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CONTENTS

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Knowledge and Attitudes of Medical Faculty Intern Students in the Diagnosis and Treatment of Hypertension: A Cross-sectional Study

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Abstract: Hypertension is an important public health problem with high morbidity and mortality and is seen in approximately 1 in 3 people worldwide. Hypertension can cause direct or indirect labour losses, but despite this, awareness in the society remains at low levels. This research examined hypertension awareness in medical school students who will graduate. The research is a cross-sectional descriptive study. The study population consisted of 55 students studying in the last year of Erzincan Binali Yıldırım University Faculty of Medicine. For the study, a questionnaire consisting of 26 questions was sent to the students electronically (Google forms), which was prepared by the researcher and questioned the presence of hypertension risk factors in the participants and the participant's knowledge and attitudes about the diagnosis and treatment of hypertension. Of 55 students, 53 (96.3%) were reached. Of the participants, 23 (43.4%) were female, and 30 (56.6%) were male. When the risk factors in the Turkish Hypertension Consensus Report were questioned among the participants, it was seen that the most common risk factor had a stressful personality with 66% (n=35). Of the participants, 18 (34%) had not measured blood pressure in the last six months. Of the participants, 73.6% stated that they could managea hypertension patient's diagnosis, follow-up and treatment with their current knowledge. It was seen that the average of correct answers given by the participants to 6 information questions about hypertension was 3.55±1.21.While most of the participants think that they can diagnose, treat and follow up the hypertension patient, the remaining 26.4% think that they are insufficient. It is a situation that should be questioned well what makes them think they cannot follow hypertension patients. Interventions should be planned in medical education so that all students who will graduate are competent in hypertension. ©2023 NTMS.

Keywords: Hypertension; Essential Hypertension; Medical Education; Primary Healthcare.

1. Introduction

Although there may be different cut-off values in the guidelines of different countries, hypertension is defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg [1, 2]. Hypertension is an important public health problem that is common in Turkey and worldwide and causes high mortality and morbidity if not controlled [3].

Hypertension is directly related to cardiovascular diseases, cerebrovascular diseases, kidney diseases and deaths at young ages. For example, it is the most

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significant cause of congestive heart failure [4]. Conditions such as heart attack, kidney failure, and cerebrovascular events may be the first reason for presentation in patients with hypertension, who unfortunately cannot be diagnosed early. The Framingham study found underlying hypertension in 91% of newly diagnosed heart failure cases [4].

Hypertension is present in about one in three people worldwide; therefore, approximately 9.4 million people die each year [5]. According to the World Health Organization data, hypertension is responsible for 45% of deaths due to cardiovascular diseases and 51% of deaths due to stroke [5]. According to the same report, Turkey is located in a high-risk region for ischemic heart diseases [5].

In the Prevalence, Awareness And Treatment of Hypertension in Turkey (PATENT) study conducted in 2003, the prevalence of hypertension was found to be 31.8% [3]. This rate was 42.3% for those between the ages of 35-64, in other words, those who provide economic added value to the country and who work [3]. In the PATENT 2 study conducted in 2012, the prevalence of hypertension regressed some what and was found to be 30.3% [6]. Although the hypertension awareness rate has increased from 40.7% to 54.7%, it is still insufficient [6].

According to the Turkey Burden of Disease Study conducted in Turkey, cardiovascular diseases are the most common cause of both deaths and the main diseases that constitute the disease burden in Turkey [7]. In the same study, the mortality rate attributed to hypertension, which can be prevented by controlling hypertension, is 25.2% of all deaths [7].

Although hypertension rarely causes symptoms in the early stages, it begins to affect vital organs such as the brain, heart and kidneys. Follow-up and treatment of patients who cannot be diagnosed early may be difficult in the future. For this reason, the World Health Organization has used the "silent killer" analogy for hypertension [5]. In the Turkish Hypertension Consensus Report, it is recommended to measure blood pressure at every examination in adults for early diagnosis [2].

Although approximately 95% of hypertension patients are considered as primary hypertension, the mechanism of hypertension formation in these patients is not fully known [8, 9]. However, obesity, excessive salt consumption, alcohol, smoking, sedentary life and genetic factors are thought to be effective [2, 9]. For this reason, it is necessary to question these risk factors in every hypertension patient and to eliminate these causes.

Only 5% of all hypertension cases are secondary hypertension, and it is not cost-effective to examine every patient for suspected secondary hypertension[2]. It can be done easily in primary care in 95% of the patients; Complete blood count, urinalysis, fasting blood sugar, sodium, potassium, uric acid, lipid profile, creatinine and electrocardiography are sufficient.

The lack of a referral system in primary health care services in our country is seen as an important deficiency in managing health services [10]. Therefore, all first-line physicians should be well-equipped in the diagnosis and treatment of hypertension and should refrain from referring newly diagnosed hypertension cases to an upper level.

There is a need for experienced and trained health personnel about hypertension in every field, whether working in primary health care or 3rd level hospital. Diagnosis, follow-up and treatment of a major portion of hypertension patients can be easily done in primary health care, where the access of the patients is also easier. According to the health statistics year book published by the Ministry of Health of the Republic of Turkey in 2019, about 288 million applications were made to the 1st level health institutions 2019, while the total number of applications to the 2nd and 3rd level hospitals was about 525 million [11]. For this reason, training health professionals who will work in primary health care, especially physicians, gain significant importance. Although there are studies in the literature that measure the knowledge and attitudes of physicians and other health professionals actively working in the field, few studies examine the status of physician candidates who have not yet started to work professionally. Physicians may have gained these abilities through their own efforts after they started to work actively. This study may be useful in terms of showing whether the medical education of physician candidates about hypertension is sufficient or insufficient.

Our study aimed to examine the knowledge and attitudes of physicians, those who have reached the end of their education period and will work in primary health care after graduation about hypertension.

2. Material and Methods

2.1. Materials

A patient group consisting of 30 patients (16 females-14 males) diagnosed with SLE and followed in Erzurum Atatürk University Health Research and Application Center Directorate Physical Medicine and Rehabilitation Department Polyclinic and 20 healthy individuals (10 females-10 males) without any systemic disease was included in our study. Informed Consent Form was signed by the patient and control group who agreed to participate in the study.

Blood samples collected for this study, which was approved by Erzurum Atatürk University Faculty of Medicine Ethics Committee, were used for RNA isolation (Roche) and gene expression studies (Roche Light Cycler 480 Real-Time) in Atatürk University Faculty of Medicine Laboratory of Medical Biology Department.

2.2. Methods

The population of the study consisted of 55 students studying in the last year of Erzincan Binali Yıldırım University Faculty of Medicine. For the study, a questionnaire consisting of 26 questions was sent to the students electronically (Google forms) prepared by the

researcher, questioning the presence of hypertension risk factors in the participants and the participant's knowledge and attitudes about the diagnosis and treatment of hypertension. Each question was coded as to be answered, and the students could not move on to the next question with out answering one question. Therefore, no missing data were detected.

The participants were questioned about a risk factor for hypertension in the Turkish Hypertension Consensus Report: BMI>30, smoking and alcohol use, regular exercise, presence of sleep apnea, family history, diet, and stressful personality. The presence of each of the risk factors was evaluated as 1 point, and the answer to the question "Do you exercise regularly" was "I do not usually do", "I do not do it at all"; and "I eat unhealthily" and "I eat very unhealthily" to the question "How do you think your diet is in general?" giving 1 point was evaluated, and hypertension risk score was calculated.

Knowledge score was created by calculating 1 point for each correct answer and 0 points for each wrong answer given to the 6 questions asked about the knowledge level of the participants.

2.2.3. Statistical analysis

SPSS 18.0 package program was used for the statistical analysis of the data. The statistical significance level was taken as $p<0.05$. Ethics committee approval was obtained for the study from the local ethics committee with the decision dated 25/10/2021 and numbered 11/05. Descriptive statistics were used to evaluate the data, Pearson correlation analysis was used to compare two numerical data, and one-way ANOVA was used when the normally distributed and variable variances were equal between the groups.

3. Results

Of 55 senior medical students, 53 (96.3%) studying in the 2020-2021 academic year were reached. Of the participants, 43.4% (n=23) were female, and 56.6% (n=30) were male. The BMI of 3 (5.6%) individuals, two men and one woman, was>30. Other data of the participants are given in Table 1.

Table 1: Age, height, weight and BMI data of the participants.

	Min	*Max*	*Mean*	*SD*
Age	22	27	24,17	0,97
Height	151	187	170,55	8,14
Weight (kg)	45	100	69,53	12,10
BMI	18	31	23,81	3,10

The participants questioned the risk factors in the Turkish Hypertension Consensus Report, and it was observed that none of the participants had witnessed sleep apnea. Other risk factors query is as in Table 2. The most common risk factor had a stressful personality with 66% (n=35). This is followed by not exercising regularly. Of the participants, 39.6% said they usually do not exercise regularly and 11.3% said they never do.

Table 2: Participants' risk factors.

	n	*%*
Stressful personality structure		
Yes	35	66
No	18	34
Regular exercise status		
I do it all the time	5	9,4
I usually do	12	22.6
I do	9	17
I don't usually do	21	39,6
I never do	6	11.3
Diagnosis of hypertension in first-degree relatives		
Yes	23	43.4
No	30	56.6
Presence of diabetes in first degree relatives		
Yes	22	41.5
No	31	58.5
Presence of dyslipidemia in first degree relatives		
Yes	15	28.8
No	38	71.7
Smoking status		
Yes	13	24.5
No	40	75.5
How is the diet in general?		
Very healthy	1	1.9
Healthy	8	15.1
Neither healthy nor unhealthy	36	67.9
Unhealthy	8	15.1
Very unhealthy	0	0
Presence of diabetes mellitus		
Yes	0	0
No	52	98.1
There is impaired glucose intolerance	1	1.9

Only one of the participants was diagnosed with hypertension and did not use medication, while there remaining 52 (98.1%) said they had not been diagnosed with hypertension before. In comparison, 34% (n=18) of the participants did not have their blood pressure measured in the last six months.

The questions were asked to determine the participants' attitudes toward the diagnosis and treatment of hypertension, and their answers are given in Table 3.

While 17% (n=9) of the participants stated that the diagnosis, follow-up and treatment of hypertension could be made entirely in primary care, 73.6% stated that they could completely manage a hypertension patient with their current knowledge.

While 60.4% (n=32) of the participants gave the correct answer to the question of "how much salt should be taken daily", 39.6% (n=21) gave the wrong answer.

Participants were asked “Should medication be started immediately in cases where systolic blood pressure is

>140 and diastolic blood pressure is >90", 86.8% (n=46) of the participants gave the answer "no", while 13.2% (n=7) gave the answer "yes".
When the questions about the drug choices of the participants were examined, it was seen that 49.1% (n=26) of the participants did not know the first group of drugs to be started in the treatment of hypertension, and 43.4% (n=23) could not choose the appropriate combinations.

Table 3: Attitudes of the participants about the diagnosis and treatment of hypertension.

	I totally agree	*I mostly agree*	*I agree*	*I mostly disagree*	*I totally disagree*
The blood pressure of every patient who comes to be examined should be measured.	26.4% (n=14)	45.3% (n=24)	20.8% (n=11)	5.7% (n=3)	1.9% (n=1)
Diagnosis, follow-up and treatment of hypertension can be done completely in primary care.	17% (n=9)	45.3% (n=24)	28.3% (n=15)	7.5% (n=4)	1.9% (n=1)
Primary care physicians should assume more responsibility in the management of hypertension.	32.1% (n=17)	32.1% (n=17)	22.6% (n=12)	13.2% (n=7)	0 (n=0)
I can diagnose, follow and treat a hypertension patient with my current knowledge.	3.8% (n=2)	26.4% (n=14)	43.4% (n=23)	24.5% (n=13)	1.9% (n=1)

Likewise, 60.4% (n=32) of the participants thought that patients whose target blood pressure values could not be reached with monotherapy should be referred to a cardiologist or internal medicine specialist.
To the question of "How should the target blood pressure values in the elderly be compared to young adults", 26.4% (n=14) of the participants gave the answer "lower", while 73.6% (n=39) gave the answer "higher".
Considering the scores obtained by the participants from the knowledge questions, it was seen that the knowledge score average was 3.56±1.25 (Table 4). No significant correlation was found between knowledge score and risk score (p=0.756).

Table 4: Participants' mean risk and knowledge scores.

	Min	*Max*	*Mean*	*SD*
Risk score	0	7	2.92	1.74
Knowledge score	1	6	3.55	1.21

A significant relationship was found between the current information, diagnosis, follow-up and treatment of hypertension, and knowledge score (p=0.035).

4. Discussion

In our study, 73.6% of the participants (3.8% totally agree, 26.4% mostly agree, and 43.4% agree) stated that they could manage a hypertension patient's diagnosis, follow-up and treatment processes. Although this rate is high, higher rates can be expected for a disease seen in one out of three people in the community.
Hypertension is a public health problem seen in approximately one in three people worldwide and brings a great burden to societies both in the field of health and economically [5]. Hypertension is a disease that physicians at all levels can follow up on. One of every three applications to health facilities in our country is made to primary care facilities. Therefore, the perception that hypertension should be managed only in 2nd and 3rd-level hospitals is wrong. On the other hand, the patient may not go to the referred place due to the workload, the difficulty of finding a queue in the hospital, etc. This causes a delay in the treatment of the patient. A study conducted in the USA observed that between 3% and 18% of the referred patients did not go to the referred specialist [12].
The referral process is essential in guiding patients correctly, preventing unnecessary applications to higher-level health institutions, using health resources more effectively, increasing patient satisfaction and providing health services more efficiently [10]. An effective referral system increases the quality of every step of the health system. However, there is no effective referral system in our country. This deficiency poses a severe problem regarding the effective planning and management of health services. For this reason, it is necessary to establish a referral system and provide necessary training in primary health care services [10].
Risk factors such as smoking and alcohol use, obesity, stressful personality structure, family history, excessive salt consumption, dyslipidemia, insufficient physical activity, diabetes, and sleep apnea should be questioned, reasons that may suggest secondary hypertension should be reviewed, and a detailed physical examination should be performed. In ourstudy, it was observed that the participants had approximately three risk factors among these risk factors. The most common risk factor was having a stressful personality, with 66%. Exposure to

psychological stress and the resulting sympathetic discharge can increase blood pressure by causing vasoconstriction. When exposure to stress is prolonged, the susceptibility to hypertension increases [13, 14]. In experiments on borderline hypertensive mice, mice regularly exposed to stress developed hypertension [15]. In a study conducted on medical school students in Mersin, 58.3% of the students were found to have mild to severe stress, and stres increased significantly from the 3rd term [16]. In another study conducted in Malaysia, this rate was found to be 56% and it was mentioned that medical school education is a stressful process [17]. Although the stress situation has not been evaluated with a universal scale and its causes have not been questioned, the increased exam stress, future anxiety and uncertainties, especially with graduation, may have increased the stress coefficient in students.

Another frequently observed risk factor was sedentary life. Of the participants, 39.6% said they usually do not exercise regularly, and 11.3% said they never do. Regular exercise can reduce systolic blood pressure by 5-7 mmHg, which is about the same as the benefit of monopharmacotherapy [18, 19]. This is why almost all hypertension guidelines recommend regular exercise [2, 9, 13, 20]. Unfortunately, studies show that medical students' physical activity levels could be much higher [21-23]. In a study conducted by Gömleksiz et al. in a medical school in Elazig, it was found that 67.5% of the students did not exercise regularly[22]. This situation is more evident especially in 5th and 6th-semester students, probably due to the medical speciality exam to be taken after graduation [22]. This is expected since we included students who will graduate in our study.

Although awareness has increased compared to previous years, it cannot be said that there is a full awareness of hypertension in our society [3, 6]. Although uncomplicated hypertension measuring device is sufficient for the diagnosis, it was seen that 45.3% of the participants who were found to have hypertension in the PATENT 2 study needed to be made aware of this condition [6]. Almost all of the participants in our study stated that they did not have hypertension. However, although they have been in the clinics for the last 1 year, they probably have a sphygmomanometer wherever they work, and they have repeatedly measured the blood pressure of others with in the scope of practical training, approximately one-third of the participants have not had their own blood pressure measured in the last six months. We find this data important. Despite all these opportunities, even in the group of physicians who should have the highest awareness of blood pressure in society, awareness needs to be sufficient. A study conducted with physicians in Japan showed that middle-aged physicians had sufficient theoretical knowledge about hypertension but were more reluctant to treat patients than younger physicians [24]. This finding has been particularly associated with changing medical education [24]. One of the best examples of such a program was experienced in Canada. As a result of the Education Program (CHEP) initiated in 1999 to improve hypertension management and reduce the burden of cardiovascular disease in Canada, 84.4% of antihypertensive prescriptions and hypertension in the Canadian population between 1996 and 2003 showed a 65.1% increase in the number of individuals diagnosed [25]. A study conducted on 340 patients registered in a family health center in Muğla found that 36.4% (n=124) of the participants never had their blood pressure measured, and their remaining 63.6% had it measured periodically [26]. From this point of view, it is a remarkable finding that the awareness of hypertension in the average population is some what better than that of medical school students. In this respect, there is a need for medical education practices that will increase awareness.

