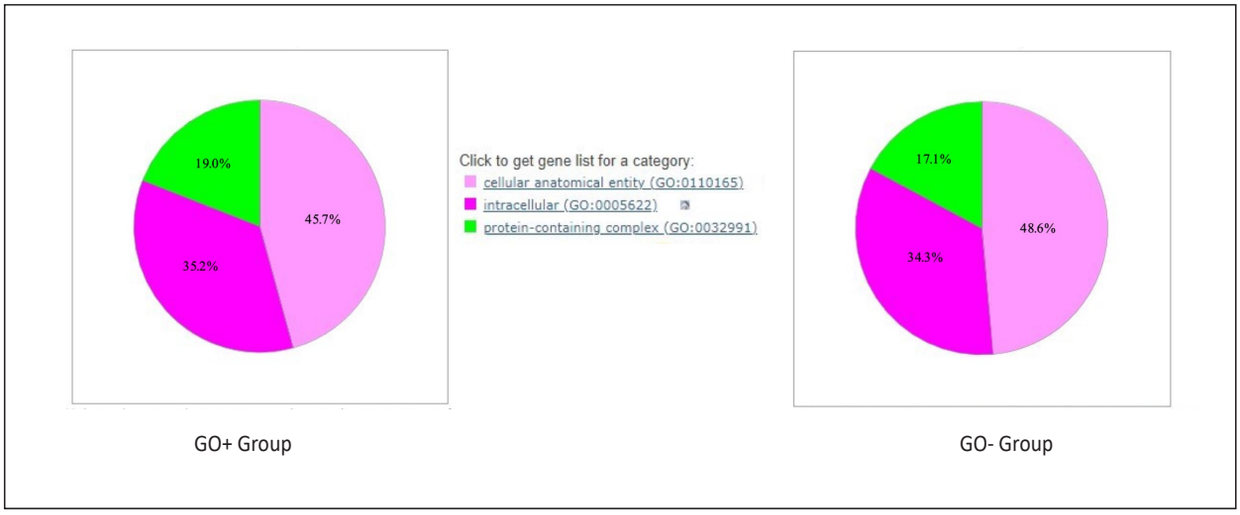

Figure 3. *Cellular component distribution of proteins by Panther Analysis*

4. DISCUSSION

DIGO is a well-known common side effect of systemically used drugs, including nifedipine. NIGO has been reported to possess the highest prevalance rate among other CCBs (24). Despite extensive in vivo and in vitro studies, the underlying pathological mechanism causing GO in affected individuals has not been fully defined as well as it is still unclear why some of the individuals using nifedipine develop GO while some others do not (25). In addition, the growth pattern of the same individual varies in different gingival regions. Recent studies have reported several associated factors such as genetic predisposition, decrease of cellular folate uptake, increased levels of certain cytokine and growth factors in overgrown gingiva (26, 27). Moreover, the severity degree of periodontal inflammation may play a role in the pathogenesis and onset of GO. Under nifedipine medication, GO may occur besides the existing periodontitis; also GO may be an initiative factor for periodontitis due to the poor oral hygiene and development of periodontal inflammation resulting with the increase in pocket depth. This cause-effect relation stil has not been illuminated (28).

Specifically, the proteome is defined as proteins that are produced in an organism under certain conditions, in a particular cell type or at a particular time. The main objective of proteomics is the rapid and quantitative characterization

of proteins. Proteomics provides biological information for many pathologies and is used to detect biomarkers of many tumours and inflammatory diseases, as well as to determine the expression of numerous proteins in cells (29).

The formation of wide and detailed expression profile database for NIGO is strongly requested and needed for better understanding its pathogenesis, discovering the potential biomarkers underlying the process and allowing an illumination for this unclear periodontal pathology. So far, the pathogenesis of NIGO has been studied in saliva, serum, gingival tissues and GCF with previously selected specific biomarkers, cytokines or genes (30, 31). However, a direct protein analysis of the overgrown gingival tissues is yet to be obtained to understand the underlying mechanism. The proteomics technique enables to uncover the biological substances and cell ingredients that may have a role in pathways leading to the clinical presentation of NIGO. Gingival tissue is the only biological material in which proteomics can offer the accurate molecular analysis.

In this cross-sectional study, the proteomic expression profiles related to overgrown gingival tissues were examined together with non-overgrown tissues of the same patients under systemic nifedipine treatment. The LC-MS/MS technique was chosen to evaluate and examine the protein content of the gingival tissues in detail, providing a basis for further studies on DIGO and NIGO, in particular.

Proteomics has been applied in medical sciences especially for the investigation of cancer and organ fibrosis (32, 33). The use of LC-MS/MS technique for proteomics has been performed in the field of periodontology recently. Tsuchida et al. (34) studied on GCF samples of healthy and chronic periodontitis individuals and identified 619 proteins of which several potential biomarkers for periodontal disease were included. McKnight et al. (35) identified 518 proteins of human gingival and periodontal ligament fibroblasts and compared with each other according to their functions. Yang et al. (36) examined the alveolar bone and surrounding soft tissue to further enlighten the regenerative process and healing. They revealed ECM associated proteins and showed soft and hard tissue interacted proteins during healing process (36). Reichenberg et al. (37) investigated the proteome map of human periodontal ligament fibrils obtained from extracted teeth and detected a total of 117 proteins with 9 different functions. The proteomic profiles of healthy and pathologic gingival pocket tissues were compared by Monari et al. (38) to analyze periodontal disease pathogenesis in more detail. They identified proteins associated with cell proliferation and apoptosis (38).

Currently, there is only one in vitro study in the literature on DIGO, in which proteomics was performed for gingival fibroblast cultures. Jung et al. (16) examined and quantified the proteins expressed by cultured gingival fibroblasts treated with cyclosporin-A at a concentration of 10 µM for 48 hours, and reported 17 proteins associated with cell proliferation.

In our study, proteomics was applied to analyze and compare the protein content of overgrown and non-overgrown gingival tissues of same patients under systemic nifedipine treatment aiming to recognize differently expressed proteins. So far, highly sensitive gel free LC-MS/MS approaches have not been performed on gingival tissues intending on research of NIGO.

The proteins identified in higher quantities in the GO+ group were found to be mainly associated with cell proliferation, growth and development. Among those, protein kinases are enzymes that regulate the biological activity of proteins (39). Pyruvate dehydrogenase kinase isozyme which is upregulated in GO+ group, has the protein kinase activity phosphorylating proteins resulting in enzyme activity and location changes. Pyruvate dehydrogenase kinase is crucial for maintaining energy homeostasis under certain conditions of overfeeding. Moreover, inappropriate suppression of this enyzme activity may promote the development of metabolic diseases. It controls the conversion of pyruvate, coenzyme A (CoA) and nicotinamide adenine dinucleotide (NAD) to acetyl-CoA, NAD + hydrogen (NADH) and CO_2, thus linking fatty acid metabolism, glucose metabolism and tricarboxylic acid cycle each other (40). Pyruvate dehydrogenase kinase, a mitochondrial enzyme, is activated in various forms of cancer, resulting in the selective inhibition of pyruvate dehydrogenase (41). Cell proliferation and prevention of the cell from apoptosis are the main roles of this enzyme that should be given attention to and may be correlated with overgrown gingival tissues. Macrophage migration inhibitory factor, striatin, myeloid-derived growth factor and thioredoxin domain containing protein are associated with fibroblast proliferation, growth factor mediated cell survival, tissue growth, tumor development and angiogenesis which were found to be upregulated in the GO+ group. Such proteins have been studied continously in a number of cancer and metabolic disease researches as their role in tissue growth and cell proliferation is remarkable (42). Therefore, connection of the above mentioned proteins with GO mechanism should be further examined. Derlin accounts for cell signaling and protein binding activity and regulates indirectly the insulin-like growth factor receptor signaling pathway. Insulin-like growth factor was reported to be related with GO (43). Derlin protein was also analyzed in organ fibrosis and cancer and was reported to have indirect interaction with renal fibrosis and lung adenocarcinoma (44). Also, fibrosis of gingival tissues occurs as a clinical presentation of DIGO. Annexin plays a role in wound healing and inflammatory response. As a result of the imbalance of the injury-repair response, the number of fibroblasts remains elevated leading to collagen deposition (45). Besides this important function, Annexin acts as a responder to drugs in organisms (46). This function appears to be spectacular indicator for overgrown tissues under systemic nifedipine use. Jung et al. (16) reported downregulated levels of Annexin in cyclosporin-A treated gingival fibroblasts in vitro. This finding contradicts with our result in which the GO+ group showed high levels of Annexin protein. Peroxiredoxin has a

role in response to oxidative stress which protects the cell against damages of reactive oxygen species, inhibiton of cell apoptosis, organization of the ECM and management of cell proliferation (47). These activities indicate that peroxiredoxin may account for NIGO, hence significantly increased level of peroxiredoxin was determined in the GO+ group. Parallel to our finding, Jung et al. (16) reported elevated levels of peroxiredoxins in cyclosporin-A treated gingival fibroblasts.

Proteins related with cell apoptosis and growth regulation were detected higher in the GO- group. One of them was integrin-linked protein kinase, that takes place in growth factor signaling pathway and tumor necrosis factor-mediated signaling pathway (48). The initiation of tumor necrosis factor pathway results in downregulation of cellular processes such as transcription. Thus, this function may explain the increased level of this protein in the GO- group. CDKN2A-interacting protein has a role in signaling pathways involved in cell growth and apoptosis and regulates these processes in either up or down regulatory ways (49). Programmed cell death protein is responsible for down-regulation of cell proliferation, up-regulation of apoptosis and inflammatory response (50). Since this protein appeared to be upregulated in the GO- group, it warrants further research in the pathogenesis of NIGO.

5. CONCLUSION

The defined proteins expressed at different levels in both groups may contribute to the pathogenesis of NIGO. Further quantitative confirmation of these proteins by Western Blot is needed to understand their regulatory roles. Our study is the first which compares the proteomic profile of over-grown (GO+) and non-overgrown (GO-) gingival regions of the same individuals under systemic nifedipine treatment. In conclusion, although limited number of proteins were discovered, the obtained data support that up-regulated or down-regulated proteins may play roles in NIGO pathogenesis. The formation of a strong and detailed proteomic expression profile knowledge for NIGO would be valuable to understand the pathogenesis and progression of this clinical phenomenon and to establish new therapeutic strategies.

Acknowledgement

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Conflicts of interest

No potential conflict of interest was reported by any of the authors in this study.

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Clinical and Experimental Health Sciences

NF-κB and COX-2 Relation Between Endometrial Cancer and the Clinicopathological Parameters

Ahmet Cem Iyibozkurt[1], Baris Ertugrul[2,3], Elif Sinem Iplik[4], Bedia Cakmakoglu[2]

[1] Florence Nightingale Hospital, Department of Obstetrics and Gynecology, Istanbul, Türkiye.
[2] Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, Istanbul, Türkiye.
[3] Istanbul University, Institute of Graduate Studies in Health Sciences, Istanbul, Türkiye.
[4] Acibadem University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Türkiye.

Correspondence Author: Bedia Cakmakoglu
E-mail: bedia@istanbul.edu.tr

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ABSTRACT

Objective: Our study examines nuclear factor kappa B (NF-κB) and cyclooxygenase-2 (COX-2) polymorphisms in the most common gynecological cancer type, endometrial cancer, and the relationship between disease parameters and these polymorphisms.