“Should primary care physicians take more responsibility in the management of hypertension in the current situation?” was asked, and 86.8% (n=46) of the participants answered either completely agree, mostly agree or agree. However, when asked, "Can you diagnose, follow up and treat a hypertension patient with your current knowledge?” this rate was low to 73.6% (n=39). When asked the participants, “Can the diagnosis, follow-up and treatment of hypertension be completely done in primary care" 90.6% (n=48) said they agree, mostly agree or agree. Although one-quarter of the participants feel inadequate in the follow-up of hypertension, it is a pleasing development that at least most of them agree that this follow-up can be done entirely in primary care and that the majority of them believe that primary care should take on more responsibility. In a study conducted on family physicians in Bursa, the most common chronic disease that physicians encountered was hypertension, with 24%.

In contrast, physicians were asked about the diseases they had the most difficulty managing the patient, and hypertension ranked 4th after diabetes mellitus, psychiatric diseases and rheumatological diseases [27]. It is an exciting finding that although hypertension is the most common chronic disease physicians see, they feel inadequate in managing it. On the other hand, when the theoretical knowledge scores of the participants are examined, it is seen that the mean score is 3.55±1.21 out of 6 points. In addition, a significant relationship was found between the knowledge score and thinking that one could follow hypertension (p=0.035). Although the theoretical knowledge inquiry was not made on a Standard scale, essential questions such as some risk factors, drugs that can be started in the first group and drugs that cannot be combined were asked.

Although the participants' theoretical knowledge is sufficient, it is a situation that should be questioned well. What makes them think that they cannot follow hypertension? This may be due to the fact that medical faculties in our country give less importance to practical education than theoretical courses. The reason for this may be the conversion of education to the online form and the disruption of practical training due

to the COVID-19 pandemic. More emphasis should be placed on the follow-up of common diseases such as hypertension in medical education, and students should be trained on systematic practical approaches. It is very essential to train physicians who can meet the health needs of society, with the training of trainers if necessary [28].

5. Conclusions

Although there are studies on hypertension attitudes and behaviours in the literature, especially with physicians working in the field, studies with physicians who will graduate and have yet to starts to work in the field are limited. In our study, one-third of the participants did not have their blood pressure measured in the last six months, and while most of them think that primary care should take a more active role in the follow-up of hypertension, some of them feel inadequate. Further studies with higher sample sizes questioning the reasons for this finding are needed. It should not be forgotten that hypertension is a public health problem, and effective diagnosis, follow-up and treatment by physicians from all levels will favour societies. Even in managing a disease such as hypertension, which is very common in society and whose diagnosis, follow-up and treatment processes are simple, physician candidates are reluctant to take an active role.

Limitations of the Study

There are some limitations of our study. One of the most important limitations is the sample size. However, the number of students could be higher because our university is newly established. The results cannot be generalized due to the small number of students and the single-centre study. Nevertheless, most of the students were reached. Another limitation is that the attitudes and knowledge levels of the participants about hypertension were not made using a standardized general form since a suitable questionnaire could not be found in the literature review. Still, questions were asked about the main topics in hypertension guidelines and a general frame work was tried to be drawn [29-31]. In addition, the participants were given training on the subject after the study, so pre-post could not be done. There is a need for such studies in the future in order to measure the effectiveness of the education provided.

Strengths of the Study

Although there are many studies on hypertension in the literature, as far as we can see, we have not come across a study on physician candidates who are about to complete their medical education but have yet to starts working actively. Knowing whether physicians' knowledge and attitudes about hypertension are formed by their medical education by developing themselves in the field can help us to see our deficiencies in medical education [24].

There are two major limitations in this study that can be addressed in future research. First, the sample size is larger. Second, showing that the number of interleukins involved in sle disease is higher.

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Conflict of Interests

All authors declare there is no conflict of interest**.**

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Author Contributions

EG: Conceptualization, Methodology, Formal analysis, Writing-Original Draft. MY: Conceptualization, Methodology, Writing-Review and Editing.

Ethical Approval

Ethics committee approval was obtained for the study from the Erzincan Binali Yıldırım University Clinical Research Ethics Committee with the decision dated 25/10/2021 and numbered 11/05.

Data sharing statement

All data underlying the results are available in the article, and no additional source data are required.

Consent to participate

Consent was obtained from those who participated in the study.

Informed Statement

Informed consent was obtained from those who agreed to participate in the study.

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Electrocardiographic Evaluation of Patients with Crimean-Congo Hemorrhagic Fever

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Abstract: Infectious diseases can affect the myocardium directly or through cytokines. Disruption of cardiac depolarization and repolarization is associated with the development of arrhythmia. In this study, we aimed to evaluate electrocardiographic (ECG) parameters in patients with Crimean-Congo Hemorrhagic Fever (CCHF). 42 patients hospitalized with the diagnosis of CCHF were included in the study. Heart rate, PR interval, P dispersion, QRS duration, QT interval and corrected QT, T peak T end, Tp-e/QT ratio, Tp-e/QTc ratio, and QT dispersion parameters were calculated from 12-lead ECGs at the time of admission and discharge. The mean age of the patients in the study was 45.8±16.9 years. ECG parameters were found to be similar at admission and discharge (all p values>0.1). Major events such as life-threatening bleeding, significant hypotension, and shock were not observed in any of the patients. Platelet and white blood cell values were significantly increased at discharge compared to admission (78.3 vs 197.6x103, p=0.01 and 2.8 vs 5.4x10^3, p=0.006 respectively). In patients with CCHF, there was no significant change in ECG polarization parameters at the onset of the active infection process and during hospitalization period and these parameters found to be within normal limits. ©2023 NTMS.

Keywords: Crimean-Congo Hemorrhagic Fever, electrocardiography, Tp-e, Tp-e/QT, P dispersion

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is an acute viral hemorrhagic fever caused by the nairovirus infection transmitted by tick bites, crushing infected ticks, or contact with viremic blood and tissues [1-3]. It is

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now endemic in many different regions of Africa, Asia, and Eastern Europe. The most important clinical features are fever and in the most severe cases, shock and hemorrhage. As in other viral diseases, cardiac involvement may occur in CCHF and cardiac involvement may have a role in the pathogenesis of shock and may also influence the prognosis. Myocardial damage is associated with the severity of the disease, and it has been shown that cardiac dysfunction may occur in severe and fatal cases of CCHF [3]. The direct effect of the virus or cytokine-mediated hyperinflammatory response may play a role in myocardial damage in viral infections as in the CCHF [4, 5]. 38% of CCHF cases have a severe course and the mortality is between 10-30% [1-3].

Many methods such as blood tests (troponin, and creatine phosphokinase), electrocardiography (ECG), echocardiography (ECHO), cardiac magnetic resonance imaging (MRI) and cardiac biopsy can be used in demonstrating the development of myocardial damage. Among these tests, ECG plays an important role because it is inexpensive, noninvasive, reproducible, can be performed practically at the bedside, and results in a short time. ECG is an important test to see arrhythmias in patients but it is not clear whether this myocardial involvement causes atrial and ventricular arrhythmias in this patient group.

QT and corrected QT(QTc) intervals, QT dispersion and T peak-T end (Tp-e) which are indicators of electrocardiographic depolarization and repolarization distribution, are associated with the development of ventricular arrhythmias [6-9]. P-wave dispersion reflects the prolongation of intra and inter-atrial conduction time and is associated with the development of atrial arrhythmias [10]. To our knowledge, the literature presents no studies examining the atrial and ventricular parameters in the ECG and the development of arrhythmias related to these parameters in CCHF patients. In our study, we aimed to evaluate these electrocardiographic parameters in CCHF patients.

2. Material and Methods

2.1. Study Population

Patients hospitalized with suspected CCHF in the infectious diseases clinic between April 2020 and September 2020 were included in the study. The definitive diagnosis of CCHF infection was established by the typical clinical and epidemiological findings, in addition to the detection of CCHF-specific IgM by ELISA method or detection of the genomic segments of the virus by the reverse transcription-polymerase chain reaction (RT-PCR) method. Patients with no definitive diagnosis of CCHF were excluded from the study. The study population consisted of 42 CCHF patients with a definite diagnosis and all were hospitalized for treatment. Patients with heart failure, coronary artery disease, chronic renal failure, those using antiarrhythmic drugs, and patients with a history of arrhythmia and conduction disorder were excluded from the study. The study protocol was approved by the local institutional ethics committee, and written informed consent was received from each patient.

2.2. Electrocardiographic analysis

For electrocardiographic examination, 12-lead ECG (20 mm/mV and 50 mm/s) records at admission and before discharge were used. (Cardiofax V, Nihon Kohden Corp., Tokyo, Japan) PR interval, QRS duration, heart rate, QT interval and QTc were recorded. The QT interval was defined as the time from the beginning of the QRS complex to a point at the T-wave returned to the isoelectric line. The R-R interval was measured by averaging the time between three QRS complexes. Using this interval we calculate the heart rate and QTc interval with Bazett's formula. Patients with low-amplitude T-waves and U waves in their electrocardiograms were excluded from the study. QT dispersion was defined as the difference between the maximum and minimum QT duration recorded in all leads in ECG. The Tp-e interval can be measured using both the tail and tangent methods, but the tail method has been shown to provide a better prediction of mortality than the tangent method [9, 11]. Therefore, we used the tail method in the present study. According to this method, Tp-e interval was defined as the time from the peak of T-wave to the end of T-wave to a point where it reaches the isoelectric line [12]. The Tp-e interval was measured from the V2 and V5 leads. Tp-e/QT ratios, and Tp-e/QTc ratios were calculated from these measurements. P wave dispersion was defined as the difference between the maximum and minimum P wave duration recorded in all leads in ECG [10]. All the measurements were calculated by two independent cardiologists.

2.3. Treatment

Treatment for CCHF is primarily supportive. Treatment includes oxygenation and hemodynamic support, fluid balance and correction of electrolyte abnormalities, managing bleeding complications and appropriate treatment of secondary infections. Also intravenous immunoglobulin (IVIG) treatment can be given in patients with a poor clinical course. However, there were no patients who received IVIG treatment in our study. Therefore, in our cohort there were no patients who received any drugs causing ECG changes.

2.4. Statistical analysis

All statistical analyses were made by using the SPSS software (Version 22.0, SPSS, Inc., Chicago, IL). Visual (histograms, probability graphs) and analytical methods (Kolmogorov-Smirnov/ Shapiro-Wilk test) were used to determine whether the variables were normally distributed. Continious variables were presented using mean±standart deviations. Wilcoxon test was used to compare the differences of the CCHF patients' admission and discharge parameters. Chi-square test was used to compare categorical variables. P value<0.05 was considered statistically significant.

3. Results

The mean age of the patients in this study was 45.8±16.9 years. Six (14.3%) of the patients were female. Mean hospitalization duration was 6.3±1.6 days. Four patients (9.5%) had hypertension and 3 patients (7.1%) had diabetes. These four patients were not using antiarrhythmic drugs. Major events such as life-threatening bleeding, significant hypotension and shock were not seen in any of the patients. The average body temperature was 37.2±0.8 °C at admission. Mean troponin and International Normalized Ratio (INR) values at admission were 1.12±0.3, and 4±2.2 ng/dl, respectively, and were within normal limits. All patients received symptomatic and supportive treatment. Additional platelet replacement and/or antiviral therapy and/or whole blood replacement were performed if clinically indicated. Mean platelet and white blood cell counts increased significantly at discharge when compared to admission (78.3 vs 197.6x10^3, p=0.01; 2.8 vs 5.4x10^3, respectively, p=0.006). Hemoglobin level at admission was similar to discharge (13.3 vs 13.6 mg/dl, p=0.772). Serum sodium, potassium, calcium, magnesium and creatinine levels were within normal limits and were similar at admission and discharge (Table 1).

In electrocardiographic measurements heart rate (73.9 vs 68.1 bpm, p=0.233), PR interval (150 vs 149.3 msec, p=0.803), P wave dispersion (30.7 vs 30 msec, p=0.739), QRS duration (89.3 vs 90.7 msec, p=0.414), QT interval (381.4 vs 398.6 msec, p=0.155), QTc interval (417.6 vs 422.4 msec, p=0.900), lead V2 Tp-e (87.9 vs 88.6 msec, p=0.796), lead V5 Tp-e (78.6 vs 84.3 msec, p=0.103), lead V2 Tp-e/QT (0.23 vs 0.22, p=0.272), lead V5 Tp-e/QT (0.20 vs 0.21, p=0.249), lead V2 Tp-e/QTc (0.22 vs 0.21, p=0.226), lead V5 Tp-e/QTc (0.19 vs 0.20, p=0.124), and QT dispersion (33.5 vs 26 msec, p=0.132) values were similar at admission vs discharge and the values were within normal range (Table 2).

Table 1: Admission and discharge laboratory findings of the study population.

Variables	Admission (n=42)	Discharge (n=42)	p value
Body Temperature (°C)	37.2±0.8	36.3±0.5	**0.01**
Serum creatinine, mg/dL	0.86±0.38	0.77±0.16	0.285
Serum sodium, mmol/L	133.9±3.1	133.2±2	0.100
Serum potassium, mmol/L	3.8±0.35	4±0.34	0.181
Serum calcium, mg/dL	8.6±1.7	8.4±1.3	0.374
Serum magnesium, mg/dL	1.6±0.25	1.9±0.27	0.073
WBC, x 10^3/mm^3	2.8±1.3	5.4±1.7	**0.006**
Platelets, 10^3/mm^3	78.3±55.3	197.6±63.7	**0.001**
Hemoglobin, g/dL	13.3±4.2	13.6±1.4	0.772

Abbreviations: WBC: white blood cell.

Table 2: Admission and discharge electrocardiographic findings of the study population.

ECG Variables	Normal values	Admission (n=42)	Discharge (n=42)	p value
Heart rate, bpm	60-100	73.9±14	68.1±8.7	0.233
PR interval, msec	120-200	150±19.2	149.3±20.9	0.803
P wave dispersion, msec	<36	30.7±11.4	30±10.4	0.739
QRS duration, msec	70-100	89.3±7.3	90.7±8.3	0.414
QT, msec	350-450	381.4±48	398.6±36.9	0.155
QTc, msec	350-450	417.6±33.8	422.4±34.4	0.900
QT dispersion, msec	10-71	33.5±11.5	26±12.1	0.132
Tp-e lead V2, msec	50-100	87.9±13.7	88.6±10.3	0.796
Tp-e lead V5, msec	50-100	78.6±8.6	84.3±10.2	0.103
Tp-e/QT, lead V2	0.13-0.29	0.23±0,04	0.22±0.02	0,272
Tp-e/QT, lead V5	0.13-0.29	0.20±0.02	0.21±0.03	0.249
Tp-e/QTc, lead V2	0.11-0.28	0.22±0.04	0.21±0.03	0.226
Tp-e/QTc, lead V5	0.11-0.28	0.19±0.02	0.20±0.02	0.124

Abbreviations: ECG: electrocardiography, QTc: rate-corrected QT interval, Tp-e: T wave peak-to-end interval.

4. Discussion

In our study, atrial and ventricular ECG parameters did not change and arrhythmia was not observed at the onset of active infection and during hospitalization in patients with Crimean-Congo Hemorrhagic Fever. In addition, these parameters remained within normal limits throughout the active infection and at discharge. Myocardial involvement in CCHF patients can affect

the course and severity of the disease [3, 13]. As in other viral myocardial infections, this may be caused by the direct effect of the infecting virus on cardiac tissues, the attack of immune cells against infected cardiomyocytes, or cytokine-mediated damage [4, 14-16]. Electrocardiographic and echocardiographic myocardial impairment has been shown to be related to mortality due to CCHF [3, 13]. ECG is not a part of the routine clinical follow-up of CCHF patients, but it has been shown that ECG changes may have a potential role in predicting disease mortality [13]. Studies have shown that electrocardiographic (ECG) T-wave inversion, bundle branch block and echocardiographic left ventricular wall motion abnormality and pericardial effusion can be observed even in non- critical patients [3, 13, 17]. In this study, we aimed to investigate the changes in ECG parameters and the development of arrhythmia in CCHF patients and to evaluate the suitability of ECG as a routine screening test.

It is not clearly known whether myocardial involvement contributes to the development of atrial or ventricular arrhythmias in these patient groups. It may be concluded that in these patient groups for whom arrhythmia has not been reported yet, ECG changes may be predictive of the severity and mortality of the disease rather than the development of arrhythmia.

Considering the literature, the fact that atrial and ventricular ECG parameters, which may predict the development of arrhythmia, have not been studied before, makes our study valuable. The additional contribution of ECG to the routine follow-up of these patients is also controversial. QRS duration, QT interval and QTc, Tp-e, Tp-e/QTc, QT dispersion, which are indicators of electrocardiographic ventricular depolarization and repolarization, did not change in our patient population at admission and discharge. In addition, P wave dispersion, which is a sign of atrial conduction delay, and PR distance, which is an indicator of atrioventricular conduction duration, were also within normal limits and were similar at admission and discharge. As a result of these findings, it can be thought that this patient group are not prone to the development of atrial and ventricular arrhythmias during the active phase of the disease. In addition, troponin levels, which are indicative of myocardial damage, were also within normal limits in the whole group. Serum electrolyte levels may also affect myocardial depolarization and repolarization in CCHF patients. Electrolyte abnormality may develop and impair myocardial electrical distribution in cases such as hypotension, acute renal failure and shock. In our patient group, major events such as life-threatening bleeding, hypotension and shock, electrolyte abnormality or acute renal failure were not observed. The low platelet levels of the patients at admission reached normal levels with platelet replacement and supportive treatments. Also, the low white blood cell count at admission increased before discharge. Bradycardia may occur in approximately 4% of CCHF patients [18]. The mechanism of this situation, which is mostly temporary, is not clear. It has also been suggested that it may develop through direct myocardial injury or cytokine induced. In our study, the mean heart rate was 73.9 bpm at admission and 68.1 bpm at discharge, and only 3 (7.1%) patients had asymptomatic sinus bradycardia and persisted at discharge.

It has been reported in the literature that viral hemorrhagic fever virus infections such as Hantavirus infection, Lassa fever, and dengue fever can cause electrocardiographic changes [19-21]. Although non-specific ECG abnormalities such as ST segment and T wave changes and ST-segment elevation have been detected in Lassa fever infection, it has been revealed that these changes are not related to the clinical severity of the disease [19]. Sinus bradycardia and conduction defects were observed in dengue fever infection, and these changes were reported to be usually temporary [20]. In addition, ECG abnormalities have been reported in hemorrhagic fever with renal syndrome (HFRS) caused by Hanta virus. However, it has been reported that ECG changes in HFRS occur in critically ill patients, especially in the oliguric stage [21]. Among these ECG abnormalities, prolonged QT interval, long and sharp T waves and bradycardia are more prominent. It was thought that secondary causes such as hypopituitarism, hyperthyroidism and renal failure that may increase the severity of the disease in HFRS may explain the ECG abnormalities [21-23]. As a result of these findings, it can be interpreted that severe ECG changes in viral hemorrhagic fever virus infections occur during the critical stages of disease. In our study, it was observed that the parameters that can be accepted as electrocardiographic findings of atrial and ventricular arrhythmia did not change during admission and discharge.

5. Conclusions

Atrial and ventricular electrocardiographic parameters were in normal limits and did not change at the onset of active infection and during hospitalization in patients with Crimean-Congo Hemorrhagic Fever. The benefit of routine ECG follow-up in this patient group for determining myocardial involvement and arrhythmia seems controversial. The use of ECG can be considered in selected patient groups in order to determine cardiac involvement in patients with a poor clinical course. However, studies evaluating ECG parameters in patient groups with severe clinical course and/or mortality with proven myocardial involvement are needed.

Limitations of the Study

The main limitation of our study was the small number of patients. We evaluated myocardial involvement only by troponin level and patients were not routinely performed echocardiography. The inability to compare with MRI and/or cardiac biopsy, which are more sensitive methods in detecting myocardial damage, is another limitation of our study. Failure to observe a clinical major adverse event in our patient group during

follow-up led to the absence of a serious patient group. Considering that prominent ECG changes occur in patient groups with hemodynamic disorders and secondary clinical aggravation, this may be considered as a limitation.

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Conflict of Interests
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Author Contributions
E.A, M.Ö and S.İ.Y. conceived and planned the hypothesis and wrote the manuscript. O.E.T. and G.C. performed the calculations. H.A. and Ö.K. are responsible for the data and supervised data analyses. All authors supported writing of the manuscript. E.A, M.Ö and S.İ.Y. designed and directed the current topic. All authors provided critical feedback and helped shape the research, analysis and manuscript. E.A directed the final version and is responsible for final approval of the submitted manuscript.

Ethical Approval
Ethical committee approval was received from the Ethics Committee of Erzurum Region Training and Research Hospital (Approval Date: 2020; Approval Number: 2020/17-183).

Data sharing statement
All data relevant to the study are included in the article

Consent to participate
All participants read the consent form and understand the study being described.

Informed Statement
Informed consent was obtained from all participants included in the study.

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Detection of Human Brucellosis by Brucellacapt and Rose Bengal Test in the Endemic Area

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Abstract: Although brucellosis is the most common zoonosis in the world, it remains an infectious disease that has not received sufficient attention. There are a few tests detecting brucellosis by serum. Rose Bengal Test is an advantageous one. Our aim with this study was to compare Rose Bengal and Brucellacapt tests in serum samples and draw attention to the advantages of the test. Between June 2019 and June 2021, 7827 serum samples sent to the public health laboratory with a provisional diagnosis of brucellosis were evaluated. The Rose Bengal and Brucellacapt test (Vircell, Spain) was used to diagnose infection. Samples with antibody titer ≥1/160 were considered positive. Of the 7827 samples included in the study, 354 (4.6%) of the total 7677 serum samples tested were RBT positive, 118 (3.1%) of the 3776 samples tested were BCAP positive, and 118 (3.3%) of the 3626 samples tested were both RBT and BCAP positive. It was found that seropositivity was significantly higher in female patients ($p<0.10$). RBT and BCAP test positivity were most frequently observed in the 25-34 year age group. Considering brucellosis cases in terms of seasonal changes; 10.7% of cases were found in spring, 52.4% in summer, 20.9% in fall, and 16% in winter. In suspected cases, RBT is still considered the ideal detection method because it is easy to use, inexpensive, sensitive, and provides rapid results. It was concluded that the BCAP test is suitable for diagnosis due to its ability to detect blocker and high titer antibodies. ©2023 NTMS.

Keywords: Brucellacapt; Brucellosis; Endemic Area; Public Health; Rose Bengal; Seroprevalence.

1. Introduction

Brucellosis is an ancient disease that remains the most common zoonosis in the world. *Brucella* spp. Gram-negative coccobacilli are defined as non-sporulating immotile, pleomorphic, facultative intracellular bacteria. The species *B. melitensis* and *B. abortus* are most commonly isolated in our country [1]. The main methods of transmission of brucellosis are direct contact with the blood or uterine fluids of sheep, goats, cattle, pigs, and camels, as well as ingestion of raw animal products that have been contaminated and unpasteurized milk. In addition, brucellosis is one of the most common laboratory-acquired bacterial infections worldwide [2]. In our country, brucellosis cases occur more frequently in the Eastern and

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Southeastern Anatolia regions, where animal husbandry is intensive [3]. According to the data of the Public Health Institution of Turkey, the number of brucellosis cases in our country as of 2017 is 6457 and the morbidity rate is 7.99 per 100,000. According to the same data, the last case of death caused by brucellosis was observed in 2008 [4]. The disease can affect any organ or system of the body. The symptoms are numerous and nonspecific and include fatigue, weakness, back or joint pain, sweating, anorexia, weight loss, and depression. Fever, mild lymphadenopathy, hepatomegaly, or splenomegaly are the physical findings that may be seen [5].

The diagnosis of brucellosis is usually made by bacteriologic and serological methods. Cultures, Rose Bengal agglutination test (RBT), standard agglutination test (SAT), enzyme-linked immunosorbent test (ELISA), Coombs test, and Brucella Capt (immunocapture-agglutination).Technique are used for serological diagnosis, and polymerase chain reaction (PCR) and real-time PCR are used for molecular diagnosis [6]. Blood, bone marrow, and tissue cultures are used as the gold standard for the diagnosis of brucellosis [7]. The method is invasive and has low sensitivity. For these reasons, clinicians prefer methods that provide indirect evidence of brucellosis rather than these invasive methods [8]. The Rose Bengal test is commonly used as a screening test for brucellosis, but it is not sufficient by itself for diagnosis because of its high false positivity rate. Brucellacapt testing may help detect disease in patients with a long evolutionary time that cannot be detected by SAT. After a successful treatment, a decrease in specific antibody titers is more rapid in Brucellacapt than in the other tests [9].

This study aimed to retrospectively investigate and compare serum samples, RBT, and Brucellacapt (BCAP) test results from patients with a prior - diagnosis of brucellosis sent to the public health laboratory in our province, which is a highly endemic region for brucellosis (The number of reported cases in 2017 was 6404) [14].

2. Material and Methods

Ethical approval for this registration was granted by the Van Regional Training and Research Hospital Clinical Research Ethics Committee, dated 30.09.2021 with the decision number 2021/17.

2.1. Patients

In our study, patients sent from family medicine and community health centers to our public health laboratory between June 2019 and June 2021 were retrospectively included. RB and BCAP results of 7827 cases, demographic information of patients, and seasonal distribution of cases it has been taken into consideration.

2.2. Analyses

Patient blood samples were centrifuged at 4000 rpm for 10 minutes, and their serums were separated. For RBT, RBT antigen (50 µL) was mixed with patient serum (50 µL), and agglutination formation was observed by rotating for 4 minutes. Samples with agglutination were classified as positive, and those without agglutination were classified as negative.

BCAP test; microagglutination test according to the Sandwich-Elisa model for the determination of Brucella antibody titer and the determination of total Brucella (IgM/IgG/IgA). All reagents, test plates, and serum samples were brought to room temperature before testing. Eight wells to A-H, including positive and negative controls, were placed on the plate layer for titration 95 µL of special dilution was added to the first well (A) and 50 µL of special dilution was added to the other wells. 5 µL of the positive/negative control serum was added to the wells for the positive and negative controls. 5 µL of the patient serum was added to the first well and mixed three or four times with a micropipette. 50 µL of this sample was added to well (B), and the same procedure was continued until well H. At the end of this procedure, 50 µL of antigen was added to all wells, and the plating layer was mixed with circular movements. The plate was sealed with protective tape in a special box and incubated for 18 to 24 hours at 37°C with moist cotton. The results were evaluated to a titration of 1/1280, where the first well was 1/40. The shape of a blue dot in the well was considered negative and the homogeneous image of the well was considered positive. Antibody titers >1/160 were considered positive.

2.3. Statistical Analysis

In the analysis of the results obtained in the study, the program "IBM SPSS Statistics for Windows, Version 20.0 software (IBM Corp., Armonk, NY, USA)" was used for the analysis. Descriptive statistics for quantitative variables (mean, standard deviation, largest, smallest), frequency tables for qualitative variables (number, n; percentage, %), cross tables, and the chi-square test and Fisher exact test for independent groups were used. Statistical significance was assessed using a margin of error of <0.10 and a confidence level of 0.90.

3. Results

This study includes 7827 suspected brucellosis patients, 5640 (72.1%) were female and 2187 (27.9%) were male. The mean age was 39.06±16.70 (38.97±16.18 for women; 39.30±17.96 for men; age range 1-99). In the samples, both tests were not performed simultaneously. In 7827 serum samples, 7677 (98.08%) RBT, 3776 (48.24%) BCAP, and 3626 (46.32%) samples were tested for both RBT and BCAP (Figure 1).

Looking at the RBT and BCAP test positivity rates of cases by sex, we found that 67.5% and 62.7% of cases involved women, respectively. These rates were higher than those of men (32.5% and 37.3%, respectively), and this fact was considered statistically significant (RBT: p=0.045, BCAP: p=0.092) (Table 1).

Table 1: Distribution of Rose Bengal Test and Brucellacapt Test seropositivity by gender.

	Rose Bengal Test				Brucellacapt Test			
	Negative		Positive		Negative		Positive	
Gender	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Female	5308	72.5	239	67.5	2559	70	74	62.7
Male	2015	27.5	115	32.5	1099	30	44	37.3
Total	7323		354 (4.6%)		3658		118 (3.1%)	

Figure 1: Distribution of Brucella test species.

When the RBT and BCAP positivity rates were deciphered according to the age groups of the cases, a statistically significant difference was found between the groups (RBT: p=0.080, BCAP: p=0.060). RBT and BCAP test positivity were most frequently observed in the 25-34 age group, with 23.2% and 24.6%, respectively (Table 2).

Table 2: Distribution of Rose Bengal Test and Brucellacapt Test seropositivity by age group

Age	Rose Bengal Test				Brucellacapt Test			
	Negative		Positive		Negative		Positive	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
<18	596	8.1	45	12.7	270	7.4	15	12.7
18-24	898	12.3	47	13.3	431	11.8	21	17.8
25-34	1736	23.7	82	23.2	827	22.6	29	24.6
35-44	1484	20.3	70	19.8	753	20.5	18	15.2
45-54	1091	14.9	43	12.1	573	15.7	12	10.2
55-64	928	12.7	39	11	500	13.7	14	11.9
>65	590	8	28	7.9	304	8.3	9	7.6
Total	7323		354		3658		118	

Positivity for both tests was detected in 354 (4.6%) of 7677 serum samples with RBT, 118 (3.1%) of 3776 samples with BCAP testing, and 118 (3.3%) of 3626 samples with both RBT and BCAP testing (Table 3). Of the 118 samples with positive BCAP test, agglutination was detected at 1/320 titer in 93 (78.81%) and 1/160 titer in 25 (21.19%) in 93 (21.19%). There is a relationship between the Dect and BCAP test results at the level of 0.75 level and this relationship was found to be statistically significant (p=0.000). When Brucella cases were evaluated in terms of seasonal changes, 10.7% of cases were detected in spring, 52.4% in summer, 20.9% in autumn, and 16% in winter (Figure 2).

4. Discussion

In our study, 4.6% RBT positive, 3.1% BCAP positive, and 3.3% both RBT and BCAP positive results were detected. The final diagnosis of brucellosis is made by bacteriologic methods. However, serological diagnostic methods are among the priority choices for the diagnosis of brucellosis, because of the long time required to grow bacteria, the variability in production rates, the risk of contamination in culture procedures related to the medium, and the fact that blood cultures cannot be performed in every health center [10, 11]. Although gold standard methods existed for the diagnosis of brucellosis, culturing Brucella species is time-consuming and not always possible to produce.

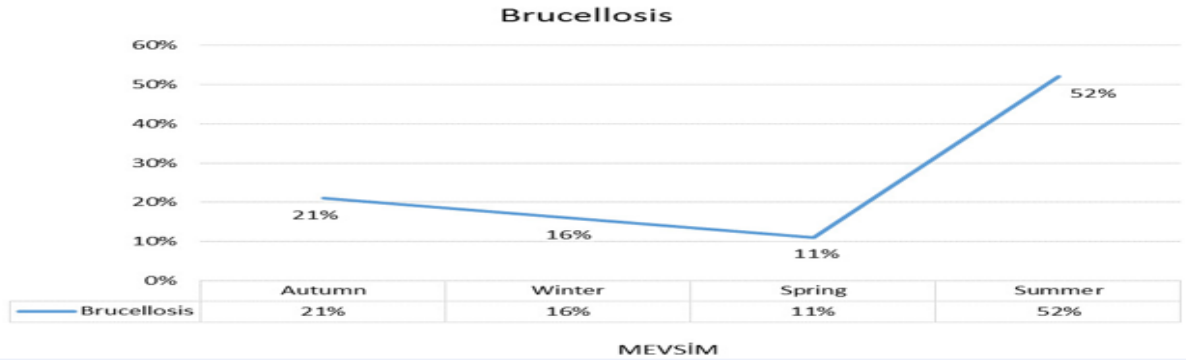

Figure 2: Seasonal distribution of Brucella seropositivity

Table 3: Comparison of Rose Bengal Test and Brucellacapt Test.

		Brucellacapt Test	
		Negative n (%)	**Positive n (%)**
Rose Bengal Test	**Negative**	3420 (94.3%)	0
	Positive	88 (2.4%)	118 (3.3%)
Total		3508	118

χ^2:2024.926; Phi:0.747; p:0.000.