Methods: In our patient group; while 109 endometrial cancer patients were examined and treated in the Department of Gynecology and Obstetrics, Istanbul Medical Faculty, and 106 healthy women without the disease were included in the control group. DNA of blood samples taken from all groups were isolated; COX-2 765C> G and COX-2 1195A> G polymorphisms were studied with NF-κB-94 ins / delATTG. Genotypes analyzed using the PCR-based restriction fragment length polymorphisms (RFLP) method were investigated in terms of the relationship between endometrial cancer susceptibility and endometrial cancer disease parameters. Results in SPSS 17 program; Student's t-tests were analyzed using Anova, Fisher's exact, and Chi-square tests.

Results: NF-κB D + and DD genotype, COX-2 765 G + and GG genotype, and COX-2 1195 AA genotype were found to be significantly more common in the endometrial cancer group compared to the control group ($p < 0.05$). However, no significant relationship was found between polymorphisms and disease parameters.

Conclusion: NF-κB and COX-2 polymorphisms are more common in women with endometrial cancer. However, the absence of a link between these polymorphisms and the prevalence or violence of the disease suggests that they often trigger cancer development.

Keywords: COX, endometrial cancer, genotype, NF-κB, polymorphism

1. INTRODUCTION

Endometrial cancer is one of the most common gynecological cancers in developed countries. In Turkey, it is also included in the first row in the incidence of gynecological cancer with cervical cancer (1). The development of the disease can be seen due to the deterioration of the balance between cell proliferation and apoptosis in the direction of loss in tumor suppression genes or increase in oncogene activation (2).

The average age of onset is 61 years, and prognostic variables include histological grade, depth of myometrial invasion, stage, cervical invasion, and metastases. The existence of these numerous criteria determines whether surgical therapy is adequate, and adjuvant treatment is used if necessary (3). To improve outcomes in the treatment of endometrial cancer, research are ongoing to split patients into subgroups based on clearer prognostic markers and to develop therapy groups tailored to them. At this point, cyclooxygenase (COX-2) and nuclear factor kappa B (NF-κB) also among the important parameters investigated. NF-κB is a primary transcription factor effective in the control of immune and inflammatory responses in the reproductive tract. One of the pathological conditions caused by loss of activity is endometrial cancers. Tumorigenesis, inhibition of apoptosis, and metastases are usually triggered by inflammatory factors (4,5). By regulating immune and inflammatory responses with cytokines and their receptors, it has been shown that cell differentiation, apoptosis and migration are involved in gene regulation mediated by cell adhesion molecules (6). Oh et al (7) showed in their studies that myometrial invasion in endometrial cancer can also develop through NF-κB. There are two isoforms of COX-2, and it has been shown in recent studies that it may be associated with malignant transformation and tumor progression in endometrial cancer (8). There are studies in the literature showing that COX-2 expression is increased in endometrial cancer and supporting that this may be a prognostic marker. Lambroupoulou et al (9,10),

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in their immunohistochemistry-based studies in 28 women with endometrial cancer, found that COX-2 expression was significantly correlated with the grade, myometrial invasion, histological type, and survival, and demonstrated the prognostic significance of COX-2 expression. It is thought that COX-2 shows its effects on cancer through NF-κB activation and apoptosis mechanism (11). NF-κB is thought to be important in this context and may be a marker for some types of endometrial cancer (12). It is known that loss of control in NF-κB activation leads to endometrial cancer by causing an increase in cell proliferation and inflammation, and suppression of apoptosis (13).

The aim of this study is to investigate the relationship between NF-κB-94 ins / delATTG, COX-2 765 C> G, and COX-2 1195 A> G gene variants in women with endometrial cancer, disease clinicopathological parameters in endometrial cancer and the frequency of these gene variants in Turkish population.

2. METHODS

2.1. Study Subjects

In this study, women who were treated by the Istanbul Faculty of Medicine, Department of Obstetrics or Gynecology or applied to the polyclinics were included in the study in two groups, after obtaining their informed consent. In the first group, 109 patients who were followed up and treated in the Gynecological Oncology Department of Istanbul Medical Faculty, Department of Obstetrics and Gynecology and were diagnosed with endometrial cancer after their operations were included. In the second group, 106 healthy individuals who were close to the average age of the patients in the age group and had no history of cancer were included. Ethical approval was obtained by the Medical Ethics Committee of Istanbul University Faculty of Medicine on 11.11.2010 (Number: 2009/2897-92). All demographic, clinical and pathological information of the individuals in the patient and control groups were transferred to a database and recorded in the study.

2.2. DNA Isolation

Blood samples from each individual were collected in EDTA tubes. Samples were isolated using the Invitrogen Purelink Genomic DNA Kit.

2.2.1. Lysate Preparation from Blood Samples

The temperature of the water bath or heatsink is set at 55°C. A sterile microcentrifuge tube is filled with 200 µl of fresh or frozen blood. Fill the sample with 20 µl of the Proteinase K included with the kit. Then, 20 µl of the RNase A supplied with the kit is added to the sample, vortexed vigorously, and incubated at room temperature for 2 minutes. To create a homogenous solution, add 200 µl of PureLink Genomic Lysis/ Link solution and thoroughly mix by vortexing. To enhance protein digestion, incubate for 10 minutes at 55°C. To the lysate, add 200 µl of 96-100 percent ethanol. Vortex the mixture for 5 seconds.

2.2.2. Binding to DNA

Add the Genomic Lysis/Link solution and ethanol-supplemented lysate (640 µl) to the PureLink spin column. Colon 1 min. Centrifuge at 10,000xg at room temperature. The collection tube is discarded and placed in a new PureLink Collection tube supplied with the kit. The DNA wash phase begins.

2.2.3. Washing Step

500 µl of Washing Solution I prepared with Ethanol is added to the column. The columns are centrifuged at 10,000xg for 1 minute at room temperature. The collection tube is discarded and placed in a new PureLink Collection tube supplied with the kit. 500 µl of Washing Solution II prepared with Ethanol is added to the column. Column at room temperature at maximum speed for 3 min. centrifugation is done. The collection tube is discarded.

2.2.4. DNA Elution Step

In a sterile 1.5 ml microcentrifuge tube, put the column. Fill the column with 25-200 µl of PureLink Genomic Elution Solution. At room temperature for 1 minute. After incubation, centrifuge at highest speed for 1 minute. Purified genomic DNA is included in the tube. To retrieve additional DNA, a second elution step is done into a fresh sterile 1.5 ml microcentrifuge tube with the same amount of elution solution. At room temperature, the column is centrifuged at maximum speed for 1.5 minutes. Purified genomic DNA is included in the tube. The column will be removed.

2.3. Polymorphism Analysis

In genomic DNA samples, alleles of COX-2 765C> G, COX-2 1195A> G and NF-κB – 94ins / del regions were amplified by polymerase chain reaction (PCR). A total of 25 µl PCR mix was prepared for the amplification of each of the DNA samples. This PCR mixture was prepared to have 100-200 ng of DNA, 1µl of each primer, 1mM dNTP, 1.5mM $MgCl_2$ and 1.0U Taq DNA Polymerase. Amplification reactions performed in Thermal Cycler. Amplification of PCR condition for both COX-2 regions was 94°C for 3 min for the initial denaturation step, followed by 33 cycles of denaturation at 94°C (15 sec), annealing at 58°C (30 sec), and extension at 72°C (60 sec). The final extension step was at 72°C for 7 min. Primer sequences for 765C>G polymorphism of COX-2 gene were forward – 5'-AGGCAGGAAACTTTATATTATTGG-3' and reverse – 5'-ATGTTTTAGTGACGACGCTTA-3'. The primers for 1195G>A polymorphisms of COX-2 gene were forward – 5'-CCCTGAGCACTACCCATGAT-3', reverse – 5'-GCCTTCATAGGAGATACTGG-3'. The primer sequences used for NF-B-94 ins/del were selected as forward-5' – TTTAATCTGTGAAGAGATGTGAATG-3', reverse – 5'-GCCTTCATAGGAGATACTGG-3'. Enzymatic digestions were performed with AciI, PvuII and Van91I enzmes for COX-2

765C>G, COX-2 1195A>G and NF-κB – 94ins / del polymorphisms respectively.

2.4. Statistical Analysis

In the statistical analysis of this study, SPSS 11.0 package program was used. Statistical significance was taken as $p < 0.05$. Chi-square (χ^2) and Fisher tests were used to evaluate the differences in the frequency of genotypes and alleles between groups. Student's t-test and Annova were used to compare demographic data between groups. Allele frequencies were made according to the gene counting method. Fisher's exact and χ^2 tests were used to investigate the relationship between endometrial cancer disease parameters and polymorphisms.

3. RESULTS

3.1. Demographical Data

The demographic information of the study groups is shown (Table 1). For the control group, the mean and standard deviation of age distribution was found 52.81± 8.4, and it was found 55.25 ± 8.37 for the patient group. There was no statistically significant difference between the control and patient groups in terms of age distribution (p= 0.07).

Table 1. *Demographic data of the study group*

	Patient (N=109)
Age of menarche, age ±SD	13.39 ± 1.64
Last menstrual time	51.40 ± 4.83
Oral contraceptive use (%)	
Yes	76.9
No	23.1
Family planning (%)	
Yes	41.3
No	58.7
Diabetes (%)	
Yes	25.7
No	74.3
Hypertension (%)	
Yes	44.6
No	55.4
Hystology (%)	
Endometrioid	87.5
Adenocarcinoma	2.5
Serous	5.0
Clear cell	2.5
Indifferentiated	2.5
Grade (%)	
I	61.5
II	23.1
III	15.4

SD: standart deviation

3.2. COX-2 765 C → G genotype distributions

COX-2 765 C → G genotype distributions in control and patient groups are shown in Table 2. COX-2 765 CC genotype distribution was significantly different in the control group compared to the patient group (χ^2= 4.55; p= 0.033; OR= 0.35; 95%CI= 0.13-0.94). COX-2 765 GG genotype distribution was significantly different in the control group compared to the patient group (χ^2= 16.29; p= 0.000; OR= 3.10; 95%CI=1.77-5.41) COX-2 765 CG genotype distribution was significantly different in the control group compared to the patient group (χ^2= 7.83; p= 0.45; OR= 0.45;95%CI= 0.26-0.79).

These findings were significantly different between the control and patient groups (Table 2).

3.3. COX-2 1195A → G genotype distributions

COX-2 1195A → G genotype distributions in control and patient groups are summarized in Table 3. COX1195 AA genotype distribution was significantly different in the patient group compared to the control group (χ^2= 8.78; p= 0.003; OR= 2.35; 95%CI= 1.33-4.14). COX1195 GG genotype distribution was significantly different in the patient group compared to the control group (χ^2= 3.96; p= 0.064; OR= 1.03; 95%CI= 1.00-1.07) COX1195 AG genotype distribution was significantly different in the patient group compared to the control group (χ^2= 12.78; p=0.000; OR= 0.35; 95%CI= 0.19-0.62)

3.4. NF-ĸB genotype distributions

Genotype distributions of NF-κB genes were found significantly different between control and patient groups (Table 4). NF-κB WW genotype distribution was significantly different in the patient group compared to the control group (χ^2= 5.73; p= 0.017; OR= 0.51; 95%CI= 0.29-0.88) NF-κB DD genotype distribution was significantly different in the patient group compared to the control group (χ^2= 34.11; p= 0.000; OR= 8.94; 95%CI= 3.96-20.2) NF-κB WD genotype distribution was significantly different in the patient group compared to the control group (χ^2= 8.23; p= 0.004; OR= 0.43; 95%CI= 0.24-0.77)

3.5. COX-2 765: 1195 Haplotype frequency

Haplotype frequencies of COX-2 765: 1195 were shown in Table 5. Despite the fact that the association between endometrial cancer disease parameters and statistically significant polymorphisms is analyzed in Table 6, there is no statistically significant relationship between disease parameters and polymorphisms.

Table 2. *COX-2 765 C → G genotype distributions in control and patient groups*

COX-2 *765 C→G* Genotypes	Control N:106		Patient N:109		P value	χ^2	OR	95% CI
	N	%	N	%				
CC	15	14.1	6	5.5	0.03*	4.55	0.35	0.13-0.94
GG	36	34	67	61.5	0.000**	16.29	3.10	1.77-5.41
CG	55	51.9	36	33.0	0.005**	7.83	0.45	0.26-0.79
Alleles								
C	85	40.1	48	22.02	0,000**	16.43	0.32	0.18-0.56
G	127	59.9	170	77.98				

*OR: odd ratio, CI: confidence interval, (one asterisk * indicates for p<.05; two asterisks ** indicate for p<.01).*

Table 3. *COX-2-1195A → G genotype distributions in control and patient groups*

COX-2-*1195A→G* Genotypes	Control N:106		Patient N:109		P value	χ^2	OR	95% CI
	N	%	N	%				
AA	56	52.8	79	72.5	0.003*	8.78	2.35	1.33-4.14
GG	0	0	4	3.7	0.064	3.96	1.03	1.00-1.07
AG	50	47.2	26	23.8	0.000**	12.78	0.35	0.19-0.62
Alleles								
A	162	76.42	184	84.4	0.03*	4.36		
G	50	23.58	34	15.6				

*OR: odd ratio, CI: confidence interval, (one asterisk * indicates for p<.05; two asterisks ** indicate for p<.01).*

Table 4. *NF-ĸB genotype distributions in control and patient groups*

NF-ĸB Genotypes	Control N:106		Patient N:109		P value	χ^2	OR	95% CI
	N	%	N	%				
WW	51	48.1	35	32.1	0.017*	5.73	0.51	0.29-0.88
DD	8	7.5	46	42.2	0.000**	34.11	8.94	3.96-20.2
WD	47	44.4	28	25.7	0.004**	8.23	0.43	0.24-0.77
Alleles								
W	149	70.28	98	44.96	0.0000**	28.20		
D	63	29.72	120	55.04				

*OR: odd ratio, CI: confidence interval, (one asterisk * indicates for p<.05; two asterisks ** indicate for p<.01).*

Tablo 5. *COX-2 765:1195 haplotype frequencies*

Haplotype	Frequency				
	Total	Patients	Controls	χ^2	*p* – value
COX-2 765:1195					
GA	0.530	0.638	0.418	21.019	$4.54.10^{-6}$ **
CA	0.275	0.206	0.347	10.691	0.0011**
GG	0.161	0.141	0.181	1.27	0.2598
CG	0.034	0.014	0.054	5.189	0.0227*

*(one asterisk * indicates for p<.05; two asterisks ** indicate for p<.01).*

Tablo 6. *Relationship between endometrial cancer disease parameters and statistically significant polymorphisms*

Polymorphism Type	*COX-2 755 G+*	*COX-2 765 GG*	*COX-2 1195 AA*	*NF-κB D+*	*NF-κB DD*
Endometrial Cancer Clinicopathological parameter					
Type1 and Type2	**0.59**	**0.87**	**1.00**	**0.35**	**0.54**
Presence of deep invasion	**0.42**	**0.99**	**0.95**	**0.75**	**0.61**
Early Stage (1-2) vs Advanced Stage (3-4)	**0.50**	**0.73**	**0.43**	**0.44**	**1.00**
Presence of lymphovascular area invasion	**1.00**	**0.49**	**0.64**	**0.71**	**0.35**

(results are given as p value, all results were found as statistically not significant; p <.05)

4. DISCUSSION

In our study, it was found that NF-κB-94ins / del polymorphisms increased the risk for endometrial cancer in patients carrying the del allele. Besides, the susceptibility to endometrial cancer was higher in COX-2 765 C> G gene polymorphism in individuals carrying homozygous GG or G allele and in COX-2 1195 A> G gene polymorphism for homozygous AA individuals. One of the strengths of our study is that the patients used in our study had well-characterized data and that the age range determined for the patient and control group was kept close. The endometrium patients determined for the patient group consisted of patients who are diagnosed with endometrium because of pathology.

One of the weaknesses of this study may be the low number in the patient and control groups. In our study, we found no difference in both NF-κB and COX-2 polymorphism rates in type 1 and type 2 endometrial cancer groups. This shows that polymorphisms are more related to the occurrence of cancer itself than the type of endometrial cancer. It has been reported that NF-κB suppression is associated with a decrease in tumor incidence and small tumor size in animal model studies (14). It is possible to interpret that the incidence of cancer may be affected by NF-κB gene polymorphisms. Andersen et al (15) stated in their study that NF-κB-94del polymorphism increased the risk for colorectal cancer 1.45 times. When compared with our study, it is seen that the results are consistent with each other, those carrying the Del allele in the NF-κB gene are thought to be riskier in terms of endometrial cancer. The relationship between NF-κB and endometrial cancer was first demonstrated in the study of Vaskivuo et al (16). NF-κB is a regulator with shown antiapoptotic function in many cells (17). NF-κB is also present in the endometrium during the normal menstrual cycle and increased NF-κB levels have been shown to suppress apoptosis. Increases in IL-1beta and COX2 transcription, induced by increased levels of NF-κB, have been reported to suppress the proapoptotic properties of TNF alpha (17,18). It can be thought that increased levels of NF-κB due to NF-κB polymorphisms may cause loss of control of cell proliferation and subsequently cancer by creating an antiapoptotic environment. In the period following cancer formation, NF-κB stimulates the increase of matrix metalloproteinases in malignant cells and accelerates the processes of tumor invasion, metastasis, and neovascularization (19,20). Vaskivuo et al (16) found that NF-κB levels in individuals with endometrial cancer decreased in their study and suggested that NF-κB did not have an important role in the spread of endometrial cancer. In our study, a relationship was found between NF-κB polymorphisms and the incidence of endometrial cancer, but when this relationship was evaluated in terms of clinicopathological data of the disease, no significant difference was found between disease types (16). PTEN inactivations have also been shown to trigger an increase in NF-κB activity (11).

The relationship between COX-2 gene polymorphisms and cancer has also been investigated in some studies. In a study conducted on individuals with colorectal cancer, it was found that individuals carrying the A allele at 1195 G> A polymorphism had a higher risk of cancer (21). In another study conducted with colorectal cancer patients, it was shown that the distribution of the 765GG genotype was high in patients (22). While COX-2 expression is seen in colorectal cancer tissue, the absence of expression in surrounding normal tissue is a sign that COX-2 plays an active role in tumor development (23). Nevertheless, some publications are stating that 1195 A> G polymorphisms are not associated with colon cancer (24). Our study is original in that it is the first study evaluating the relationship between endometrial cancer and COX-2 1195 G> A polymorphisms, and as a result, 1195 AA genotype was found to be significantly higher in the patient group. In studies conducted with other cancer types, the high incidence of cancer due to COX-2 1195 A> G has been found responsible for the increase in COX-2 gene transcription (21). COX-2 overexpression has been associated with its potential for resistance to apoptosis, adhesion, angiogenesis, and metastasis. Increased cancer susceptibility can be seen with the change caused by the mutation in the COX-2 gene. Another study that supports the results of our study is the decrease in cancer risk seen in the presence of the G allele (22). It has been suggested that the result of changes in the COX-2 gene may have different consequences in Europe, mixed races, and Chinese; for example, esophageal cancer has increased in the Chinese but does not change in Europeans (22,23). In our study, the significantly higher detection of COX-2 1195 A> G and COX-2

765 C> G polymorphisms in women with endometrial cancer indirectly supports the role of COX-2 in the development of this cancer.

Although we showed the connection between the detected NF-κB and COX-2 polymorphisms with endometrial cancer, no significant relationship was found between these polymorphisms and type 1 and 2 endometrial cancer. Likewise, no significant difference was found between these polymorphisms and the presence of deep invasion-lymphovascular area invasion or in terms of the frequency of early-advanced stage cancer. This brings to the fore the idea that NF-κB and COX-2 polymorphisms have a role in the development of type 1 or type 2 endometrial cancer, but do not directly affect the invasion degree or stage of cancer. Since these clinicopathological parameters we mentioned are also related to the aggressiveness of the disease, it can be suggested that the presence of these polymorphisms does not affect the aggressiveness of the disease. As a result, increased NF-κB and COX-2 activity has been demonstrated in colon cancer so far, and the relationship between endometrial cancer and NF-κB and increased COX-2 has been suggested (11,24-26). NF-κB and COX-2 activation may give the endometrium cancer cell a survival advantage, and it appears to do so by regulation of apoptosis (4). NF-κB may be providing this regulation through different mechanisms, since no link was found between NF-κB and absence of PTEN, kRAS mutation, and beta-catenin changes in these studies. The significant relationship between endometrial cancer and NF-κB-94 ins / del ATTG polymorphism, COX-2 765C> G and COX-2 1195A> G polymorphisms we found in our study support the mentioned findings and mechanisms. Some of the studies in the literature investigating the COX and NF-κB relationship with endometrium cancer, have showed that polymorphisms contributing the endometrium cancer development (27,28). Cavalcanti et al (29) showed that – 765G polymorphism of the COX-2 gene was associated with an increased risk for endometriosis in Brazilian fertile women, and also increased expression of COX-2 relative to the control group was shown in the patient group.

5. CONCLUSION

Our study is the first study to reveal the relationship between these polymorphisms and endometrial cancer. The absence of a significant relationship between polymorphisms and the clinicopathological parameters of the disease suggests that these polymorphisms are more important in terms of cell cancer, and they have less effect on the spread of the disease after cancer.

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Authors' Contributions: ACI: writing, sample collection and experimental process; BE: writing and experimental process; ESI: experimental process and statistical analysis; BC: statistical analysis and supervising.

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Clinical and Experimental Health Sciences

Effect of Non-surgical Periodontal Therapy on Salivary Melatonin Levels

Kubra Kundak[1], Aysen Yarat[2], Basak Dogan[3], Leyla Kuru[3]

[1] Marmara University, Institute of Health Sciences, Department of Periodontology, Istanbul, Türkiye.
[2] Marmara University, Faculty of Dentistry, Department of Basic Health Sciences, Istanbul, Türkiye.
[3] Marmara University, Faculty of Dentistry, Department of Periodontology, Istanbul, Türkiye.

Correspondence Author: Leyla Kuru
E-mail: lkuru@marmara.edu.tr
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ABSTRACT

Objective: Melatonin, a hormone secreted predominantly by pineal gland in a circadian manner, has antioxidant and anti-inflammatory effects. The current research is conducted to explore the influence of non-surgical periodontal therapy (NSPT) on levels of salivary melatonin in subjects with gingivitis and periodontitis.

Methods: Sixty systemically healthy participants were included in this study; the groups are as follows: gingivitis (G), chronic periodontitis (CP), generalized aggressive periodontitis (GAP) and periodontally healthy (H). NSPT was applied to G group patients for 2 sessions, to CP and GAP group patients for 4 sessions. Plaque and gingival indices, probing depth (PD), bleeding on probing (BOP), and clinical attachment level (CAL) were documented at baseline and 3 months post – treatment and early morning salivary samples were collected. ELISA was used to detect melatonin levels in saliva. Pittsburgh Sleep Quality Index (PSQI) questionnaire was performed to evaluate of sleep quality of patients.