Reasons for this may include misdiagnosis of the patient and thus incorrect treatment, the number of bacteria in the blood, late diagnosis, or in adequate laboratory facilities [12]. Serological tests are frequently preferred in routine laboratories because they are faster, more reliable, and more sensitive in diagnosing brucellosis [13]. According to the statistics of the Ministry of Health for 2017, the number of active brucellosis cases in our country is 6457, with Kars (82.4), Iğdır (76), Hakkari (62.4), Bitlis (61.2), Batman (60), Van (57.6) and Siirt (45) reported as the provinces with the highest incidence of brucellosis [14]. In this study, which is an endemic area for brucellosis in our province and around the comparison of studies has been done with the reason that the test BCAP, and RBT with the highest incidence of brucellosis in our lab previously watched the province and neighboring provinces in the various family medicine and community health centers and patient samples sent to questionable for the detection of brucellosis in BCAP, and RBT tests have been made.

The RBT is the leading serological test for the diagnosis of brucellosis in endemic regions. The RBT has a high diagnostic value in patients with no contact or history of disease, but a lower value in cases of previous or recurrent infections [15]. For this reason, diagnosis with RBT alone is not sufficient for initial or recurrent infections in Brucella endemic areas.[16] The BCAP test, which is considered one of the current serological detection methods, is based on the "sandwich ELISA" method. In this test; the wells, of the plate are coated with antibodies (Coombs antibodies) developed against human IgG, IgM, and IgA antibodies. Thanks to the BCAP test, all three antibodies can be detected in the patient's serum sample. The titers of this test are a good indicator of infection activity, regardless of the stage of brucellosis. BCAP titers show a marked decrease after successful antibiotic therapy compared with standard tube agglutination titers. After a successful treatment, a decrease in specific antibody titers can be reversed after treatment because Brucellacapt is faster than other tests [17].

In our study, RBT positivity was detected in 4.6% of serum from 7677 patients with suspected brucellosis. In some studies conducted in our country to determine the seroprevalence of brucellosis; RBT positivity was found to be 4.8% in Afyon, 4.2% in Manisa, and 3.6% in Isparta and Kırşehir [18-21]. Our study is largely similar to existing studies in terms of RBT positivity. Although the province where the study was conducted and its surroundings are located in an endemic region in terms of brucellosis, it is expected to be higher. However, in contrast to the studies in which high positivity was found, lower positivity was found in our study because the serum samples tested were not possible patient serum samples but serum samples sent for general screening purposes from primary health care facilities.

From December 2018 to December 2019, BCAP positivity was detected in the serum of 18 patients (3.85%) in 467 patients sent from various outpatient clinics or services with suspected brucellosis in a study conducted in Istanbul. Infection was observed more frequently in the 0 to 18-year-old age group and persons older than 50 years. In addition, seropositivity was found to be significantly higher in male patients [22]. In our study, 3.1% of the serum from 3776 patients with suspected brucellosis was positive by BCAP test. Considering the age groups, the most common positivity in our study was in the 25 to 34-year-old age group (24.6%). In contrast to other studies, our study shows that the positivity rates of RBT and BCAP tests

according to sex, are 67.5% and 62.7% in women, respectively [22, 23]. Although brucellosis is a disease that does not differ by age and sex, it is expected that positivity rates in our study are higher, especially in our region where the incidence is high due to occupational risk, in women who take care of animals and spend more time producing of milk and dairy products. In another study evaluating RBT, SAT, and Brucella capt results, the RBT screening test was found positive in 115 (10.8%) of 1060 serum samples. When BCAP results were evaluated, 86 (74.8%) samples were found to be agglutinated with a titer of 1/320 or more. The positivity rate for both tests was lower in women than in men. The age and seropositivity rates for both RBT and BCAP were higher in the age group of 55-64 years [23]. In another study conducted by Kaya et al. determined the positivity rates of 74 (74.0%) RBT and 84 (84.0%) BCAP in the serum samples of 100 patients with suspected brucellosis.[24] In another study conducted by Karameşe and Acar, RBT and BCAP tests were applied to 107 serum samples sent by internal medicine, infectious diseases, and pediatrics clinics with a provisional diagnosis of brucellosis. According to the data obtained, the RBT test was positive in 96 (89.7%) of 107 patients, and the BCAP test was positive in 102 (95.3%) [25].

In a study examining the distribution of STA test positivity rates by months, it was seen that it increased in March, peaked in August, and decreased to the initial rates as of October. The difference between August and September, when the positivity rates are the highest, was found statistically significant. To interpret it seasonally; the increase that begins with spring peaks at the end of summer and the beginning of fall. In our study, brucellosis cases were evaluated in terms of seasonal changes; 10.7% in spring, 52.4% in summer, 20.9% in fall, and 16% in winter. Thus, this study, conducted in the same endemic region as ours, and our study have similarities in terms of seasonal positivity. It is hypothesized that these seasonal changes are due to the periods when livestock production is intensive and at the same time, more raw milk and dairy products are consumed.

5. Conclusion

Diagnosis of brucellosis is important in endemic areas. Therefore, correct detection methods should be preferred to make the correct diagnosis. In suspected cases, RBT can be considered an ideal detection method because it is easy to use, inexpensive, sensitive, and provides rapid results. On the other hand, it was concluded that the BCAP test would be suitable for diagnosis due to its ability to detect blocking antibodies and to detect high antibody titers.

Although significant success has been achieved with the national mobilization initiated in 1984 in our country to control and eradicate Brucellosis in humans and animals, the eradication of this disease can be made possible by comprehensive support of this mobilization. Especially in the rural areas of our country, it is not possible to have the animals controlled by veterinarians. To reduce the frequency of transmission of brucellosis in endemic regions, it is important to inform the people living there that they should not be consuming raw milk and dairy products as often as necessary.

Limitations of the Study

The main limitation of this study was its retrospective design. The relatively small sample size and lack of a multicenter study were also limiting factors. In addition, the inability to include blood culture results in the study was a limiting factor for our study.

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Conflict of Interests

The authors declare that there is no conflict of interest and no financial support was provided for the study.

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Author Contributions

Concept-EA; Design-YD; Supervision-EA, YD; Resource-YD; Materials-SK, AFG; Data Collection and/or Processing-SK, AFG; Analysis and/or Interpretation-EA; Literature Search-YD; Writing-YD; Critical Reviews-EA, YD.

Ethical Approval

Ethical approval for this study was granted by the Van Regional Training and Research Hospital Clinical Research Ethics Committee with decision number 2021/17 on 30 September 2021.

Data sharing statement

The material used in the study and without the permission of the authors.

Consent to participate

Not applicable.

Informed Consent

The authors accept their responsibilities in the study. There is no conflict of interest between the authors.

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The "Medical Cost" of Robot-Assisted Laparoscopic Surgery in Endometrial Cancer in Terms of Anesthesia Comparison with Traditional Laparoscopic Surgery

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Abstract: Minimally invasive surgeries have important advantages such as lower morbidity, shorter hospital stay and earlier return to routine life. In this study, we investigated the results of Robot-assisted laparoscopic surgery (RALS) and conventional laparoscopic surgery (TLS) in terms of anesthesia management and postoperative process in patients with endometrial cancer. Patients over 18 years of age with an American Society of Anesthesiologists (ASA) score II-III who were operated for endometrial cancer with TLS or RALS between January 2020 and March 2022 were included. Patients' age, ASA scores, duration of surgery and anesthesia, peroperative fluid management, urine output, bleeding, postoperative Visual Analogue Scale (VAS) scores, post-anesthesia care unit (PACU) hospitalizations and hospital stay were obtained from the standard anesthesia record form. A total of 75 patients, 44 patients in the RALS group and 31 patients in the TLS group, were included in the study. Peroperative intravenous fluid volume RALS average 1500 ml, TLS average 2450 ml ($p<0.0070$), bleeding amount RALS 80 ml and TLS average 350 ml ($p< 0.0001$), RALS group was statistically lower than TLS group. The mean hospital stay was shorter in the RALS group compared to the TLS group ($p<0.0070$). Our result support that RALS decreases the medical cost of surgical treatment of endometrial cancer patient, providing lower peroperative intravenous fluid, bleeding and urine output volumes and shorter mean hospital stay in RALS group. ©2023 NTMS.

Keywords: Anesthesia; Cancer of Endometrium; Laparoscopic Surgery; Postoperative Pain; Robotic-Assisted Surgery.

1. Introduction

Endometrial cancer, which is the most common gynecological malignancy, is the fourth most common cancer in women [1]. The standard treatment procedure is bilateral salpingo-oophorectomy and total hysterectomy [2]. The surgical approach to endometrial cancer has changed over the years. While open abdominal surgery used to performed in endometrial cancer treatment, currently TLS and RALS, as minimally invasive alternatives, are the choice of surgical treatment. Minimally invasive techniques are recommended instead of laparotomy in tumors confined to the uterus (Grade 1A) [3], which have important advantages such as lower morbidity, a shorter hospital stay and earlier return to routine life. TLS has been used in gynecologic oncology for a long time and there are a lot of reported experiences in the literature, but, nowadays RALS plays a leading role in the development of minimally invasive surgery because

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it provides better surgical comfort, higher-quality imaging, and more technical advantages. The most common uses of RALS are gynecological and urological surgeries [4]. Besides its advantages, the cost-effectiveness of RALS is controversial [5]. Although many publications compare RALS and TLS, current literature lacks a "medical cost" perspective regarding RALS and TLS in endometrial cancer. In this clinical study, to investigate "medical cost" of both procedures as we compared the results of anesthesia-related processes such as peroperative fluid management, transfusion requirement, analgesic strategies, postoperative complications, PACU admission ratio and hospital stay in endometrial cancer patients who operated with RALS and TLS.

2. Material and Methods

This retrospective observational study was conducted by the University of Health Sciences Bakırköy Dr Sadi Konuk Hospital and was approved by the Clinical Research Ethics Committee (Proctocol No: 2023/34). Between January 2020 and March 2022, patients over 18 years of age with an ASA score of II-III who were operated on by the TLS or RALS method for endometrial cancer in the Gynecological Oncology Clinic were included in the study. Patients with ASA IV, missing data, or who switched to open surgery were excluded. The patients age, ASA score, duration of surgery and anesthesia, peroperative fluid management, urine output, bleeding, postoperative Visual Analogue Scale (VAS) scores, and PACU application were obtained from the standard anesthesia record form, and the remaining data on preoperative and postoperative hemoglobin (Hb) values, length of hospital stay, and patients with advanced lymph node excision were obtained from the hospital's electronic database (Probel, İzmir, Turkey).

2.1. Anesthesia Management

Before the operation, patients fasted for at least 6 hours according to the anesthesia protocol of our clinic. Standard monitoring (blood pressure, electrocardiography, oxygen saturation and capnography) was applied to all patients before the surgical procedure. For continuous intra-arterial blood pressure monitoring, a catheter was placed in the radial artery using the Seldinger method after the Allen test. In both techniques, the patient's arms were fixed by closing them to the sides, and no venous intervention could be performed after positioning. For this reason, intravenous (iv) vascular access (18,16 G) was opened before the position and accesses were provided with extension lines. Intravenous crystalloid fluid infusion of 3-5 ml/kg/hr was started in the operating room and 250 crystalloid bolus infusions were initiated if mean arterial pressure decreased (<60 mm-Hg) and heart rate increased (>90/min) during surgery. Patients were given midazolam (1.5 mg) for premedication, paracetamol (1 g)+tramadol (100 mg) for preventive analgesia. For anesthesia induction, 1-2 mg/kg of propofol, 2 µg/kg of fentanyl, and 0.6 mg/kg of rocuronium were given. Sevoflurane (Minimum alveolar concentration 0.5-1) and remifentanil (0.05-0.5 µg/kg/min) were used for anesthesia maintenance. For mechanical ventilation, pressure regulated volume control (PRVC) mode was selected and 6-8 ml/kg tidal volume, 3-7 cm/H_2O Positive end-expiratory pressure (PEEP), fresh gas flow 1-4 lt/min, I: E=½, frequency 12-16 /min (End-tidal CO_2 between 35-45 mmHg) was set. Paracetamol (1 g)+tramadol (100 mg) was repeated for postoperative analgesia and 3-5 mg of morphine was given. Patients with intraoperative massive blood transfusion, unstable hemodynamics, vasoactive/inotropic drug administration, and respiratory distress were referred to the PACU, and patients with a modified Aldrete sedation score >9 after follow-up in the recovery room were referred to the service.

2.2. Surgical Procedure

The patients, who underwent detailed preoperative examination and imaging, were operated on by the same surgical team after the board's decision. The traditional two-dimensional laparoscopic surgical system (Richard WOLF, Knittlingen, Germany) was used for TLS and da Vinci Si (Intuitive Surgical, Sunnyvale, California, USA) system was used for RALS. In TLS, while the camera was placed from the navel, both lateral ports were placed 3 cm medial to the anterior superior spina iliaca. In RALS, the ports are placed in a single plane around the hub. First, a hysterectomy and bilateral oophorectomy were performed. Advanced pelvic and paraaortic lymph node dissection was decided by the surgical team, according to the intraoperative frozen pathology results.

2.3. Statistical Analysis

GraphPad $V_{5.0}$ (San Diego, California, USA) program was used. Homogeneity was evaluated with the Shapiro-Wilk test. Since the data of the study were not homogeneous, the two groups were compared with the Mann-Whitney U test. Categorical variables (number of patients admitted to PACU or undergoing advanced lymphatic dissection) were compared with the Chi-square test. Median and interquartile range (Q_{25-75}), numbers and percentages were used in statistical representation. $p<0.05$ was considered statistically significant. The length of hospital stay was determined as the primary outcome of the study. The median hospital stay (IQR) values of the laparoscopic and robotic groups were calculated as 7±4 and 4±4 days, respectively. According to these values, the power of the study was calculated at 80% (Gpower 3.1, Düsseldorf, Germany).

3. Results

A total of 75 patient data sets, 44 RALS and 31 TLS groups, were analyzed. The mean age of the patients in the RALS group was 59 years, 58 years in the TLS

group, and the mean ASA score was II in both groups. There was no statistical difference between groups regarding patient ages and ASA scores (p=0.7920, p=0.4140 respectivelly) (Table 1).

The mean preoperative hemoglobin level was 12.9 g/dL in RALS group and 12.4 g/dL in the TLS group. The mean operative time was 250 minutes in RALS group and 300 minutes in TLS group. The number of patients who underwent advanced lymph node dissection was 21 (47.7%) in RALS group and 9 (29.0%) in TLS group. The number of patients admitted to PACU was 8 (18.2%) in the RALS group and 6 (19.4%) in TLS group. The postoperative mean VAS score was 4 (3 - 4) in both groups. There were no statistical significant differences between the groups regarding the above mentioned parameters, preoperative Hb level, operative time, total anesthesia time, number of PACU admissions, and postoperative VAS scores (p=0.0820, p=0.6508, p=0.1651, p=0.8630, p=0.6283, p=0.6283, respectively) (Table 2).

Statistical analysis of peroperative intravenous fluid volume, bleeding volume and urine output volume, mean postoperative hemoglobin levels, mean hospital stay and mean postoperative Hb levels revealed significant differences between the groups. The mean peroperative crystalloid fluid volume was found to be significantly lower in RALS group than in TLS group (1500 ml and 2450 ml respectively, p<0.0001). The mean peroperative bleeding volume was also lower in RALS group than TLS group (80 ml and 350ml ml, respectively, p<0.0001). The mean volume of peroperative diuresis was 120 ml in RALS group and 200 ml in TLS group (p<0.0001). The mean hospital stay was 4.0 days for RALS groups and 7.0 days for TLS group (p:0.0070). The mean postoperative hemoglobin level was 11.3 g/dL in RALS and 10.5 g/dL in TLS (p:0.0019). Study findings of both groups are listed in Table 2. The median (Q_{25-75}), number of patients, percentages, and p values are shown in Tables 1 and 2.

Table 1: Demographic data of patients in both groups.

	Group TLS (n=31)	**Group RALS (n=44)**	**p Value**
	Median (Q_{25-75})	Median (Q_{25-75})	
AGE (year)	58 (47-68)	59 (50-66)	0.7920
ASA score	2 (2-3)	2 (2-3)	0.4140

TLS: Traditional Laparoscopic Surgery, RALS: Robot-Assisted Laparoscopic Surgery.

Table 2: Perioperative data of patients in both groups.