Results: At baseline, significant difference in gingival index, PD, BOP and CAL values was detected among all groups ($p<0.05$). Following NSPT, clinical measurements improved in G, CP, and GAP groups significantly ($p<0.05$). While salivary melatonin concentration of all groups was similar at baseline ($p>0.05$), a significant elevation in the level of salivary melatonin was found only in the G group after NSPT ($p<0.05$). PSQI scores differed significantly among groups ($p<0.05$).

Conclusion: The salivary melatonin levels in the presence of gingivitis and periodontitis varied at baseline and elevated following NSPT parallel to the improvement in clinical parameters.

Keywords: Melatonin, gingivitis, periodontitis

1. INTRODUCTION

Melatonin, a natural hormone, is produced and secreted in a circadian way by retina, ovaries, gastrointestinal tract, leukocytes, lymphocytes, bone marrow and skin but predominantly the pineal gland (1). Melatonin is engaged in numerous physiologic processes, as well as regulation of circadian rhythm, control of body temperature and immunomodulation (2, 3). As melatonin controls the circadian rhythm, a two-way relationship between melatonin level and the quality of sleep is considered important (4). After its secretion, melatonin circulates in the bloodstream and diffuses passively into saliva (5, 6). Following recent discovery of its immune enhancing, anti-inflammatory and antioxidant properties against reactive oxygen species (ROS), melatonin was thoroughly studied in the medical and dental fields (6, 7). Furthermore, it has been established that melatonin may have an essential role in type I collagen synthesis and promotion of bone formation (6, 8, 9). Moreover, the remarkable efficiency and diversity of melatonin's physiological regulatory actions draw attention to prospective benefits of using it in the therapeutic prevention and treatment of bone disorders (10-12).

Periodontal disease is an inflammatory chronic condition commenced by microbial dental biofilm affecting periodontium and can be broadly categorized as gingivitis and periodontitis. Gingivitis is a biofilm induced inflammatory condition expressed by redness of gingiva, edema, and the lack of periodontal attachment loss (13). Periodontitis is a chronic inflammatory condition of periodontium that results in periodontal attachment loss, bone loss and inevitably tooth loss when not treated (14). Accumulation of microbial dental plaque containing periodontopathogen bacteria activates host immune response which results in secretion of proosteoclastogenic factors, inflammatory cytokines,

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and matrix metalloproteinases as well as generation of free radicals and ROS (15, 16). Furthermore, it has been reported that increased ROS levels in periodontal disease are associated with oxidative damage to the periodontal tissues and a decrease in antioxidative defense mechanisms (17-19).

Non-surgical periodontal therapy (NSPT) including oral hygiene instructions, scaling and root planing aims to stop the development of periodontal disease and to ensure optimal health and function by elimination of chronic inflammation (20). Thus, it also restores the oxidant/antioxidant balance within the periodontium (21).

The relationship between periodontal status and levels of salivary melatonin is yet to be determined. The current research was conducted to explore the influence of NSPT on salivary melatonin levels in individuals with gingivitis and periodontitis.

2. METHODS

The ethical approval was obtained from the Ethics Committee of Marmara University, Faculty of Medicine, Istanbul, Turkey on 05.10.2018 (#09.2018.0673).

2.1. Study Population

Sixty systemically healthy participants consisting of 15 subjects with gingivitis (G group), 15 subjects with chronic periodontitis (CP group), 15 subjects with generalized aggressive periodontitis (GAP group), 15 volunteers with periodontal health (H group) participated in this study (22) (According to the new periodontal disease classification (23) 11 of CP patients are diagnosed as Stage III Grade B, 4 of them are Stage III Grade C; whereas 13 of GAP group patients are diagnosed as Stage III Grade C and 2 of them are Stage IV Grade C). Inclusion criteria were as follows; (i) systemically healthy, (ii) non-smoker, (iii) no antibiotic usage within 3 months, (iv) no history of periodontal treatment within 6 months, (v) no pregnancy or lactation, (vi) presence of at least 20 teeth, and (vii) no medication. Informed consent was obtained from all individuals prior to inclusion to the trial. Exclusion criteria included conditions such as systemic disorders, pregnancy, lactation, smoking habits, and medications. Flowchart of the study is demonstrated in Figure 1.

2.2. Clinical Periodontal Examination

Panoramic radiography and intraoral photographs were taken from each participant. Following medical and dental history, thorough periodontal examination was performed by the same clinician (KK). Plaque index (PI) (24), gingival index (GI) (25), probing depth (PD), bleeding on probing (BOP), and clinical attachment level (CAL) were documented on six aspects per tooth via a periodontal probe (PCPUNC15, Hu-Friedy, Ins. Co., USA) at baseline and 3 months post – treatment.

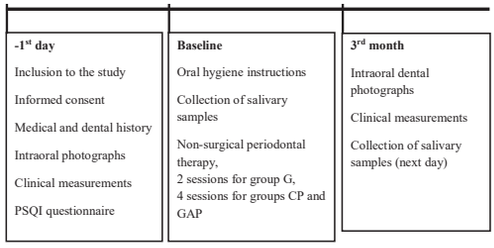

Figure 1. *Flowchart of the study.*

2.3. Salivary Sample Collection

All participants were instructed to refrain from consuming sustenance after 12:00 am prior to the salivary sample collection at baseline and 3 months following the treatment. All subjects were admitted to the clinic at 8:30 am on the day of sample collection. The unstimulated salivary samples were collected via spitting into a sterile glass beaker after accumulation on the floor of the mouth (26). The samples were then transferred to sterile tubes and were stored at −80°C until the day of assay.

2.4. Non-surgical Periodontal Therapy

At baseline, oral hygiene instructions were given, scaling and root planing with ultrasonic and hand instruments (WOODPECKER® Cavitron, Guilin Woodpecker Medical Ins. Co., China, EverEdge®; Gracey, 5/6, 7/8, 11/12, 13/14, Hu-Friedy Ins. Co., USA), was performed. Subjects in the G group received 2 sessions of NSPT, while patients in the CP and GAP groups underwent 4 sessions of NSPT under local anesthesia.

2.5. Determination of Melatonin Levels

On the day of assay, frozen salivary samples were thawed at room temperature and centrifuged at 3000 rpm for 20 min, the supernatant was taken and transferred immediately to a new propylene tube. The melatonin levels were determined by radioimmunoassay per manufacturer's instructions (RE54041, IBL GmbH, Hamburg, Germany).

2.6. Assessment of Sleep Quality

For assessment of sleep quality, subjects were instructed to complete Pittsburgh Sleep Quality Index (PSQI) questionnaire (Figure 2) at baseline. According to the PSQI, scores varies from 0 to 21 and scores higher than or equal to 5 indicate low quality of sleep (27).

2.7. Statistical Analysis

Demographic and clinical data were entered in a spreadsheet application and statistical analysis were completed using Statistical Package for the Social Sciences (SPSS® 20.0, Chicago, IL, USA). A p value<0.05 was considered as statistically significant. To test the normality, the Kolmogorov-Smirnov

test was done. Data were analyzed first by *Kruskal–Wallis* test among groups, and when significant difference was detected, *Mann–Whitney U* test with *Bonferroni* correction was applied for pair-wise comparison. *Wilcoxon* test was performed to analyze differences between the two time intervals.

Pittsburgh Sleep Quality Index

During the past month;

1. When have you usually gone to bed? ……
2. How long (in minutes) has it taken you to fall asleep each night? ……...
3. What time have you usually gotten up in the morning? ……
A. How many hours of actual sleep did you get at night? ……
B. How many hours were you in bed? ……

4. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s)				
5. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
6. During the past month, have you had trouble staying awake while driving, eating, or engaging in social activity?				
7. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
8. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	**C1 …**
Component 2	#2 (<15min (0), 16-30min (1), 31-60min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	**C2 …**
Component 3	#4 Score (>7(0), 6-7(1), 5-6(2), <5(3)	**C3 …**
Component 4	(total # of hours asleep)/(total # of hours in bed) x 100	**C4 …**
Component 5	# sum of scores 5b to 5j (0=0, 1-9=1, 10-18=2, 19-27=3)	**C5 …**
Component 6	#6 Score	**C6 …**
Component 7	#7 Score + #8 Score (0=0; 1-2=1; 3-4=2; 5-6=3)	**C7 …**

Add the seven component scores together = Global PSQI score …

Figure 2. *PSQI questionnaire (24).*

3. RESULTS

3.1. Demographic Data and Clinical Parameters

Demographic data are presented in Table 1.

Table 1. *Demographic data of healthy individuals and patients.*

	H group n=15	G group n=15	CP group n=15	GAP group n=15	*p*
Gender N (%)					
Female	9 (60)	9 (60)	6 (40)	8 (53.3)	0.658*
Male	6 (40)	6 (40)	9 (60)	7 (46.7)	
Age (years)					
Mean ± SD	30.27 ± 5.90	26.20 ± 5.16	38.27 ± 5.86†‡	32.47 ± 4.26‡	<0.0001**
Min – Max	23.0 – 45.0	22.0 – 40.0	30.0 – 51.0	27.0 – 40.0	

H: Healthy, G: Gingivitis, CP: Chronic Periodontitis, GAP: Generalized Aggressive Periodontitis, SD: Standard deviation
**Chi-square test, **Kruskal-Wallis test; †different from H group, p<0.05; ‡different from G group, Mann-Whitney U, p<0.05*

Table 2. *Clinical parameters of study population at baseline and 3rd month.*

		H group n=15 Mean±SD Min-Max Median	G group n=15 Mean±SD Min-Max Median	CP group n=15 Mean±SD Min-Max Median	GAP group n=15 Mean±SD Min-Max Median	*p**
PI	**Baseline**	0.12 ± 0.059 0.02 – 0.25 0.10	1.72 ± 0.30† 1.18 – 2.21 1.70	2.02 ±0.37†‡ 1.43 – 2.69 1.94	1.75 ±0.43† 1.05 – 2.48 1.56	<0.0001
	3rd month	-	0.12 ± 0.095 0.03 – 0.32 0.095	0.17 ± 0.17 0.01 – 0.68 0.12	0.23 ± 0.15 0.02 – 0.47 0.25	0.232
	p**	-	0.001	0.001	0.001	
GI	**Baseline**	0.12 ±0.059 0.03 – 0.22 0.11	1.67 ± 0.27† 1.14 – 2.19 1.66	1.84 ± 0.18† 1.44 – 2.11 1.89	1.97 ± 0.16†‡ 1.69 – 2.22 1.98	<0.0001
	3rd month	-	0.13 ± 0.10 0.04 – 0.42 0.13	0.22 ± 0.10‡ 0.02 – 0.48 0.21	0.34 ± 0.14‡ 0.08 – 0.61 0.30	<0.0001
	p**	-	0.001	0.001	0.001	
PD	**Baseline**	1.91 ±0.14 1.71 – 2.17 1.92	2.59 ± 0.29† 2.22 – 3.44 2.50	3.64 ± 0.64†‡ 2.99 – 5.54 3.53	4.43 ± 0.51†‡§ 3.72 – 5.82 4.34	<0.0001
	3rd month	-	2.08 ± 0.24 1.70 – 2.58 2.06	2.62 ± 0.40‡ 2.09 – 3.64 2.63	3.00 ± 0.37‡§ 2.56 – 4.01 2.88	<0.0001
	p**	-	0.001	0.001	0.001	
BOP	**Baseline**	5.80 ± 2.59 1.71 – 2.17 5.95	68.25 ± 17.94† 39.88 – 91.70 74.44	82.69 ± 16.33†‡ 44.05 – 100.00 89.4	90.97 ± 9.70†‡ 68.70 – 100.00 95.1	<0.0001
	3rd month	-	11.37 ± 6.18 4.17 – 19.64 10.12	12.38 ± 3.25§ 4.94 – 16.67 13.58	17.29 ± 6.36‡§ 7.30 – 25.93 17.36	<0.0001
	p**	-	0.001	0.001	0.001	
CAL	**Baseline**	1.92 ± 0.15 1.71 – 2.17 1.92	2.59 ± 0.30† 2.20 – 3.45 2.50	3.88 ± 0.64†‡ 3.23 – 5.79 3.72	4.84 ± 0.62†‡ 4.05 – 5.99 4.83	<0.0001
	3rd month	-	2.12 ± 0.24 1.77 – 2.60 2.06	3.10 ± 0.52‡ 2.40 – 4.48 3.18	3.90 ± 0.51‡§ 3.18 – 4.64 3.73	<0.0001
	p**	-	0.001	0.001	0.001	

*H: Healthy, G: Gingivitis, CP: Chronic Periodontitis, GAP: Generalized Aggressive Periodontitis, PI: plaque index, GI: gingival index, PD: probing depth, BOP: bleeding on probing, CAL: clinical attachment level, SD: Standard deviation*Kruskal-Wallis test, **Wilcoxon test, †different from H group, Mann-Whitney U, p<0.05; ‡different from G group, Mann-Whitney U, p<0.05; §different from CP group, Mann-Whitney U, p<0.05*

The clinical measurements of study population at baseline and 3rd month are displayed in Table 2. At baseline, PI and GI values of the treatment groups were significantly greater than the H group, as expected (p<0.001). PD, BOP and CAL values of the G group were detected to be higher than the H group and lower than the CP and GAP groups, significantly (p<0.001). Following NSPT, all clinical values improved significantly in all treatment groups (p<0.01). Following treatment, GI, PD, BOP and CAL values were found to be the highest in the GAP group, followed by the CP and G groups, respectively (p<0.001). At 3rd month, PI values were found to be similar in all groups (p>0.05).

As presented in Table 3, at baseline, significant difference in salivary melatonin concentrations among groups was detected (p<0.01), where the lowest concentration of salivary melatonin was found in the G group (p<0.05). After NSPT, melatonin level in saliva increased only in the G group, significantly (p<0.05). Change in melatonin level in saliva was significantly different in the G group (p<0.001)

Regarding the PSQI scores, a significant difference was detected in sleep quality among groups. As displayed in Table 4, the G group presented greater PSQI scores than the H and GAP groups (p<0.05), and also the highest percentage (%53.3) of patients with bad sleep quality.

Table 3. *Salivary melatonin levels at baseline, 3 months after NSPT and change (Δ)*

		H group n=15 Mean±SD Min-Max Median	G group n=15 Mean±SD Min-Max Median	CP group n=15 Mean±SD Min-Max Median	GAP group n=15 Mean±SD Min-Max Median	*p**
Melatonin pg/mL	**Baseline**	3.21 ± 3.99‡ 0.60 – 11.69 0.91	0.92 ± 0.82 0.36 – 3.75 0.71	2.14 ± 1.26‡ 0.79 – 5.13 2.00	1.56 ± 0.80‡ 0.34 – 3.04 1.44	0.002
	3rd month	-	3.35 ± 3.25¶ 0.47 – 11.58 2.63	2.22 ± 1.76 1.00 – 7.31 1.68	1.50 ± 0.78 0.49 – 3.18 1.16	0.234
	Δ (0-3)	-	2.42 ± 3.27 0.03 – 10.86 0.94	0.08 ± 1.48‡ -1.74 – 4.52 0.18	-0.06 ± 0.93‡ -2.12 – 1.46 0.15	<0.0001

H: Healthy, G: Gingivitis, CP: Chronic Periodontitis, GAP: Generalized Aggressive Periodontitis, PI: plaque index, GI: gingival index, PD: probing depth, BOP: bleeding on probing, CAL: clinical attachment level, SD: Standard deviation

**Kruskal-Wallis test, ‡different from G group, Mann-Whitney U, p<0.05; ¶different than baseline, Wilcoxon test, p<0.05*

Table 4. *PSQI scores of all participants at baseline.*

		H group n=15	G group n=15	CP group n=15	GAP group n=15	*p*
PSQI						
Mean ± SD		2.93 ± 1.87‡	5.47 ± 2.83	3.80 ± 2.54	3.13 ± 2.42‡	0.048*
Min – max		0.00 – 6.00	1.00 – 11.00	0.00 – 9.00	0.00 – 8.00	
Median		3.00	5.00	3.00	3.00	
Sleep quality	**Good N (%)**	11 (73.3)	7 (46.7)	9 (60)	11(73.3)	0.368**
	Bad N (%)	4 (26.7)	8 (53.3)	6 (40)	4 (26.7)	

H: Healthy, G: Gingivitis, CP: Chronic Periodontitis, GAP: Generalized Aggressive Periodontitis, PI: plaque index, GI: gingival index, PD: probing depth, BOP: bleeding on probing, CAL: clinical attachment level, SD: Standard deviation

**Kruskal-Wallis test, p<0.05; **Chi–Square test; ¥ different from G group, Mann-Whitney U test, p<0.05*

4. DISCUSSION

The principal cause of periodontal disease is microbial dental biofilm which triggers inflammatory process on host immune response. Periodontal diseases are worsened by an overproduction of ROS leading to cellular damage (28). Melatonin, a hormone produced and secreted in a circadian manner mainly by the pineal gland, has been discovered in saliva (6) and recently received remarkable attention in periodontal research because of its anti-inflammatory and antioxidant properties. Recent findings have focused on melatonin levels in different periodontal conditions such as periodontal health, G, and periodontitis, and revealed decreased levels of melatonin in both saliva and serum in periodontal diseases (7, 29-32). Furthermore, the only study evaluating the effect of NSPT on melatonin levels discovered that NSPT resulted in marked improvement of low salivary melatonin levels in subjects with periodontitis (33). However, the outcome of NSPT in relation to salivary melatonin levels of individuals with different periodontal diseases is yet to be explored. For all we know, the current study is the first study conducted to evaluate the influence of NSPT on salivary melatonin levels in subjects with gingivitis and periodontitis in accordance with PSQI scores.

Periodontal status and healing can be affected by existing systemic disorders and environmental factors such as smoking, antibiotic usage, previous periodontal treatment, pregnancy, and lactation (34-36). Melatonin diffuses into the blood immediately after its secretion (2) . Ophthalmic diseases, spinal cord injuries, liver and kidney diseases may also alter melatonin levels as well as some medications such as beta-blockers, nonsteroidal anti-inflammatory drugs, antiepileptic drugs, and antidepressants (37). Therefore, in this study participants with conditions affecting the periodontal status and/or melatonin levels were excluded.

NSPT aims the establishment of biologically acceptable root surfaces, resolution of gingival inflammation, reduction in PD, gain of CAL through oral hygiene instructions, supragingival and subgingival calculus removal and root surface debridement (20, 38-40). At baseline, clinical measurements showed significant difference among groups parallel to the severity of the periodontal disease, as expected. PI scores of all treatment groups were greater than the healthy individuals at baseline ($p<0.05$), however, plaque control of all patients improved significantly following NSPT which can be explained by the efficacy of oral hygiene instructions. NSPT resulted in improvement of GI and PI scores, reduction in PD and BOP and CAL gain, in all treatment groups ($p<0.05$), as supported by previous studies (41, 42).

Data obtained from this study indicated that the salivary melatonin levels were lower in the presence of periodontal disease than the healthy individuals, similar to the studies investigating melatonin levels in periodontal health and disease (7, 29-32, 43), although no significance was reached in the present study. Almughrabi et al. (32) compared salivary melatonin levels among healthy individuals, G, CP and GAP patients and reported an association between melatonin concentration and severity of periodontal disease, where the lowest melatonin level in salivary samples was detected in GAP patients and the highest in healthy individuals. Similar to this finding, our result demonstrated the highest melatonin level in healthy individuals. However, the lowest salivary melatonin levels observed in the G group contradicted with previous studies (31, 32). This may be explained by lower scores of PSQI of the G group indicating bad quality of sleep. Cutando *et al.* (31) reported that concentrations of melatonin in saliva decreased as the periodontal status deteriorated. Parallel with this result, a tendency to decrease in melatonin from health to severe periodontitis was found in our study, although not significant ($p>0.05$). Further investigations with larger study population are required to confirm this relationship.

After successful NSPT, salivary melatonin level significantly elevated only in the G group ($p<0.05$) without any substantial change in the CP and GAP groups ($p>0.05$). However, the first study investigating impact of NSPT on melatonin levels by Bertl *et al.* reported elevated melatonin levels in salivary samples in periodontitis following NSPT (33), contradicting with the data from the present study. For all we know, current research is the first in evaluating the influence of NSPT on salivary melatonin levels in subjects diagnosed as gingivitis. In our study, improvement of salivary melatonin levels following NSPT in the G group suggested that gingival inflammation can be resolved and reversed by non-surgical treatment approach in subjects with gingivitis whereas in subjects with periodontitis, in order to achieve complete periodontal healing surgical treatment might be necessary. Further clinical trials with enlarged study population are needed to discover the influence of NSPT on melatonin levels in the presence of different periodontal diseases and the role of melatonin in periodontal pathogenesis.

The small-scale sample size, the lack of evaluation of other antioxidant and/or oxidant biomarkers, the lack of melatonin treatment as an adjunct to NSPT, and the lack of PSQI scores at 3rd month post-treatment limited the study.

5. CONCLUSION

The current study indicates that salivary melatonin may be a factor in periodontal pathogenesis and NSPT may induce improvement of decreased melatonin levels in the presence of periodontal disease. Further investigations with larger scale are required to fully elaborate the three-way association between melatonin levels in saliva, its effect on periodontal condition and NSPT.

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Conflict of Interest

None.

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Clinical and Experimental Health Sciences

Copeptin As A Diagnostic PH Marker in Acute Pulmonary Embolism

Necmi Baykan[1], Sule Yakar[2], Seda Ozkan[3], Ahmet Sen[4], Omer Salt[5], Polat Durukan[6]

[1] Nevsehir State Hospital, Department of Emergency Medicine, Nevsehir, Türkiye.
[2] Kayseri City Training and Research Hospital, Department of Emergency Medicine, Kayseri, Türkiye.
[3] Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine,Department of Emergency Medicine, Istanbul, Türkiye.
[4] Van Education and Research Hospital, Department of Biochemistry, Van, Türkiye.
[5] Trakya University School of Medicine, Department of Emergency Medicine, Edirne , Türkiye.
[6] Medical Park Hospital, Department of Emergency Medicine, Gaziantep, Türkiye.

Correspondence Author: Necmi Baykan
E-mail: drnecmibaykan@gmail.com
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ABSTRACT

Objective: The present study aimed to investigate the effectiveness of copeptin levels in detecting increased pulmonary artery pressure and right ventricular dysfunction in patients with acute pulmonary embolism.