	Group TLS (n=31)	**Group RALS (n=44)**	**p Value**
	Median (Q_{25-75})	Median (Q_{25-75})	
Operation Time (min.)	300 (180-300)	250 (190-335)	0.6508
Anesthesia time (min)	310 (195-328)	290 (240-350)	0.3747
Length of stay in hospital (days)	7.0 (4.0-10.0)	4.0 (3.0-5.0)	0.0070
Peroperative crystalloid fluid (ml)	2450 (1650-2900)	1500 (1200-2000)	<0.0001
Peroperative bleeding (ml)	350 (200-500)	80 (50-100)	<0.0001
Peroperative diuresis (ml)	200 (150-320)	120 (100-150)	<0.0001
Preoperative Hemoglobin, gr/dL	12.4 (11.4-13.3)	12.9 (12.0-13.6)	0.0820
Postoperative hemoglobin, gr/dL	10.5 (9.9-11.2)	11.3 (10.8-12.0)	0.0019
PACU patient (%)	6 (19.4%)	8 (18.2%)	0.8630
Number of patients who underwent advanced lymph node dissection (%)	9 (29.0%)	21 (47.7%)	0.1651

TLS: Traditional Laparoscopic Surgery, RALS: Robot-Assisted Laparoscopic Surgery

The approximate instrument cost for a standard endometrial cancer case for RALS is around 25.113TL which includes 81.313TL/18 usage prograps forceps (4517 TL), 72.344 TL/15 usage large needle (4822 TL), 88.626/14 usage Maryland bipolar (6330 TL), Seal canula 472 TLx4 (1888 TL), blades obturator 655 TL, aim drape 1.364 x 4 (5456 TL), clinch 357 TL, grasper 431 TL, scissors 357 TL, dressing, 300 TL.

The approximate instrument cost for a standard endometrial cancer case for TLS is around 13.575 TL which includes 10 mm trocar 223 TLx1.5 mm trocar 223x3 TL (669 TL), veres needle 54 TL, camera cover (50 TL), and a laparoscopic ligasure 5mm-37cm. 9000 TL, grasper 431 TLx2 (862 TL), laparoscopic dissector 357 TL, scissors 357 TL, laparoscopic clinch 357 TL, Aspiration 168 TL, endoclip 10mm 582 TL, endoclip 5mm 819 TL, and dressing 300 TL.

4. Discussion

In this retrospective clinical study, anesthesia related clinical results of RALS and TLS in endometrial cancer patients. Our results revealed that RALS group has shorter operative time, a lower peroperative fluid requirement, a lower peroperative bleeding volume, a higher postoperative hemoglobin level, and a shorter hospital stay than the TLS group.

Laparoscopic surgery is the beginning of minimally invasive surgery and together with technological developments, RALS has become the choice of surgical treatment in many medical disciplines. Better 3D vision, a wider working area, better mobility and ergonomics for the surgeon, and not reflecting movements such as the surgeon's hand tremor to the patient could be listed as thecnological superiority and advantages of RALS over TLS. On the other hands, RALS has some disadvantages. It needs additional surgical training process. Possible longer operating room occupation and expensive robotic instruments may be costly than standart open surgery or laparoscopic surgery. However, it is necessary to think multidimensionally while making cost calculations. The comparison of these two methods should not only be about the surgical aspect and expenses, but the medical and social aspect. That is why our study focused on "medical cost of the procedures" in which we compared the results of anesthetisa aspects of RALS and TLS in endometrial cancer. Because endometrial cancer is one of the most common disease that both robotic and standart laparoscopic techniques are used for surgical teratment, these groups of patients were chosen as in our study.

We have studied a total of 75 patients' data, 44 patients were in the RALS group and 31 patients were in the TLS group. When we analyzed our results, we found important differences between both groups, and these differences may affect the "medical cost" of the procedures. One of our results is that the RALS group had significantly shorter hospital stays. This is a prominent component in cost calculations. Aside from hospital charges, a prolonged hospital stay increases the risk of nosocomial infections and reduces quality of life. Another discussion about the cost of the surgical procedure is the occupation time of the operating room. Preparing the patient and docking the robotic system often increases the overall operation time and operating room occupation. In our study, we could not find a statistical difference between the groups in terms of both the duration of surgery (mean 300 min vs 250 min. in TLS and RALS, respectively, p=0.6508) and total anesthesia time (mean 310 min vs 290 min in TLS and RALS respectively, p=0.3747). While it was not statistically significant, the shorter mean operative time in RALS is clinically important because we believe that a shorter operative time may reduce the complication rates and decrease the medical cost of the surgery.

In addition, RALS can be preferred in terms of patient satisfaction and comfort. In a study comparing robotic surgery and open surgery in terms of perioperative drug use and cost in endometrial cancer surgery by Agarwal et al. it has been shown that less replacement fluid is used in the RALS group, the duration of hospital stay is shortened, and the cost is accordingly lower [6]. Another important result of our study is that perioperative blood loss and the need for crystalloid fluid, blood, or blood product replacement were less in the RALS group. The studies comparing open surgery, TLS, and RALS in literature usually report less intraoperative blood loss, a shorter hospital stay, and a longer operation time in robotic surgery [6-8]. Chuan et al. attributed less blood loss during RALS to better stability of robotic instruments, which reduces hand tremor and potential damage, better 3D vision, and a larger visual field that facilitates detection of vascular and vital structures during surgery [9].

In a recent systematic review involving robot-assisted laparoscopic surgery versus conventional laparoscopic surgery in randomized controlled trials, it was concluded that despite its higher cost, RALS did not result in statistically improved treatment outcomes, except for lower blood loss. [10].

Our results showed a shorter Median (Q_{25-75}) operative time in the RALS group than TLS (250 vs 300 minutes respectively). Similar to our results, in a randomized study (n=99) for the staging of endometrial cancers, the operative time was found to be 139 minutes (86-197 minutes) in the RALS group and 170 minutes in the TLS group (126-259 min.) [11]. This result of our study is consistent with studies that found that RALS has a shorter operation time than TLS in endometrial cancer surgery [12, 13]. Anesthesia preparation time is excluded from this evaluation. In our study, we have compared both surgical time and total anesthesia time which includes preparation time as well. Unlike TLS, preparation time is longer in RALS because of need for special patient positioning, docking, and after docking, positional manupilations cannot be done during RALS. The deep Trendelenburg position given to the patient may cause difficulty in ventilation, increased intracranial pressure, edema formation around the conjunctiva, nasopharynx, and larynx, subcutaneous emphysema, and peripheral nerve injuries in both the lower and upper extremities. There was no difference between the anesthesia management performed with awareness of these risks and the number of patients referred to PACU in both groups. The total time (preparation and operation time) in robotic surgery may

be longer due to the initial insertion and final removal of the robot arms.

In a meta-analysis study covering the years 2000-2016, in line with our results, it was concluded that robotic surgery has advantages such as less blood loss, less need for transfusion and less hospital stay in 739 robotic and 815 laparoscopic endometrial cancer surgeries [14].

However, they shared in the same meta-analysis that the cost was too expensive to limit clinical application. One of our limitations is that we did not collect detailed data on financial calculations for each patient, but the approximate instrument cost of a standard case is more expensive for RALS than TLS (25,113 TL and 13,575 TL, respectively). In general, we would like to emphasize the risks of complications that may occur with antibiotic use, blood and blood product use, IV fluids, opioids, nonopoidal analgesics, antiemetics, and drug use, rather than making price comparisons on the basis of materials used. The protracted hospitalization and hospital stay should not be ignored.

Staging of endometrial carcinomas is performed surgically [15,16]. Pelvic and paraaortic lymph node evaluation is the main component of the surgical staging procedure for many gynecological malignancies, including endometrial and ovarian carcinoma [17,18]. The approach to lymph node evaluation is controversial, with options such as pelvic-aortic lymph node dissection and sentinel lymph node biopsy. In a study conducted by Japanese researchers, the life expectancy of patients who underwent pelvic and periaortic lymphadenectomy was longer than that of patients who underwent only pelvic lymphadenectomy [19]. In TLS and RALS approaches, the ports are different [20, 21]. In RALS, access to the paraaortic lymph nodes is easier, as the ports are usually located above the navel. In our study, lymph node dissection was performed on 48% and 29% of the patients in the RALS and TLS groups, respectively. However, this difference is not statistically significant. We attributed this result to the fact that the same team performed the surgery.

Postoperative pain is one of the reasons for preferring the surgical method. Reduction of pain facilitates postoperative mobilization, reduces atelectasis, and increases patient comfort. Since minimally invasive procedures were performed in both groups in our study, there was no difference between the groups in terms of postoperative VAS scores. Other publications in the literature also support that there is no difference between the two methods for postoperative pain and antiemetic use [21, 22].

Limitations of our study; retrospective evaluation of our patients, lack of detailed demographic data (BMI, additional features), absence of intraoperative hemodynamic data, blood gas and laboratory data, inability to re-measure postoperative VAS scores in different periods, lack of data such as material prices, cost of patients to the hospital in terms of cost evaluation.

5. Conclusion

In this retrospective study, we compared the results of both surgical techniques, and our results support the conclusion that RALS decreases the medical cost of surgical treatment for endometrial cancer patients by providing lower peroperative intravenous fluid, bleeding, and urine output volumes, higher postoperative mean hemoglobin values, and shorter mean hospital stay in RALS group. However, larger series and multicenter studies are needed.

Limitations of the Study

Retrospective evaluation of our patients, lack of detailed demographic data (BMI, additional features), absence of intraoperative hemodynamic data, blood gas and laboratory data, inability to re-measure postoperative VAS scores in different periods, lack of data such as material prices, cost of patients to hospital in terms of cost evaluation.

Future Insight

We believe that larger-scale prospective randomized studies will contribute more clearly to science on this subject.

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Conflict of Interests

There is no conflict of interest between the authors in this study.

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Author Contributions

Both authors mentioned in the study contributed to data collection, study planning, methodology and article writing.

Ethical Approval

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Informed Consent

Informed consent forms were obtained from all patients.

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Investigation of the Effect of Using a Metronome or Song on the Lay Rescuers' Quality of Chest Compressions

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Abstract: This study aimed to determine the quality of chest compressions performed by lay rescuers assisted by a healthcare worker over the telephone and investigate the effect of metronome use or the Stayin' Alive song on the quality of these chest compressions. This study was conducted prospectively at the emergency department of a tertiary hospital. The lay rescuers were assisted by an emergency medicine specialist over the telephone to perform chest compressions using the CPR Lilly PRO+ simulator. Three groups were formed, and the same participants performed three cycles of chest compressions over the telephone for two minutes: first without any external stimulus (Group 1), then using a metronome as an external stimulus (Group 2), and finally by listening to the Stayin' Alive song as another external stimulus (Group 3). The obtained data were analyzed with IBM SPSS v. 23.0. There was no statistically significant difference between the three groups in terms of the target number of chest compressions ($p=0.404$). However, the compression depth and chest compression fraction statistically significantly differed between the groups ($p<0.05$). If lay rescuers who have not received basic life support training in Turkey are assisted by healthcare workers over the telephone, they can achieve a sufficient number of chest compressions. However, considering the inadequate compression depth, chest compressions applied by lay rescuers with telephone assistance do not seem to be effective in our country. ©2023 NTMS.

Keywords: Metronome; Chest Compressions; Emergency Medicine; Stayin' Alive Song; Resuscitation.

1. Introduction

One of the most important factors affecting the return of spontaneous circulation in out-of-hospital cardiac arrests (OHCA) is early and effective chest compressions (CCs) [1]. However, in OHCA cases, bystanders are often hesitant to intervene with patients. In a previous study, it was shown that cardiopulmonary resuscitation (CPR) in patients with OHCA was performed by non-healthcare professionals or emergency rescue service personnel at a rate of approximately 49/100.000 in the European Union [2]. In order to increase these rates, many training programs are organized, during which methods for high-quality CCs are also practiced. One of the methods to achieve an appropriate number of CCs is the use of a metronome [3, 4]. This is an instrument that makes beats at regular intervals to provide a stable rhythm. When performing CCs, the operator's synchronization with the metronome makes it easier to reach the target number of compressions [5].

In the literature, there are also studies examining CCs accompanied by songs to achieve the targeted number of CCs [6-8]. The oldest and best-known song that has

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been the subject of many studies on reaching the target number of CCs is "Stayin' Alive" by the Bee Gees [8]. Various researchers have attempted to achieve the targeted number of CCs during CPR using the metronome, Stayin' Alive, and other songs [9-11]. However, to the best of our knowledge, in Turkey, no study has been conducted to investigate the harmony of lay rescuers with this song when performing CCs. Therefore, this study aimed to determine the quality of CCs performed by lay rescuers assisted by a healthcare worker over the telephone prior to the CPR process and compare the use of a metronome and the Stayin' Alive song in terms of their effects on the quality of these CCs.

2. Material and Methods

2.1. Study Design and Participant Selection

This study was conducted prospectively at a tertiary emergency department after obtaining approval from the local ethics committee (meeting no:10, decision no:18). The study was performed in accordance with the tenets of the Declaration of Helsinki. G∗Power 3.1 (Heinlich-Heine Universität, Düsseldorf, Germany) was used to determine the sample size [12]. The sample size was calculated at the 95% confidence interval, 80% power, α 0.05 error, and 0.39 effect size. Assuming a 10% loss, the number of participants required for the study was found to be 94.

As lay rescuers, volunteer non-healthcare professionals aged >18 years who had not received basic life support (BLS) or advanced cardiac life support (ACLS) training, had no speech or hearing problems, were able to understand and speak Turkish, and had no contraindications to performing CCs (upper extremity amputation-injury, history of ischemic heart disease, third trimester pregnancy, etc.) were included in the study. Excluded from the sample were those who had previously received BLS or ACLS training, healthcare professionals, and individuals who could not perform CCs for any reason.

2.2. Study Interventions

A temporary information station was set up to reach the participants by telephone. Before moving on to the area where CCs would be performed, the participants were informed about the study scenario, in which they would talk to an emergency medicine specialist with more than five years' professional experience and encounter a simulator simulating a patient with no pulse. They were asked to phone once they entered the simulation area to talk to the healthcare worker. It was explained that all the necessary information would be given during this telephone call and that there would be no further conversation.

During the simulation, once the telephone call was made, the emergency medicine specialist gave the participants a standard instruction on how to perform CCs: "depress the thorax 5-6 (2-2.4 inch) cm deep from the midpoint of the thorax at a rate of 100-120 per minute"[1]. With the phone left on speaker mode, the participants were asked to perform two minutes of CCs without any external stimulus. After a 15-minute rest, the simulation was repeated by giving the same instruction, which was, this time, followed by the use of a metronome (103 beats/min) without the participants' knowledge. After the participants performed CCs accompanied by the metronome for two minutes, they rested for 15 minutes, and the same procedure was repeated using the Stayin' Alive song by the Bee Gees as the external stimulus (103 beats/min).

2.3. Data Collection

The simulator used was CPR Lilly PRO+(3B Scientific GmbH Ludwig-Erhard-Straße 20. 20459 Hamburg. Germany), which was available in our clinic. This new high-quality CPR training manikin from 3B Scientific allows practitioners to measure, monitor, and analyze CPR performance. CPR Lilly PRO+ helps measure practitioner performance by connecting with the free CPR Lilly App on the tablet to which it is connected (Lenovo Tab M10 TB-X306F 4GB + 64GB 10.1" IPS Tablet ZA7W0007TR) to track CPR performance and provide objective feedback on high-quality CPR training practice. In the test mode of the CPR Lilly application, the number of CCs performed by the practitioner during CPR, the mean depth of CCs, chest recoil, correct or incorrect hand positions, and chest compression fraction (CCF) can be measured. We formed three study groups and obtained data on the telephone-assisted CCs performed by each practitioner over two minutes first without an external stimulus (Group 1, n=94), then using the metronome as an external stimulus (Group 2, n=94), and lastly using the Stayin' Alive song as an external stimulus (Group 3, n=94). We also recorded the age, sex, and educational status of the participants.

2.4. Statistical Analysis

Statistical analyses were performed using IBM SPSS v. 23.0 (IBM Corp., Armonk, NY, USA) software. The Shapiro-Wilk test was used to check the normality of data distribution. Categorical variables were expressed as frequency and percentage, and continuous variables as median and interquartile range. The Friedman test and Cochran's Q test were used for non-parametric data. Statistical significance was accepted as $p<0.05$.

3. Results

Ninety-four individuals [47 (50%) men] were included in the study. The median age was 25.0 years. When the educational status of the participants was examined, it was determined that 40.4% (n=38) were graduates of secondary schools (Table 1).

Table 2 presents the comparison of the CPR parameters of the participants according to the groups. Accordingly, there was a statistically significant difference between the groups in terms of compression depth and CCF ($p<0.05$) (Figure 1).

Table 1. Characteristics of the participants.