Methods: A total of 116 patients who presented to the emergency department with chest pain or dyspnea and were diagnosed with acute pulmonary embolism and 30 healthy controls were included in the study. Plasma copeptin levels of patients and healthy control group were measured. Right ventricular functions and pulmonary artery pressures were evaluated in echocardiography of patients diagnosed with acute pulmonary embolism.

Results: Copeptin levels were significantly higher in patients with right ventricular dysfunction than in those without right ventricular dysfunction [median 1.06(0.03–7.14) vs. 0.59(0.31–2.50), p= 0.01].

Conclusion: Copeptin can be used as a new biomarker in the diagnosis of acute pulmonary embolism and in predicting right ventricular dysfunction and increased pulmonary artery pressure in patients with acute pulmonary embolism.

Keywords: Pulmonary embolism, mortality, pulmonary wedge pressure, right ventricular dysfunction

1. INTRODUCTION

Acute pulmonary embolism (PE) is a common condition that can lead to high mortality and morbidity rates unless treated properly (1). As such, diagnostic tests with radiological and laboratory data are necessary for the detection and differential diagnosis of PE due to its nonspecific symptomatology, which can be mimicked by other conditions (2). Notably, 30%–40% of all PEs are submassive, making the rapid and accurate diagnosis of right ventricular dysfunction (RVD) crucial for the correct risk stratification of these patients (3). However, to the best of our knowledge, there has been no biochemical marker or other available means that are definitely useful in early diagnosis (2).

Under different stress levels, vasopressin, a hypothalamic hormone, is stimulated to enhance the activity of corticotropin-releasing hormone, leading to adrenocorticotropic hormone secretion and cortisol production (4). Copeptin, which is a stable peptide of the vasopressin precursor, constitutes the C-terminal portion of provasopressin and is a new neurohormone of the arginine vasopressin (AVP) system (5). Similar to arginine vasopressin, it is synthesized in the hypothalamus and released into the portal circulation of the neurohypophysis. Moreover, copeptin is secreted in molar proportions equal and reflective to those of arginine vasopressin (6). Thus, it can reflect individual stress responses at the hypothalamic level (7). Furthermore, copeptin has been reported to have prognostic value in various diseases, including acute coronary syndrome, cerebral hemorrhage, congestive heart failure, lung diseases, and sepsis (8).

Despite this, only a limited number of studies have demonstrated the relationship between copeptin and right-sided heart failure. A recent study reported that copeptin levels were increased and prognostic in pulmonary hypertension and right-sided heart failure (9). This study also reported that increased neurohumoral activity due to right ventricular failure led to increased copeptin levels in patients with pulmonary hypertension (9).

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Therefore, the aim of this study was to investigate the utility of copeptin levels in detecting increased pulmonary artery pressure (PAP) and RVD in patients with acute PE.

2. METHODS

This study was conducted in accordance with the principles of the Declaration of Helsinki and the principles of research after obtaining approval from the Ethics Board of Erciyes University (dated 10.02.2012 and no. 2012/136).

Patients older than 18 years with a diagnosis of acute PE at the emergency department were included in the study and divided into three groups. The first group comprised patients with PE and RVD, the second group comprised patients with PE but without RVD, and the third group comprised healthy controls.

In contrast, patients with severe chronic obstructive pulmonary disease, congestive heart failure, right-sided heart failure, sepsis, chronic renal failure, acute cerebrovascular disease, pneumonia, aortic dissection, pulmonary hypertension, and a history of PE were excluded from the study.

Demographics, computed tomography and transthoracic echocardiography (TTE) findings, laboratory data, and administered treatments were all recorded in the computer software. Wells scores were calculated for all the patients, and the PE severity index (PESI) and simplified PESI (sPESI) were calculated for prognostication based on their clinical characteristics and medical history on admission.

RVD was determined by TTE.

2.1. Copeptin Measurement

Exactly 3 ml of venous blood was extracted from the antecubital region into ethylenediaminetetraacetic acid-containing tubes for the measurement of serum copeptin levels. After shaking the specimen, the collected blood was transferred and centrifuged for 10 minutes at 3,500 rpm to separate the serum. Samples were then stored at −70°C until further analysis. Sandwich enzyme-linked immunosorbent assay was manually conducted, and blood levels were quantitatively measured using the EK-065-32 Human Copeptin enzyme immunoassay test kit from Phoenix Pharmaceuticals Inc.

2.2. Statistical Analysis

The Statistical Package for the Social Sciences version 24.0 software was used for statistical analyses, and P values of <0.05 were considered statistically significant. The Kolmogorov–Smirnov test was used to analyze the normality of the data, and the Shapiro–Wilk test was used for normality analysis in groups with <30 patients. The Mann–Whitney U test was used to compare two groups that did not exhibit normal distribution, whereas the Kruskal-Wallis test was used to compare three groups that did not exhibit normal distribution. Bonferroni correction was also performed in the post-hoc analysis, wherein p values of <0.017 was considered statistically significant. Furthermore, receiver operating characteristic (ROC) analysis was performed to determine the utility of copeptin levels in PE diagnosis.

3. RESULTS

A total of 116 patients with acute PE were included in the study. The mean age was 66.77 ± 14.22 years, and dyspnea (41.4%) was the most common presenting symptom. Deep vein thrombosis (12.9%), history of surgery (19%), and immobilization (19.8%) were the most common risk factors. According to the PESI scores, 31% of patients were in the low-risk group, and 69% were in the high-risk group. Based on TTE, 51.7% of the patients had RVD. The mean PAP was 44.8 ± 18.6 mmHg, of which 68 patients (58.6%) had a PAP of >40 mmHg. Five patients (4.3%) received thrombolytic therapy, and 28 patients (24.1%) died within 30 days of admission (Table 1). Furthermore, copeptin levels were higher in patients who were considered suitable for thrombolytic therapy than in patients who were not. Patients with a mortal course were notably found in the high-risk group.

Plasma copeptin levels were significantly higher in patients with acute PE than in the healthy controls [median: 0.65 (0.03–7.14) vs. 0.41 (0.09–0.97), p = 0.002] (Figure 1). Assuming a cut-off value of 0.42 for the ROC analysis of copeptin in detecting PE, the area under the curve (AUC) was 0.7, sensitivity was 84.5%, specificity was 46.7%, positive predictive value (PPD) was 87.5%, and negative predictive value (NPD) was 47.1% (Figure 2A, Table 2).

Comparison of plasma copeptin levels among patients with and without RVD, as revealed by TTE, and healthy controls showed that there was a significant difference between these groups (p = 0.001). Specifically, plasma copeptin levels were significantly higher in patients with RVD than in those without RVD [median: 1.06 (0.03–7.14) vs. 0.59 (0.31–2.50), p = 0.01] (Figure 3). Similarly, plasma copeptin levels were significantly higher in patients with PE but without RVD than in the healthy controls [median: 0.59 (0.31–2.50) vs. 0.41 (0.09–0.97), p = 0.016]. Assuming a cut-off value of 1.01 for the ROC analysis of copeptin in predicting RVD, the AUC was 0.638, sensitivity was 51.7%, specificity was 89.3%, PPD was 83.8%, and NPD was 63.3% (Figure 2B, Table 2).

Significant differences in the plasma copeptin levels of patients with a PAP of >40 mmHg, PAP of <40 mmHg, and healthy controls were also observed (p < 0.001). In patients with a PAP of >40 mmHg, plasma copeptin levels were significantly higher than those of patients with a PAP of <40 mmHg [median: 0.77 (0.03–7.14) vs. 0.59 (0.21–4.03), p = 0.046] (Figure 4). Likewise, plasma copeptin levels were significantly higher in patients with a PAP of >40 mm Hg than in the healthy controls (p = 0.012). According to the ROC analysis to determine the power of copeptin in predicting increased PAP, the AUC was 0.609, sensitivity was 54.4%,

specificity was 75.0%, PPD was 75.5%, and NPD was 53.7% (Figure 2C, Table 2).

On the other hand, there was no significant difference in the plasma copeptin levels of patients who died and survived within 30 days of admission [median: 0.68 (0.19–5.64) vs. 0.64 (0.03–7.14), p = 0.91]. Assuming a cut-off value of 0.32 for the ROC analysis of copeptin in determining the 30-day mortality risk, the AUC was 0.551, sensitivity was 96.0%, specificity was 16.9%, PPD was 25.2%, and NPD was 89.9% (p = 0.339) (Table 2).

Table 1. *Demographic, clinical, laboratory, and echocardiography findings of patients*

Age years (mean ± SD)	66.77 ± 14.22
Symptoms n (%)	
Dyspnea	48 (41.4)
Chest pain	12 (10.3)
Hemoptysis	1 (0.9)
Syncope	2 (1.7)
Palpitations	4 (3.4)
Cough	13 (11.2)
Risk factors n (%)	
Symptoms of DVT	15 (12.9)
Previous surgery	22 (19.0)
Malignancy	11 (9.5)
Coagulation disorder factor deficiency	2 (1.7)
Immobilization	23 (19.8)
CBVD	1 (0.9)
Vital signs	
Age years (mean ± SD)	66.77 ± 14.22
Systolic blood pressure (mean ± SD)	134 ± 24 (87–190)
Diastolic blood pressure (mean ± SD)	78 ± 13 (42–120)
Pulse/min (mean ± SD)	101 ± 20 (10–147)
Respiratory rate/min (mean ± SD)	21 ± 2 (14–32)
O_2 saturation (mean ± SD)	90 ± 7 (60–99)
Tachycardia n (%)	46 (39.7)
Wells score (mean ± SD)	4.03 ± 2.14
Low Risk n (%)	12 (10.3)
Medium Risk n (%)	88 (75.9)
High Risk n (%)	16 (13.8)
median PESI score (range)	3 (1–5)
Class 1 n (%)	6 (5.2)
Class 2 n (%)	30 (25.9)
Class 3 n (%)	34 (29.3)
Class 4 n (%)	25 (21.6)
Class 5 n (%)	21 (18.1)
sPESI	
Low risk n (%)	36 (31.0)
High risk n (%)	80 (69.0)
Right ventricular dysfunction in echo n (%)	60 (51.7)
PAP (mean ± SD)	44.8 ± 18.6
PAP≥40 mmHg n (%)	68 (58.6)
Thrombolytic therapy n (%)	5 (4.3)
30-day mortality n (%)	28 (24.1)

DVT: Deep Vein Thrombosis
CBVD: Cerebrovascular Disease
PAP: Pulmonary Artery Pressure

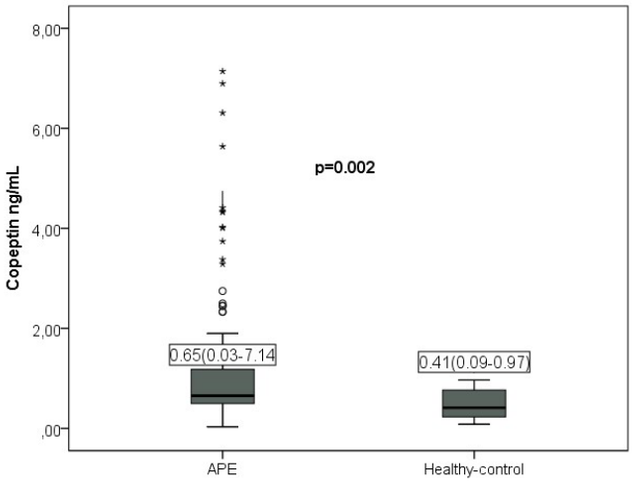

Figure 1. *Comparison of plasma copeptin levels between patients with pulmonary embolism and healthy controls. Mann–Whitney U test: p < 0.05 statistically significant*

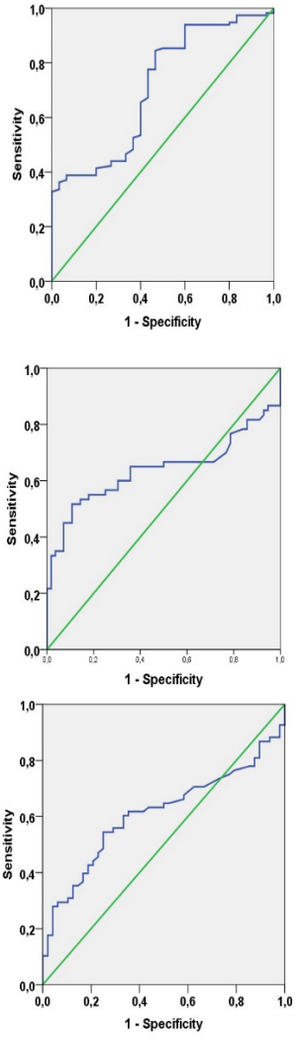

Figure 2 *a-c. Receiver operating characteristic (ROC) curve for copeptin levels in terms of detecting pulmonary embolism (a). ROC curve for copeptin levels in terms of detecting right ventricular dysfunction (b). ROC curve for copeptin levels in terms of detecting the increase in pulmonary artery pressure (c).*

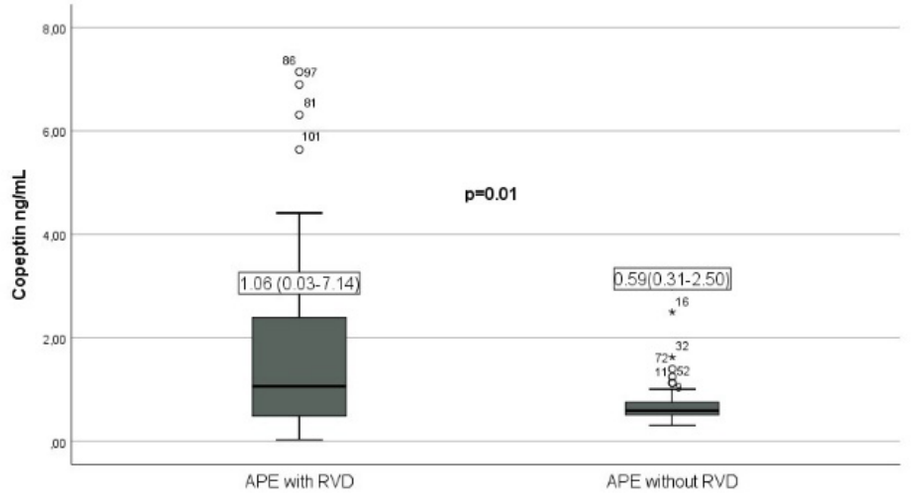

Figure 3. *Comparison of copeptin levels according to the state of right ventricular dysfunction.*

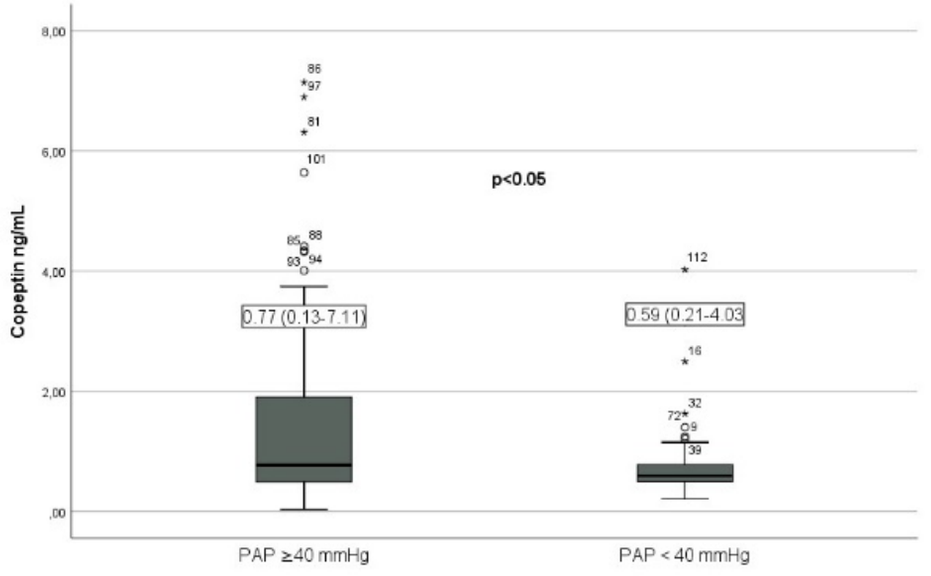

Figure 4. *Comparison of copeptin levels according to pulmonary artery pressure.*

Table 2. *Receiver operating characteristic (ROC) analysis results of copeptin levels for detecting PE, RVD, increased PAP, and mortality risk*

	Copeptin cut-off value	AUC	P	SE	95%CI	Sensitivity %	Specificity %	PPD %	NPD %
PE	>0.42	0.709	<0.001	0.052	0.607–0.812	84.7	46.7	87.5	47.1
RVD	>1.01	0.638	0.010	0.055	0.532–0.745	51.7	89.3	83.8	63.3
PAP increase	>0.73	0.609	0.046	0.052	0.507–0,712	54.4	75.0	75.5	53.7
Mortality	>0.32	0.551	0.399	0.058	0.437–0.665	96.0	16.9	25.2	59.9

PE: Pulmonary Embolism
RVD: Right Ventricular Dysfunction
PAP: Pulmonary Artery Pressure

4. DISCUSSION

Several biomarkers have been used in acute PE diagnosis and risk classification in previous studies (3,10). Contributing to these findings, the results of the present study showed that plasma copeptin levels were higher in patients with acute PE than in healthy controls. Furthermore, these levels were higher in patients with PE and RVD than in those who had normal right ventricular function.

Copeptin levels have been associated with prognosis and mortality in various cardiovascular diseases, where increasing levels reflect increasing disease severity (11). In the present study, the median plasma copeptin levels were 0.65 (0.03–7.14) in patients with PE and 0.41 (0.09–0.97) in healthy controls, showing a statistically significant difference. In a similar study conducted by Öztürk et al., plasma copeptin levels were reported to be significantly higher in patients with acute PE (n = 32) than in healthy controls (n = 24) (2). Kalkan et al. similarly found that among patients who presented to the emergency department with chest pain or dyspnea, plasma copeptin levels were significantly higher in patients diagnosed with acute PE than in those without acute PE. A previous study also reported that although troponin, D-dimer, and pro-BNP levels were elevated in patients with acute PE, copeptin was a more specific biomarker in PE diagnosis by comparison (AUC: 0.836, sensitivity: 68%, selectivity: 83.7%, PPD: 82.1%, and NPD: 70.6%) (3). Vasopressin is an antidiuretic and vasoconstrictive hypothalamic hormone that is released after stimulation by different levels of stress. Specifically, systemic vasoconstriction and renal fluid retention caused by increased plasma osmolality, decreased arterial pressure, decreased cardiac filling, or activated neurohumoral peptides (e.g., angiotensin) and baroreceptors in the carotid sinus stimulate the release of vasopressin (12). As part of the AVP system, copeptin is much more stable than vasopressin, allowing it to reflect individual stress responses at the hypothalamic level (13). As such, we believed that increased neurohumoral activity due to pathophysiological changes and individual stress responses from hemodynamic changes, chest pain, and dyspnea would lead to elevated copeptin levels in patients with PE.

In acute PE, pulmonary vasoconstriction and high PAP result in increased right ventricular afterload and dilatation, thereby leading to RVD (14). Among patients with PE, RVD is an important indicator of poor prognosis and has been demonstrated to be the most important cause of death (15). Echocardiography and certain cardiac biomarkers have been used in determining RVD (16). Excessive neurohumoral activation occurring due to thrombosis from PE leads to bronchoconstriction, hypoxia, vascular resistance, and changes in blood volume and heart contraction, all of which contribute to the development of heart failure (9). Interestingly, elevated plasma levels of vasopressin have been reported in patients with heart failure (17). Elevated plasma copeptin levels were also shown to be associated with disease severity and long-term prognosis in patients with left ventricular failure (17). In the present study, plasma copeptin levels were elevated in patients with acute PE, which was especially higher in patients with acute PE and RVD. Assuming a cut-off value of 1.01 on ROC analysis, the sensitivity, specificity, PPD, and NPD of plasma copeptin levels in detecting RVD was 51.7%, 89.3%, 83.8%, and 63.3%, respectively. According to Kalkan et al., the copeptin levels of patients with acute PE and RVD were significantly higher than in patients with acute PE but without RVD, which was consistent with our findings (3). Given the findings from our study and from previous studies, we believe that the increased neurohumoral activity due to the hemodynamic stimulus in acute PE with RVD is responsible for the significant increase in plasma copeptin levels. Therefore, serum copeptin levels can be used as a novel biomarker for detecting RVD.

Nickel et al. compared copeptin levels in patients with and without pulmonary arterial hypertension, finding that copeptin levels were markedly higher in patients with pulmonary arterial hypertension. Furthermore, they reported that patients with pulmonary arterial hypertension and high copeptin levels had poor prognosis and were at higher risk for mortality (9). Although the present study did not include patients with chronic pulmonary hypertension, the plasma copeptin levels of patients with and without acute pulmonary arterial hypertension secondary to acute PE were compared, showing that patients with increased PAP had significantly higher plasma copeptin levels. According to the present study, the 30-day mortality rate in patients with acute PE was 24.1%; however, there was no significant difference between the copeptin levels of patients who died and survived within 30 days of admission. Similarly, according to the PESI score, there was no significant difference in the copeptin levels between patients in the low-risk and high-risk groups. In a study conducted by Vuilleumier et al., the specificity and sensitivity of copeptin levels in determining the 1-month mortality risk was reported to be 20% and 83.3%, respectively (10). According to another study by Deveci et al., there were significant differences in the plasma copeptin levels between patients with acute PE who died and survived during hospitalization, as well as between patients who died and survived within 1 month of admission. Contrarily, there was no significant difference in the 3-month mortality. In the same study, a significant difference in the copeptin levels between patients in the low-risk and high-risk groups was also observed, as revealed by the sPESI scores (18). Other studies have shown that acute PE remains a major cause of mortality (19,20). Likewise, the results of the present study showed that PE was a major cause of mortality, although we failed to demonstrate the relationship between mortality and copeptin levels.

5. CONCLUSION

The present study showed that copeptin levels were significantly elevated in patients with acute PE, especially those who developed RVD. Although copeptin is nonspecific and does not have high sensitivity, it can be used as an alternative biomarker for determining RVD in patients with

acute PE. Extensive studies are warranted in the future to determine the cut-off values associated with the diagnosis, prognosis, and mortality rate, as well as to reveal the sensitivity and specificity of copeptin levels in patients with acute PE.

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Clinical and Experimental Health Sciences

Coexistence of Papillary Microcarcinoma and Hurthle Cell Adenoma: A Case of Thyroid Collision Tumor

Halil Ibrahim Tasci[1], Hilal Erinanc[2], Emin Turk[1], Erdal Karagulle[1]

[1] Başkent University, School of Medicine, Department of General Surgery, Ankara, Türkiye.
[2] Başkent University, School of Medicine,Department of Pathology, Ankara, Türkiye.