Variables (n = 94)	
Age, median (IQR)	25.0 (20.0-39.3)
Sex, male, n (%)	47 (50)
Education, n (%)	
Primary school	6 (6.4)
Secondary school	38 (40.4)
Associate degree	24 (25.5)
Undergraduate/post-graduate degree	26 (27.7)

IQR: interquartile range.

4. Discussion

In this study, with the over-the-phone instruction of a healthcare worker before CPR, lay rescuers were able to reach a sufficient compression rate (100-120/min) without any stimulus but could not achieve an adequate compression depth (2-2.4 inches). It was observed that the use of an external stimulus (metronome sound or the Stayin' Alive song) did not have an effect on the CCs rate. However, there was a change in compression depth with the use of external stimuli. The depth of compression did not significantly differ between Groups 1 and 2 and Groups 1 and 3. However, the compression depth of Group 3 was statistically significantly higher than that of Group 2. Regardless of the presence of an external stimulus, the pressures applied by the participants did not achieve a sufficient level of depth. Therefore, we consider that CPR assisted by a healthcare worker over the telephone is not applicable for lay rescuers in Turkey.

In OHCA cases, early CCs increase the chance of survival [13]. Therefore, bystanders are often expected to initiate CCs in individuals with OHCA [14]. Researchers have begun to discuss the compression abilities of lay rescuers in OHCA cases. In a study by Plata et al. On this subject, the compression rate of lay rescuers guided by telephone was found to be 82/min [15]. Ecker et al. on the other hand, observed that the lay rescuers applied an average of 74/min compressions per minute without being assisted, while the mean number of compressions increased to 99/min in the group assisted by telephone [16]. In the current study, when the lay rescuers were assisted by telephone, they applied an average of 100/min compressions, which is consistent with the target number of CCs reported in the literature.

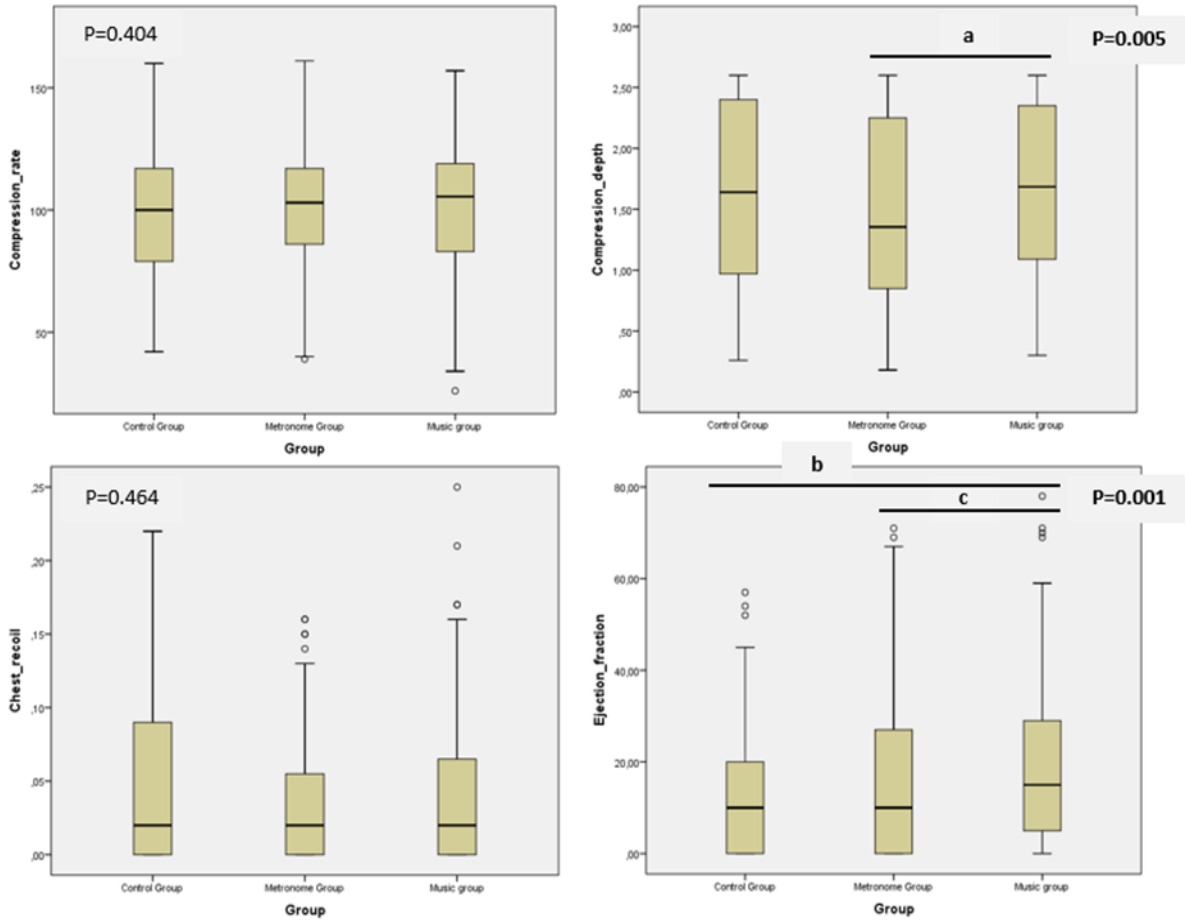

Figure 1: Box plot showing the cardiopulmonary resuscitation parameters by groups. a: 0.004, b: 0.002, c: 0.018.

Table 2: Comparison of the cardiopulmonary resuscitation parameters of the participants according to the study groups.

Variables	Group 1	Group 2	Group 3	P value	Post hoc
Compression rate, median (IQR)	100.0 (78.8-117.0)	103.0 (85.8-117.5)	105.5 (82.8-119.0)	0.404[a]	
Compression depth, median (IQR)	1.64 (0.97-2.41)	1.36 (0.85-2.26)	1.69 (1.08-2.36)	0.005[a]	Group 1 vs. 2: 0.160 Group 1 vs. 3: 0.606 Group 2 vs. 3: 0.004
Chest recoil, median (IQR)	0.03 (0-0.10)	0.02 (0-0.06)	0.04 (0-0.08)	0.464[a]	
CCF, median (IQR)	10.0 (0-20.0)	10.0 (0-27.3)	15.0 (5.0-29.0)	0.001[a]	Group 1 vs. 2: 0.871 Group 1 vs. 3: 0.002 Group 2 vs. 3: 0.018
Hand position, accurate, n (%)	43 (45.7)	38 (40.4)	44 (46.8)	0.412[b]	

Group 1: no external stimulus, Group 2: metronome, Group 3: Stayin' Alive song, IQR: interquartile range, CCF: chest compression fraction. [a] Friedman test, [b] Cochran's Q test.

In the literature, there are also studies in which verbal motivation, metronome use, and songs that can match the compression rhythm have been used to keep the number of CCs performed by lay rescuers within an effective range [17-19]. In some of these studies, it was observed that the use of a metronome or a song did not affect the average number of compressions per minute [11, 20]. Similarly, we determined that the use of a metronome or the Stayin' Alive song did not significantly affect the number of compressions per minute.

Concerning the depth of compressions performed by lay rescuers, Plata et al. determined the mean compression depth of the participants assisted by telephone to be 55 mm (2.16 inches). The authors noted that this compression depth was within the optimum range [17]. However, another study showed that telephone-assisted lay rescuers applied less pressure (1.6 inches) than necessary, and this was not adequate [21]. In our study, the lay rescuers assisted by telephone without an external stimulus could not obtain sufficient compression depth. Although the compressions accompanied by the Stayin' Alive were deeper than those performed without any external stimulus, the participants were still not able to achieve sufficient compression depth.

While performing effective CCs, the hands must be placed on the anterior chest wall appropriately. In the literature, lay rescuers assisted by video applications have been reported to be more successful in positioning their hands appropriately compared to those who were unassisted or only assisted by telephone [15, 22]. In our study, although the participants were assisted by telephone at all times, the highest rate of accurate hand positioning was 46.8%, regardless of the presence of an external stimulus. This confirms the necessity of including visual descriptors through video calls and drone assistance in BLS, as recommended by the relevant section of the European Resuscitation Council Guidelines 2021 [23].

An effective CCF can be achieved by applying CCs uninterruptedly at the right place and speed and with sufficient compression depth. For a good-quality CCs, it is recommended that the CCF value be 60% [24]. In our study, although the participants reached the appropriate number of CCs in all three cases, the CCF values were below this requirement due to the insufficient CCs depth and inaccurate hand positioning during CPR. However, CCF increased up to 15% in Group 3, probably in parallel to the increase in CCs depth. Despite this, the participants were still not able to achieve optimal CCF.

5. Conclusions

If lay rescuers who have not received basic life support training in Turkey are assisted by healthcare workers over the phone, they can perform a sufficient number of CCs. However, they cannot achieve optimal compression depth. In addition, the rate of accurate hand positioning during CPR is not high when only telephone guidance is provided. Therefore, it can be concluded that CCs applied by lay rescuers with telephone assistance do not seem to be effective in Turkey. Further studies should be conducted in which rescuers are assisted by visual methods. Based on the results of such studies, BLS training should be organized for lay rescuers.

Limitations of the Study

This study had certain limitations. First, it was carried out using a manikin. When faced with a real person who requires CCs, the reactions and practices of lay rescuers with no BLS training may change. Second, the participants were not informed that external stimuli would be applied during two sets of CPR simulations. Therefore, they may have focused only on the voice of the healthcare worker speaking on the phone. Another limitation can be considered as an English song being chosen as an external stimulus in Group 3, which may have created difficulties in adapting to a song that was not in the native language of the participants.

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Conflict of Interests

The authors declare no conflict of interest.

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None.

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Ethical approval was obtained from the Atatürk University, Faculty of Medicine clinical research ethics committee (Number: 10/18, Date: 19/12/2022).

Data sharing statement

Data and statistical analysis plan will be shared if requested.

Consent to participate

Consent was obtained from all patients for the use of data under ethical conditions.

Informed Statement

Written informed consent was obtained from all of the participants..

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Evaluation of Knowledge and Attitudes Concerning Adult Immunization in University Students Enrolled in Health-Related Departments: A Cross-Sectional Study

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Abstract: Immunization is the most effective and reliable method of protection from infectious diseases in both children and adults. This research aims at evaluating the knowledge and attitudes concerning adult immunization of students in health-related fields. A descriptive cross-sectional study was conducted among all final-year students studying health at the Erzincan Binali Yıldırım University using a questionnaire including sociodemographic data as well as knowledge and attitudes about adult vaccination. The statistical data were analyzed by number and percentage using Statistical Package for the Social Sciences, version 21.0. A total of 379 students took part in the study, and 19% of the students reported having sufficient knowledge about adult immunization. It was observed that for the participants, among the vaccines administered in adulthood, the hepatitis B and the meningococcus vaccines were the most and least familiar, respectively. When asked about knowledge and attitudes concerning vaccines administered in adulthood, the respondents proved uninformed about many vaccination practices. It was observed that the students participating in the study did not have sufficient knowledge and sensitivity about adult vaccination. In order to increase adult immunization rates, it would be appropriate to eliminate the deficiencies in this subject in pre-graduate education.

Keywords: Adult Immunization; Attitude; Knowledge; University Students.

1. Introduction

Immunization is one of the most effective and safest health services provided to prevent and protect individuals against infectious diseases through the artificial stimulation of their immune systems [1]. Life expectancy at birth and the percentage of the elderly in the population have been steadily increasing worldwide owing to the efforts to combat infectious diseases alongside improved environmental conditions and protective health services [2, 3].

Although adulthood is the healthiest period of life, the risks of infectious diseases persist. The protection of childhood vaccines in adult and old age does not last a lifetime, and the immunity provided by primary immunization decreases with age. Infections tend to be more severe with age, and the susceptibility of people who were not fully immunized in childhood to infectious diseases increases. Therefore, there is a need to continue providing immunization services for both adults and children [1].

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Students enrolled in health-related departments at universities are expected to play an active role in the health services of the future. As they are the group that require, apply, and recommend immunization in the community, their knowledge of and attitude toward adult immunization are particularly important. However, in many studies conducted with health department students, it has been observed that the lack of knowledge and practice about adult immunization and the education they receive before graduation is insufficient [4, 5].

Health workers have a considerably higher risk of exposure to vaccine-preventable diseases and a higher risk of infecting their patients; thus, shaping their attitudes toward vaccination is critical [6, 7].

This research is aimed at evaluating the knowledge and attitude concerning adult immunization among the students in health-related fields at Erzincan Binali Yıldırım University, Turkey.

2. Material and Methods

This descriptive study involved students enrolled in the health-related departments at Erzincan Binali Yıldırım University. All students were informed about the research and consented to take part in it. Thus, all final-year students in the Medicine, Pharmacy, and Health Sciences Faculties and Health Sciences Vocational School were included in the study. After obtaining the necessary permissions, a questionnaire was applied to the students between November and December 2019 by using the face-to-face interview technique. The questionnaire response time was approximately 20 minutes.

There is no validated scale that measures immunization knowledge level and attitude in adults. The questionnaire form, which is the data collection tool used in the research, was prepared by the researchers by searching the relevant literature [4, 5]. In the questionnaire used, there are 28 questions in total that determine sociodemographic information (age, gender, faculty), general information about vaccines, attitudes and behaviors. In the knowledge and perception section about vaccines, the participants were asked about known vaccines related to adult vaccination (flu, hepatitis B, hepatitis A, tetanus, human papilloma virus, pneumococcus, measles-mumps-rubella, meningococcus, varicella, herpes zoster vaccines),whether the vaccines useful in protection against disease, whether immunization is necessary for adults, whether there is an up-to-date vaccination guide for adults, and whether they find their knowledge about vaccination sufficient. In the attitudes towards vaccination section, it was asked which of the vaccines included in adult immunization they had/would have, would have performed on their family, or would recommend for their patients. Finally, 16 questions related to knowledge and attitude prepared by evaluating the previous studies and the literature were asked.

Approval for the study was granted by the Erzincan Binali Yıldırım University Human Research Ethical Committee (07.11.2019-11/13). The obtained data were analyzed using SPSS 21, expressed as numbers and percentages.

3. Results

A total of 379 students took part in the study. The students' mean age was 21.8±1.8 years (18-37), and 64.4% were women.

The students' knowledge and attitudes concerning adult immunization are shown in Table 1. The analysis revealed that 96.3% of the students regarded vaccination as useful for protection against diseases, and 93.9% described adult immunization as necessary; 17.2% of the participants acknowledged the existence of adult immunization guidelines, and 19% of the students confirmed possessing sufficient knowledge on adult immunization.

When asked which of the vaccines administered in adulthood they were familiar with, 241 participants (65.6%) reported awareness of the flu vaccine, 323 (85.2%) mentioned hepatitis B, 218 (57.5%) hepatitis A, 211 (55.7%) tetanus, 209 (55.1%) human papilloma virus (HPV), 114 (30.1%) pneumococcus, 108 (28.5%) measles-mumps-rubella (MMR), 72 (19%) meningococcus, 82 (21.6%) varicella, and 91 (24%) herpes zoster vaccines (Figure 1).

The students' attitudes and behaviors toward adult immunization are summarized in Table 2. When the students were asked about the vaccines they had or will do, it was seen that hepatitis B was 78% and tetanus was 77%. When the vaccines recommended by the students for their patients were examined, 68% were recommended for hepatitis B and 66% for tetanus.

Table 3 reflects the students' knowledge and attitudes concerning adult immunization. Based on the results, approximately 40% of the students described annual seasonal flu vaccination as necessary; the respondents' knowledge of HPV and pneumococcus vaccinations proved inadequate; approximately 60% of students stated that tetanus vaccination should be performed once every ten years; 50% of the students were unsure about recommending HPV vaccination for individuals aged over 60; and 89.7% of the students stated that it was necessary to inquire about the immunization status of individuals in risk groups and pregnant women.