Correspondence Author: Halil İbrahim Tasci
E-mail: okcu6528@gmail.com
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ABSTRACT

While thyroid cancers are usually present one type of cancer in the thyroid gland, rarely different thyroid cancers may found in one or two different lobes of the thyroid gland at the same time. A 70-years-old female patient presented with a long-standing neck swelling, especially on the left side, which was more prominent, recently increasing in size and causing shortness of breath. Due to tracheal compression and diagnosis of multi-nodular goiter, total thyroidectomy was performed. Histopathological examination revealed a thyroid collision tumor with papillary microcarcinoma on the right and hurtle cell adenoma on the left side. Due to its rarity, clinicians encountered difficulties in the diagnosis and treatment of thyroid collision tumors. We believe that to be aware of these rare entities by encouraging clinicians to report such cases enable to more solid conclusions to diagnosis and management of collision tumors.

Keywords: collision, hurthle, micropapillary

1. INTRODUCTION

The coexistence of two or more different tumor entities which have different histologic morphologies juxtaposed within the same tissue is termed as collision tumor (1). Both composite and collision tumors involve two morphologically and immunohistochemically different neoplasms coexisting within a single organ. However, collision tumors do not have the histological cellular intermingling seen in composite tumors. (1). Collision tumors may occur in stomach, liver, adrenal gland, lung, ovaries, kidney, and colon (2). Collision tumors within the thyroid gland are extremely rare and constitutes less than 1% of all thyroid malignancies. Most of the reported cases have mixed histologies of follicular or papillary and medullary carcinomas (3).

In this report, we presented a case of thyroid collision tumor including papillary microcarcinoma and Hurthle cell adeonoma.

2. CASE PRESENTATION

A 70-years-old female patient was admitted to hospital with the complaint of swelling in her neck. She had similar symptoms one year ago. Thyroid fine needle aspiration biopsy had been applied and there were no cytologic atypia. In her anamnesis, she also had coronary artery disease and obesity. Thyroid function tests were within normal limits. She was followed up medically. Recently, she admitted to our hospital with complaining her neck swelling has gradually increased, and suffer from shortness of breath.

On physical examination, thyroid gland was palpable, it was about 9x6 cm on the left side and 3x4 cm on the right side, with firm consistency, partially mobile and nodular. The trachea was slightly deviated to the right. There was inspiratory wheezing by auscultation in the lungs. On laboratory examination, thyroid function tests were as follows; free T4: 0,73 ng/dL, free T3:3 pg/ml and thyroid stimulating hormone (TSH) was 1.67 µU/ml. There was tracheal compression to the right side on anterior posterior cervical radiography (Figure 1).

In thyroid ultrasonography, the gland size was increased and the parenchyma was heterogeneous. In the middle-lower zone of the right lobe, an irregularly contoured 21x18 mm solid nodule with punctate calcifications and a 69x50x44 mm size nodule with cystic and solid areas occupying almost the entire lobe in the left lobe was observed. Due to tracheal compression and diagnosis of multi-nodular goiter, surgical excision was planned. After total thyroidectomy, the patient's dyspnea and examination findings improved. The patient

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was discharged on the second postoperative day without any complication.

In the macroscopic examination showed that an 6 mm papillary microcarcinoma in the right lobe and a 5 cm Hurthle cell adenoma on the left lobe. Histopathological evaluation of the specimen showed that adenoma was completely enveloped by thin fibrous capsule and composed of variably sized follicles lined by Hurthle cells (Figure 2). No vascular or capsular invasion was noted. Microscopically, papillary carcinomas share certain features. The neoplastic papillae contain a central core of fibrovascular tissue lined by one or occasionally several layers of cells with crowded oval nuclei. Nuclear grooving or nuclear clearing was seen (Figure 3). Immunohistochemistry showed CK19 (+), HBME-1 (+), Galectin 3 (+) in the papillary carcinoma. , CK19 (-), HBME-1 (-), Galectin 3 (-) in Hurthle cell adenoma.

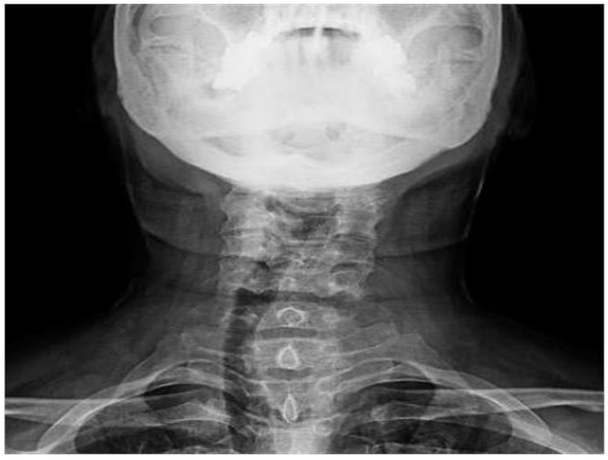

Figure 1. *Tracheal compression to the right side on anterior posterior cervical radiography.*

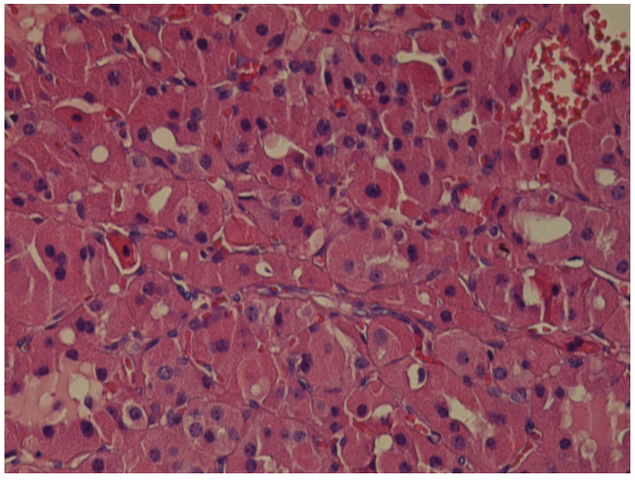

Figure 2. *Shows oncocytic follicular cells which, are characterised by large size, polygonal to square shape, voluminous granular and eosinophilic cytoplasm (HEX20).*

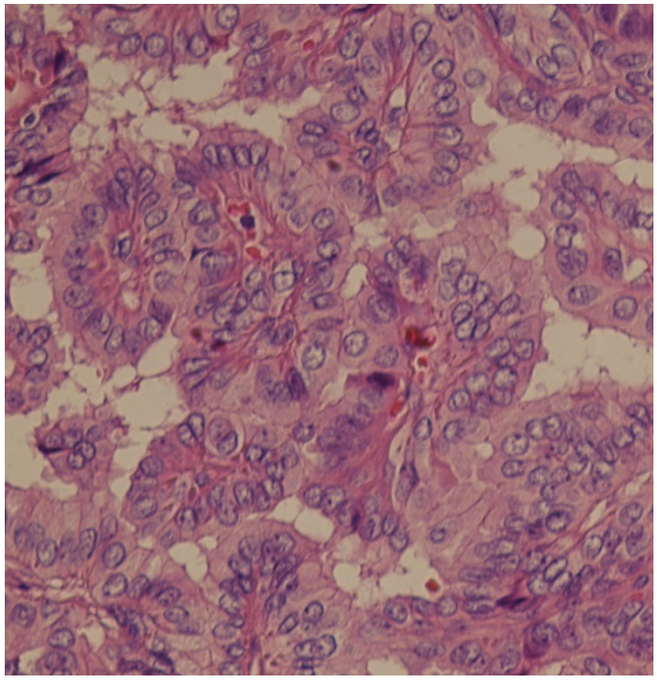

Figure 3. *Shows papillary carcinoma which is composed of multiple papillary architecture with fibrovacular core and covered with follicular cells having nuclear enlargement, nuclear groove and inclusion (HEX40).*

3. DISCUSSION

While thyroid cancers are usually present one type of cancer in the thyroid gland, different thyroid cancers may found in one or two different lobes of the thyroid gland at the same time. Collision tumors were first described by Bilroth and then modified by Warren and Gates (4). Presence of two or more tumor in the same tissue or organ at the same time are categorized into three different conditions, as mixed, composite and collision tumors (4). The term mixed tumor is used if there is a histological mixture of the two tumors in the same organ. It is thought that these tumors arise from a common cell of origin (5). However tumors arise from different cell populations in composite tumors. The term collision tumor is described as the coexistence of two adjacent but histologically and morphologically distinct malignant tumors in the same organ. Unlike the other two, in collision tumors there should be no histological mixture in the tumor mass (4). In a literature review by Ryan et al. 33 thyroid collision tumor cases were found in data from 27 different studies (6). In this case presentation we report a collision tumor case with papillary microcarcinoma and Hurthle cell adenoma, which are rarely seen in the literature.

The formation of collision tumors have been explained with some theories such as “pluripotent cell theory”, “neoplastic coercion theory” and the “chance theory”. “Pluripotent cell theory” explained that tumors with different histological structures originate from a single pluripotent stem cell. “Neoplastic coercion theory” is defined that an initial tumor can promote the development of another tumor by means of

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creating a permissive local environment. The other theory is the "chance theory", which proposes that two tumors come to occupy the same mass purely by chance (6). No single theory can fully explain the pathogenesis of these tumors in all cases and therefore, recently in the development of collision tumors a combination of theories should be considered (2). Although its pathogenesis is not known exactly, it is thought to be a familial predisposition. Some genetic mutations are thought to increase the developing of a collision tumor. Mutations in the RET oncogene, retinoblastoma (RB), p53, and BRAF gene may be associated with collision tumors of the thyroid (7).

Ryan et al reported that collision tumors are more common in women, with an average age of 53.4 (27-84) (6). Most of the patients had complaint of a mass in the neck, and metastasis was detected in the majority of the patients (23 of 33 patients) at the time of the diagnosis. Coexistence of medullary and papillary thyroid cancer was observed in the majority of cases (20 of 33 patients). Among them only 1 patient had been diagnosis with collision tumor by fine needle aspiration biopsy preoperatively (6). Our presented case was a 70-years-old female patient. She had complaint of swelling in the neck lead to shortness of breath. The combination of Hurthle cell adenoma and papillary microcarcinoma is infrequent. The case reported by Rana et al. in 2018, was the first case in the literature (8). Until now, there has been no any report in medical literature of a similar case which is presented by those. As far as we know, our case presentation would be the second one in the literature.

Because of rarity, the diagnosis and management options of collision tumor are important. The vast majority of them are diagnosed postoperatively, during histologic examination of total specimen. Fine needle aspiration biopsy was performed on our patient one year ago, but no cytological findings suggestive of neoplasia were observed. The diagnosis was made by the histopathological examination of the surgical material postoperatively.

Collision tumors are more aggressive than single primary tumors and the risk of recurrence increases (2). Since they are extremely rare tumors, there is no consensus on the treatment of collision tumors. Treatment for these tumors should be perform by implementing a combination of therapies, treating each tumor separately. However some authors advise that the treatment should mainly plan to according the more aggressive tumor type (6,9). In our patient, Hurthle cell adenoma limited to thyroid parenchyma and there were no capsular or vascular invasion. Papillary microcarcinoma was localized in the other lobe and there was no lymph node involvement. Therefore no additional treatment was required except total thyroidectomy in our case.

Due to its rarity, clinicians encountered difficulties in the diagnosis and treatment of thyroid collision tumors. We believe that to be aware of these rare entities by encouraging clinicians to report such cases enable to more solid conclusions to diagnosis and management of collision tumors.

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