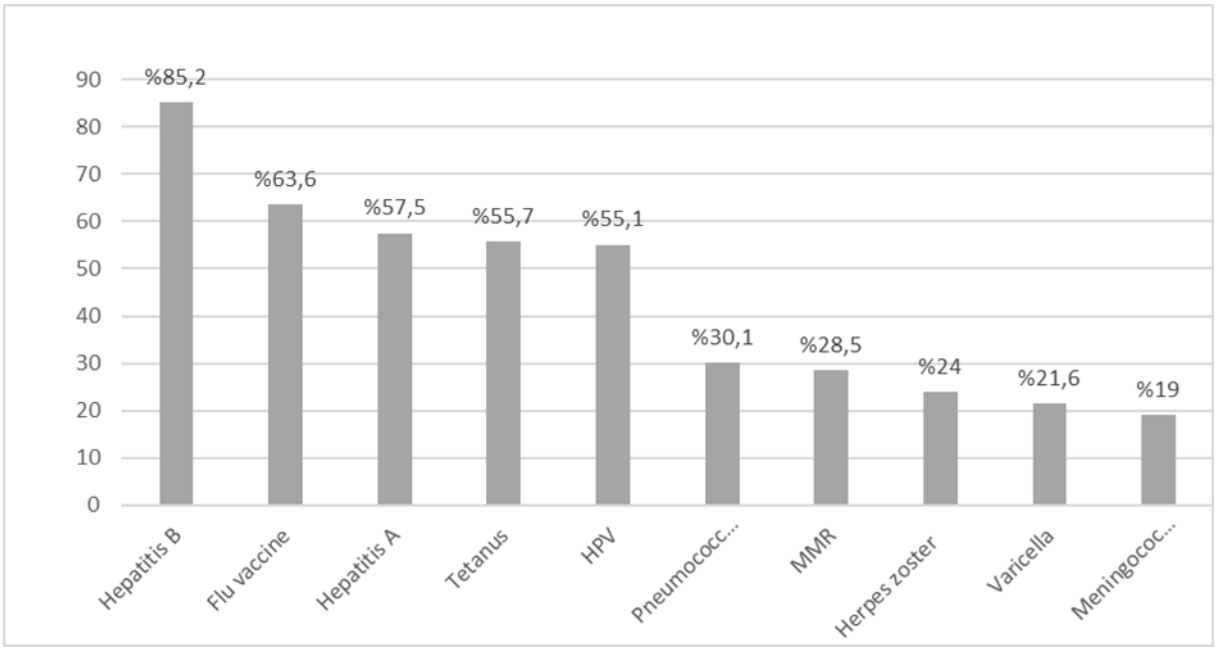

Figure 1: Distribution of known vaccines.
More than one answer was given; the percentages were calculated based on the number of responses.

Table 1: Students' knowledge and perceptions of adult immunization.

	Yes (n/%)	No (n/%)	Unsure (n/%)
Are vaccines useful in protection against disease?	365(96.3)	3(0.8)	11(2.9)
Is immunization necessary for adults?	356(93.9)	9(2.4)	14(3.7)
Is there a guideline for adult immunization?	65(17.2)	45(11.9)	269(71.0)
Do you possess sufficient knowledge concerning adult immunization?	72(19)	233(61.5)	74(19.5)

Table 2: Students' attitudes and behaviors concerning adult immunization.

Vaccines	I have had it/I would have it (%)	I would have it performed on my family (%)	I would recommend it for my patients (%)
Influenza	52	41.4	59.9
Hepatitis B	78.9	60.9	68.1
Pneumococcus	36.1	33.5	52
Tetanus	77.8	57.8	66.2
HPV	43	42	59.6

More than one answer was given to the question; the percentages were calculated based on the number of responses.

4. Discussion

Immunization continues to be a health service provided worldwide, primarily in childhood, and no sufficient importance is attached to adult immunization, which is defined as a continuation of immunization schedules [8]. This evaluation of the knowledge, attitudes, and practices concerning adult immunization among health students produced several significant findings. Although the students described immunization as necessary and beneficial, 61% regarded their knowledge of adult immunization as insufficient. Bolatkaya et al. reported that 63.7% of the participants considered their knowledge of adult vaccines deficient [8]. Similarly, Aksakal et al. reported that 62.5% of the participants acknowledged a lack of sufficient information on adult vaccines [9].

The students participating in this study were asked about vaccines administered in adulthood, and not even every third student was aware of pneumococcus, MMR, zona, varicella, and zona meningococcal vaccinations. Existing studies report low awareness rates concerning the application of these vaccines in adulthood [9-11]. When the students' attitudes and behaviors concerning adult immunization were examined, the most prominent vaccine was the vaccine against pneumococcus. Although half of the students reported that they would recommend this vaccine to their patients, less than one-third stated that they would have it administered to themselves and their families.

Table 3: Students' knowledge and attitudes concerning adult immunization.

	I agree	No idea	I disagree
	N/%	N/%	N/%
Vaccination should only be administered to particular individuals on the basis of specific factors such as age, occupation, disease etc.	169 (44.6)	33 (8.7)	177 (46.7)
Adult immunization is a means of protection	356 (93.9)	20 (5.3)	3 (0.8)
A vaccination program must be established for adulthood, similarly to childhood	324 (85.5)	42 (11.1)	13 (3.4)
It is important for all adults to be fully immunized	334 (88.1)	30 (7.9)	15 (4.0)
Vaccination is more important in children than in adults	274 (72.3)	55 (14.5)	50 (13.2)
It is important for immunization rates to be increased	307 (81)	51 (13.5)	21 (5.5)
Seasonal flu vaccination must be performed every year	147 (38.8)	109 (28.8)	123 (32.5)
HPV vaccination performed on girls aged 9-13 is the most cost-effect means of preventing cervical cancer	95 (25.1)	260 (68.6)	24 (6.3)
HPV vaccination can also be performed on boys	77 (20.3)	253 (66.8)	49 (12.9)
For adult immunization, a Td booster is recommended every 10 years, with at least one dose of tetanus	218 (57.5)	135 (35.6)	26 (6.9)
Pneumococcus vaccination must be administered in case of a splenia and hematological disease	116 (30.6)	238 (62.8)	25 (6.6)
Adults must be immunized against hepatitis B infection	336 (88.7)	32 (8.4)	11 (2.9)
Herpes zoster (Zona) immunization must be recommended to all individuals aged over 60 and with chronic disease	164 (43.3)	196 (51.7)	19 (5.0)
The immunization status of individuals aged 65 or over must be investigated	261 (68.9)	100 (26.4)	18 (4.7)
The immunization status of individuals in the risk group (occupation, disease) must be investigated	361 (95.3)	14 (3.7)	4 (1.1)
The immunization status of pregnant women must be investigated	340 (89.7)	33 (8.7)	6 (1.6)

The rates for the HPV and flu vaccines' administration to the students themselves and their families were also low. Although the US Advisory Committee on Immunization Practices recommends flu vaccination to reduce the spread of influenza among US health workers, the scale of flu vaccination among health workers is less than 50% [12]. In a study conducted with family practitioners, Baykan et al. reported low rates of practitioners having HPV vaccines administered to themselves or recommending them to their families [13]. In a study of health workers in South Korea, Yoon et al.[14] reported the pneumococcus vaccine administration rate of 1%. In contrast, in a survey study from Ankara, Turkey, Çiftçi et al.[15] reported a pneumococcus vaccination rate of 3.4% among health workers.

Another significant finding from the present study was that the students appeared uninformed about certain vaccination practices. For instance, only 40% of the surveyed group knew that seasonal flu vaccination should be performed every year. Only one in four students knew that HPV vaccination in girls is effective for preventing cervical cancer, and one in five stated that it can also be administered to boys. Fewer than half of the students stated that the Pneumococcal vaccine should be administered to various groups and that the zona vaccine should be recommended to individuals over 60. In the health-worker study conducted by Karacaer et al., 58.4% of participants stated that the seasonal flu vaccination did not have to be repeated every year [16]. Similarly, in Taştan's thesis study involving medical faculty students, approximately half of the participants stated that seasonal flu vaccination should be performed annually, and, in contrast, one in four stated that HPV vaccination was effective against cervical cancer in girls and boys [17].

5. Conclusions

The students involved in this study lacked a sufficient level of knowledge and sensitivity about adult immunization. More attention must be given to adult immunization alongside childhood immunization to improve protective health services and general health in Turkey by educating health personnel on adulthood vaccination, which is a highly effective method of raising immunization rates. Remedying this lack of

awareness before students' graduation can increase adult immunization rates in the public. In particular, the adult immunization schedule, currently available in guideline form, should be implemented in the same manner as childhood immunization and should be included in health education programs.

Limitations of the Study

Our study has some limitations. First of all, the research was conducted with the final year students of the faculties stated in the study, which does not represent all health-related departments' students. The questionnaire form, which is the data collection tool used in the research, was prepared by the researchers after searching the literature. It is not a scale whose validity and reliability have been proven.

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Conflict of Interests

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Author Contributions

Design of the study: SS. Data collection: SS, DK. Data analysis and interpretation: SS, DK. Literature search and writing the article: SS, DK. Critical revision of the article: SS. Final approval of the version to be published: SS, DK.

Ethical Approval

Ethical permission for the study has been obtained Erzincan Binali Yıldırım University Human Research Ethical Committee. (Date: 07.11.2019 Number: 11/13). The research was conducted in accordance with the principles of the Helsinki Declaration.

Data sharing statement

None.

Consent to participate

Informed consent was obtained from all participants.

Informed Statement

Informed consent was obtained from all the participitions.

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Frequency of Urinary Tract Infection and its Relationship with Disease Severity in Patients with Behçet's Disease

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Abstract: Although the importance of infections in the etiopathogenesis of Behçet's Disease (BD) has previously been reported, there are no studies in the literature concerning the frequency of urinary tract infections (UTIs) in the disease. The aim of this study was to investigate the frequency of UTIs and their association with disease severity in patients with BD. One hundred thirteen patients with BD were included in this retrospective cross-sectional study. Their files were reviewed and their symptoms on the date of admission and total urine analysis and urine culture results on that date were recorded. The frequency of UTIs and their relationship with disease severity were examined. One hundred thirteen patients with a median age of 38 (IQR: 29-47), 74.3% (n=84) of whom were women, were evaluated in the study. UTI was detected in 8.8% (n=10) of the patients. *Escherichia coli* (E. coli) was identified as the causative microorganism in 90% (n=9) and *Klebsiella spp.* in 10% (n=1) of the patients with UTIs. BD patients with UTIs were older, and UTIs were more common in those with longer disease durations (p=0.001 and p=0.005, respectively). No statistically significant relationship was detected between the severity of BD and the presence of UTIs (p>0.05). Dysuria and pyuria were detected more often in BD patients with positive pathergy test results and no UTIs (p=0.007 and p=0.038, respectively). Leukocyte esterase positivity was detected more frequently in BD patients with no urinary infections but with genital ulcers (p=0.039). Urinary system infection was detected in 8.8% (n=10) of the BD patients. Although no relationship was found between the severity of the disease and urinary system infection in the present study, we think that patients' complaints and culture results should be considered before administering treatment. ©2023 NTMS.

Keywords: Etiopathogenesis; Behçet's Disease; Urinary Tract Infection; Disease Severity; Microorganism.

1. Introduction

Behçet's disease (BD) is a chronic multisystemic vasculitis involving periods of exacerbation. It can affect the vascular, ocular, mucocutaneous, articular, gastrointestinal, and neurological systems [1]. Although BD is most commonly seen along the region of the Silk Road, extending from the Mediterranean region and the Middle East to Central and Eastern Asia, it can also be seen worldwide due to migration [2]. The etiopathogenesis involves inflammation triggered by environmental or infectious causes among genetically

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predisposed individuals. Inflammation occurs in the vascular endothelium.[3] The triggering effects of microorganisms in the development of BD have long been the subject of discussion. The first researcher to suggest that the condition might be associated with an infectious etiology was Professor Hulusi Behçet [4]. Some viral agents are known to be capable of causing UTIs However, no conclusive evidence has been found of a relationship with *Herpes simplex virus (HSV-1)*, *Cytomegalovirus*, *Epstein-Barr virus*, or *Hepatitis viruses*. Rather than active infection by the virus, inflammation has been implicated because of the altered immune response to the virus in BD. Researchers have also claimed that the cross-reaction between the heat shock proteins of some streptococcal species and human heat shock proteins may trigger an immune response in genetically predisposed patients. Toll-like receptor (TLR) can be stimulated after this cross-reaction, and T-cell expression increases [5]. Both adaptive and innate immune responses may play a role in the pathogenesis of BD. Microorganism lipopolysaccharides cause an increase in proinflammatory cytokines by stimulating the autoinflammatory response and thus interleukin-1β (IL-1β) synthesis through inflammasomes and TLR [6]. Previous studies have reported increased expression of pro–inflammatory cytokines, such as IL-1α, IL-1β, IL-6, IL-8, and tumor necrotic factor (TNF), in BD. However, low levels of IL-10, an anti–inflammatory cytokine, have been observed [7, 8]. While IL-23 increases the release of IL-17 from T-cells, IL-12 and interferon-γ (IFN-γ) cause a T-cell response in the Th1 direction. The release of IL-17 causes neutrophil accumulation in the affected organs in BD [9]. Research recently revealed that immunity against neurofilament-medium (NF-M) develops in patients with BD and that this and bacterial heat shock protein-65 (HSP) contain common epitopes [10]. Although numerous studies have investigated the pathogenesis of BD while focusing on the roles of microorganisms, none have addressed the frequency of urinary tract infections (UTIs) in BD and their effect on disease severity. The purpose of the current study was therefore to investigate the frequency of urinary infections in BD patients and their relationship with BD severity.

2. Material and Methods

2.1. Study Design

This research was designed as a retrospective, single-center, cross-sectional study.

2.2. Ethical Approval

The study was carried out in line with the principles of the Declaration of Helsinki after receipt of approval from the local ethics committee (No 5, Dated 29.12.2022).

2.3. Setting

The study was conducted between February 2020 and December 2022 among patients diagnosed with BD presenting to the dermatology clinic of our tertiary university hospital, which serves approximately 4.5 million people in Eastern Türkiye.

2.4. Participants and Study Protocol

One hundred thirteen active and inactive BD patients aged 18-70 who presented to the BD clinic between February 2020 and December 2022, were included in the study. Patients previously diagnosed with BD but with no complaints during routine control visits were considered inactive. Diagnosis of BD was based on international BD diagnostic criteria. The patients' demographic characteristics of the patients were recorded from patient registration forms. The patients' files were reviewed retrospectively, and their symptoms on the date of admission and total urinalysis and urine culture results were recorded. Our hospital's laboratory values were used as reference values for the urine laboratory tests. Analyses were carried out on automatic modular urine analyzers (model numbers H-800 and FUS-200, Dirui Industry, Changchun, China). Urinary system infection was diagnosed based on lower urinary system symptoms such as burning while urinating, increased frequency of urination, and feeling an urgency to urinate (dysuria, urgency, and frequency) as well as a leukocyte value of >10 cells/ml in urine culture and a causative microorganism value $>10^5$ colony-forming units/milliliter (cfu/ml). Patients with no growth in urine despite complaints of urinary system infection were not regarded as having UTI. Disease severity of BD was determined using Krause's BD clinical severity scoring system. Accordingly, one point was given for each mild symptom (oral or genital aphthous ulcer, arthralgia, erythema nodosum, papulopustular lesion, or folliculitis), two for each moderate symptom (arthritis, anterior uveitis, deep vein thrombosis in the legs, or gastrointestinal involvement), and three for each severe symptom (arterial thrombosis, retinal vasculitis, posterior uveitis/panuveitis, neuro-Behçet's, and bowel perforation). Once the total scores had been calculated, the patients were divided into three groups - mild (scores <4), moderate (4-6), and severe (≥7) based on the determined disease severity. Patients under 18 or who provided histories of another infection on the patient registration form, and pregnant and breastfeeding women were excluded from the study.

2.5. Statistical Analysis

All the study data were entered onto SPSS version 23 for Windows software (IBM, Chicago, IL, USA) for analysis. Categorical descriptive data were presented as frequency distribution and percentage, and continuous variables as median plus interquartile range. Chi–square and Fisher's Exact tests were used to compare categorical data between the groups, The non-parametric Mann–Whitney U and Kruskal-Wallis tests were used in the comparison of continuous data since the parametric hypothesis test conditions were not met. p values <0.05 were regarded as statistically significant.

3. Results

One hundred thirteen patients with BD, with a median age of 38 (IQR: 29-47), 74.3% (n=84) of whom women, were evaluated. UTI was detected in 8.8% (n=10) of the patients. *E. coli* was detected as the causative microorganism in 90% (n=9) of the patients and *Klebsiella spp.* in 10% (n=1). The distribution of the patient characteristics according to the development of urinary system infection is shown in Table 1.

Table 1: The distribution of patients' demographic and clinical characteristics of BD according to the presence of UTI.

Variables	No (n=13)	Yes (n=10)	P
Age, years, median (IQR)	36 (27-45)	49 (42-58)	0.001
Gender, female, n (%)	76 (73.8)	8 (80.0)	0.501
Presence of additional underlying disease, n (%)	18 (17.5)	4 (40.0)	0.102
Duration of complaints, months, median (IQR)	72 (36-144)	180 (84-315)	0.005
Age at disease onset, median (IQR)	28 (21-36)	34 (26-40)	0.125
BD organ involvement, n (%)			
Oral aphtha	73 (70.9)	6 (60.0)	0.349
Genital ulcer	22 (21.4)	1 (10.0)	0.354
Papulopustular lesion	54 (52.4)	7 (70.0)	0.234
Erythema nodosum	24 (23.3)	-	0.082
Vascular involvement	12 (11.7)	1 (10.0)	0.677
Superficial thrombophlebitis	5 (4.9)	-	
Deep vein thrombosis	4 (3.9)	-	
CNS vascular thrombosis	3 (2.9)	1 (10.0)	
Pathergy positivity	39 (37.9)	2 (20.0)	0.223
Joint signs	48 (46.6)	5 (50.0)	0.837
Arthralgia	39 (37.9)	5 (50.0)	
Arthritis	9 (8.7)	-	
Eye signs	29 (28.2)	4 (40.0)	0.325
Active	17 (16.5)	2 (20.0)	
Inactive	13 (12.6)	2 (20.0)	
CNS involvement	6 (5.8)	1 (10.0)	0.487
Disease activity, active	88 (85.4)	7 (70.0)	0.197
Disease severity index, median (IQR)			0.209
Inactive	14 (13.6)	3 (30.0)	
Mild	57 (55.3)	3 (30.0)	
Moderate	24 (23.3)	4 (40.0)	
Severe	8 (7.8)		
Number of skin and mucosal signs, median (IQR)	2 (1-3)	1,5 (0.75-2.25)	0.229
Treatment modality used, n (%)			
Drug use	97 (94.2)	9 (90.0)	0.487
Corticosteroid	2 (1.9)	-	0.830
Colchicine	95 (92.2)	9 (90.0)	0.580
Other immunosuppressive therapy	26 (25.2)	3 (30.0)	0.499
Biological agent	10 (9.7)	1 (10.0)	0.657

CNS: Central Nervous System, IQR: Interquartile range

Patients with UTIs were significantly older, and the duration of their BD complaints was significantly longer (p=0.001, and p=0.005, respectively). No significan relationship was observed between the presence of urinary system infection and the severity of BD (p>0.05). The symptom variations and laboratory findings according to the presence of UTI in patients with BD are shown in Table 2.

As anticipated, dysuria, frequency of urination, urinary leukocytes, leukocyte esterase, and nitrite positivity were significantly higher in patients with UTIs (Table 2). Leukocyte and erythrocyte counts in urine were also significantly higher in the presence of UTI (p<0.001). Relationships between the symptoms of BD in patients without diagnoses of UTI but with clinical findings of urinary system infection are shown in Table 3.

Table 2: The distribution of symptoms and laboratory findings according to the presence of UTI in BD patients.

Symptoms/n (%)	None (n=13)	Yes (n=10)	p
Dysuria	16 (15.5)	10 (100)	<0.001
Frequency	2 (1.9)	4 (40.0)	<0.001
Urgency	-	1 (10.0)	0.088
Laboratory parameters			
Median (IQR)			
Leukocyte count	7.12 (5.98-8.79)	7.21 (5.86-8.35)	0.976
Hemoglobin	14.0 (13.2-14.8)	13.0 (12.0-14.9)	0.195
Platelet count	281 (238-332)	273 (236-396)	0.746
Sedimentation	7 (5-15)	7.5 (6-25)	0.245
C-reactive protein	2.1 (1.1-5.4)	1.8 (1.0-5.1)	0.606
Creatine	0.63 (0.54-0.74)	0.6 (0.56-0.73)	0.980
Complete urinalysis			
Urine density, median (IQR)	1017 (1012-1023)	1017 (1012-1020)	0.812
Urine pH, median (IQR)	6.0 (5.5-6.0)	6.0 (5.5-6.0)	0.422
Leukocyte esterase positivity, n (%)	11 (10.7)	9 (90.0)	<0.001
Presence of protein, n (%)	3 (2.9)	1 (10.0)	0.131
Nitrite positivity, n (%)	-	2 (20.0)	0.007
Leukocyte positivity, n (%)	21 (20.4)	10 (100)	<0.001
Erythrocyte positivity, n (%)	33 (32.0)	5 (50.0)	0.210
Urine leukocyte count, median (IQR)	1 (1-2)	41 (14-123)	<0.001
Urine erythrocyte count, median (IQR)	2 (1-4)	24 (9-29)	<0.001

Table 3: The distribution of BD involvement according to clinical findings of urinary system infections in patients without such infections.

	Dysuria			**Frequency**		
BD organ involvement, n (%)	No (n=87)	Yes (n=16)	p	No (n=101)	Yes (n=2)	p
Oral aphtha	61 (70.1)	12 (75.0)	0.474	72 (71.3)	1 (50.0)	0.500
Genital ulcer	18 (20.7)	4 (25.0)	0.460	80 (79.2)	1 (50.0)	0.383
Papulopustular lesion	48 (55.2)	6 (37.5)	0.152	48 (475)	1 (50.0)	0.728
Erythema nodosum	20 (23.0)	4 (25.0)	0.542	78 (77.2)	1 (50.0)	0.413
Vascular involvement	10 (11.5)	2 (12.5)	0.591	89 (88.1)	2 (100)	0.780
Pathergy positivity	28 (32.2)	11 (68.8)	0.007	63 (62.4)	1 (50.0)	0.616
Joint signs	42 (48.3)	6 (37.5)	0.303	48 (47.5)	-	0.283
Eye signs	24 (27.6)	5 (31.3)	0.489	29 (28.7)	-	0.514
CNS involvement	4 (4.6)	2 (12.5)	0.233	6 (5.9)	-	0.886

Pathergy test positivity was significantly more frequent in patients with dysuria (p=0.007). Relationships between the symptoms of BD patients without diagnoses of urinary system infection and total urinalysis results are shown in Table 4. Urinary leukocyte esterase positivity and pathergy positivity were significantly in patients with BD with genital ulcers (p=0.039 and p=0.038, respectively).

Table 4: The distribution of BD involvement according to urinary system infection laboratory findings in patients without such infections.

	Leukocyte positivity in urine			Leukocyte esterase positivity in urine		
Behçet's disease organ involvement, n (%)	No (n=82)	Yes (n=21)	p	No (n=92)	Yes (n=11)	p
Oral aphtha	57 (69.5)	16 (76.2)	0.378	64 (69.6)	9 (81.8)	0.323
Genital ulcer	17 (20.7)	5 (23.8)	0.483	17 (18.5)	5 (45.5)	0.039
Papulopustular lesion	45 (54.9)	9 (42.9)	0.230	48 (52.2)	6 (54.5)	0.569
Erythema nodosum	20 (24.4)	4 (19.0)	0.422	20 (21.7)	4 (36.4)	0.232
Vascular involvement	10 (12.2)	2 (9.5)	0.540	10 (10.9)	2 (18.2)	0.376
Pathergy positivity	27 (32.9)	12 (57.1)	0.038	34 (37.0)	5 (45.5)	0.405
Joint signs	40 (48.8)	8 (38.1)	0.265	42 (45.7)	6 (54.5)	0.576
Eye signs	23 (28.0)	6 (28.6)	0.579	26 (28.3)	3 (27.3)	0.626

4. Discussion

Although many studies have investigated antigenic stimuli related to microorganisms in the etiopathogenesis of BD, none have specifically addressed the association between UTI frequency and the disease severity. In the light of the relationship with infectious etiology, this study was conducted to investigate the frequency of UTI and its effect on disease severity in these patients. Although no specific microorganism has been identified in the etiology of BD to date, studies have suggested that microorganisms may play an indirect triggering role in BD due to impaired immune system function. Microbiological studies have generally involved oral flora, ulcers, and skin lesions. *E. coli, Mycobacteria, Staphylococcus aureus, Borrelia burgdorferi, Streptococcal antigens, Helicobacter pylori, Mycoplasma fermentans,* and *Saccharomyces cerevisiae* have been described as potential triggering bacteria in the etiology of BD [11].

One of the studies of the possible association between BD and microorganisms involved pustular lesions. Although these have been were considered sterile in previous BD studies, subsequent research reported that *S. aereus* reproduced in 58% of these lesions [12]. A previous study reported that the occurrence of uveitis in patients after hypersensitivity testing with streptococcal antigens suggested that streptococci may be involved in the etiology.[13]Intradermal antigen tests applied with *E. coli, Pseudomonas aeruginosa,* and *Proteus vulgaris* revealed mild exacerbation in BD in another study [14]. BD has also been reported after *Streptococcus agalactiae* vaginitis [15]. Although antibiotics and antivirals are not routinely used in the treatment of BD, the improvement of symptoms observed with the use of these drugs supports the idea of an infectious etiology of BD. Another study showed that the combined use of benzathine penicillin and colchicine was more effective in ameliorating clinical symptoms compared to patients using colchicine alone [16].

In light of all these data supporting the idea of an infectious trigger in the etiopathogenesis of BD, 113 patients with BD were included in this study, with UTI being detected in 8.8% (n=10) of these. Urine culture results showed *E. coli* growth in 90% (n=9) of these patients and *Klebsiella spp.* in 10% (n=1).

UTIs can be seen in the form of simple cystitis or in complicated forms that can lead to septic shock and that have a very high incidence in the community. Involvement of the bladder and urethra is regarded as lower UTI, and that of the ureter, pelvis, and kidneys as upper UTI.[17]*E. coli* is the responsible microorganism in approximately 75-90% of cystitis or acute complicated UTIs.[18]Urine culture was performed on 29.2% (n=33) of the patients in this study, and growth was observed in only 10. *E. coli* growth wad determined in 90% (n=9) of those 10 patients, a figure compatible with the previous literature.

The diagnosis of UTI is accompanied by lower urinary systems symptoms such as a burning sensation during urination, increased frequency of urination, and a feeling of an urgent need to urinate (dysuria, urgency, and frequency), as well as >10 cells/ml leukocytes at urine testing, and $>10^5$ cfu/ml causative microorganisms in urine culture [19]. These criteria were considered when diagnosing UTI in our patients. The most common symptoms in the patients wıth UTIs were dysuria and frequency. Addıtıonally, as anticipated, leukocyte elevatıon, hematuria, leukocyte esterase, and nitrite positivity in the urine were the most common laboratory findings at total urinalysis.

Although BD causes epididymitis and sterile urethritis as well as genital ulcerations in the urogenital system, there is insufficient evidence to conclude that it exacerbates susceptibility to frequent UTIs [20]. In the present study, UTI developed more frequently in patients wıth prolonged durations of BD compared to other patients. This may be because BD can make the urogenital system more susceptible to infections in the long term or to receivıng immunosuppressive therapy for a longer period. Evaluation of the relationship between the severity of BD and the presence of UTI in this study revealed no statistical association between them. Among the BD patients with UTIs, 30% (n=3) were inactive, 30% (n=3) mild, and 40% (n=4) moderate. No statistically significant relationship was detected between BD patients with and without UTI in terms of the frequency of mucocutaneous symptoms (p=0.229). No significant relationship was also observed between the presence of UTI and the frequency of BD system involvement (p>0.05). Although 17.4% (n=18) of the patients had one or more UTI complaints, these were not considered to have UTI due to absence of growth in urine culture. Although no UTI was detected in our study, leukocyte esterase positivity was significantly higher, especially in patients with genital ulceration (p=0.039). This may be attributable to microorganisms secondarily infecting the ulcerated lesions in the genital area. A significant relationship was detected between the pathergy test positivity and the frequency of pyuria and dysuria in BD patients without UTI. Although this may be coincidental, we think that prospective studies with much greater participation should now be conducted to confirm such a relationship.

5. Conclusions

Although no statistically significant relationships were detected between the presence of UTI and the severity of the BD in the present study, microorganisms may be involved in the exacerbation of the disease. The frequency of UTI in BD patients was 8.8%. During the follow-up and treatment of BD cases, clinicians should therefore also investigate the presence of UTI complaints. We think that appropriate antibiotic therapy should be initiated depending on the urine culture results for patients with such complaints.

Limitations of the Study

The particular strength of this study is that it is the first to compare the frequency of UTI in BD, the severity of the disease, and the activity of symptoms. Another strength is that it was performed with a relatively large number of BD patients.

However, this study also has a number of limitations. The first involves the retrospective nature of the research. Second, despite the large participation in the study, the results cannot be generalized because of its single-center nature, and we think that further, multicenter prospective studies are now required.

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Conflict of Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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None.

Author Contributions

EP; The study concept and design, data collection, writing of the manuscript or critical review, and approval of the final version of the manuscript. ÖK; Statistical analysis, critical review of the literature, and approval of the final version of the manuscript.

Ethical Approval

The study was carried out in line with the Declaration of Helsinki Rules after receipt of approval from the local ethics committee (No. 5, Dated 29.12.2022).

The research was conducted in accordance with the principles of the Helsinki Declaration.

Data sharing statement

None.

Consent to participate and Informed Statement

Informed Statement was not obtained from the patients since the study was conducted retrospectively and was designed as an archive scan of all patient files.

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Evaluation of Mortality in Patients Involved In-vehicle and Out-of-vehicle Traffic Accidents

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Abstract: Traffic accidents are among the most common causes of mortality due to trauma. This study aimed to examine demographic and clinical characteristics that may affect mortality among patients who were involved in-vehicle and out-of-vehicle traffic accidents. In this retrospective study conducted with 2,120 patients, the patients were divided into two groups according to whether they had an in-vehicle or out-of-vehicle traffic accident. The patients in both groups were evaluated according to gender, Glasgow Coma Scale (GCS) scores, injury sites, and characteristics of the injured person. Then, factors that could be effective in mortality were compared between the two groups using statistical methods. Mortality occurred in 3.2% of the patients in the out-of-vehicle and 0.9% of the patients in the in-vehicle accident groups (p=0.001). There was a statistically significant difference between the two groups in relation to the GCS scores (p = 0.001). The pedestrians were the most injured individuals in out-of-vehicle traffic accidents (p=0.001). The most common injury site was the head and neck region at a rate of 24.8% (p=0.001). Mortality was higher in out-of-vehicle traffic accidents than in in-vehicle traffic accidents. Mortality was also higher among patients with low GCS scores, regardless of whether an accident occurred inside or outside a vehicle. ©2023 NTMS.

Keywords: In-vehicle Traffic Accident; Out-of-vehicle Traffic Accident; Mortality; Trauma.

1. Introduction

Traumas constitute the most common reason for mortality under the age of 40. The most common causes of trauma are traffic accidents, falling from a height, gunshot wounds, and stab wounds [1]. Traffic accidents are the most common cause of trauma, which increases mortality and morbidity.

In-vehicle and non-vehicle traffic accidents result in the deaths of thousands of people across the world every year. Motorcycle accidents and falls from vehicles are included in the category of out-of-vehicle traffic accidents. Mortality rates in in-vehicle and out-of-vehicle traffic accidents depend on many factors [2]. The mortality rate in in-vehicle traffic accidents varies according to the type of vehicle, its speed, and the type of collision. In out-of-vehicle traffic accidents, fatalities occur depending on the type, location, and type of the crashing vehicle. However, the main factor determining morbidity and mortality is whether the accident has high energy [3].

Evaluation of mortality in in-vehicle and out-of-vehicle traffic accidents is important to understand the impact of these accidents and help develop traffic safety policies. Studies carried out for this purpose are mostly studies conducted specifically for in-vehicle or out-of-vehicle traffic accidents. In this study, it was aimed to evaluate both groups together. Therefore, this study

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aimed to examine factors such as demographic data, type of accident, and injury site that could affect mortality and morbidity among in-vehicle and out-of-vehicle traffic accident victims presenting to the emergency department.

2. Material and Methods

This study was retrospectively conducted with patients who presented to the emergency department of a tertiary hospital from January 1, 2020, through December 31, 2021, following a traffic accident. The study was approved by the Local Ethics Committee (ethics committee number: B.30.2.ATA.0.01.00/66).

To obtain the related data, the hospital automation system and the physical files of the patients were screened. Patient screening on the hospital automation system was undertaken using the International Classification of Diseases codes (V39.4, V39.5, V39.6, V39.9, V79.9, V86.0, V86.1, V86.2, V86.3, V69.4, V69.5, V69.6, V69.9, V79.6, V79.4, V79.5, V49.4, V49.5, V49.6, V59.4, V59.5, V59.9, V87, V82.1, V82.9, Z04.1, V85.0, V85.1, V85.2, V85.3, and V81.1). Patients from all age groups who had been involved in in-vehicle or out-of-vehicle traffic accidents were included in the study. Pregnant women, patients with missing data, those who left the hospital without waiting for the completion of follow-up or procedures, and those who had suffered from trauma due to causes other than a traffic accident were excluded from the study. As a result of the screening, the data of 2,802 patients were obtained. However, since 581 patients had missing data and 101 left the hospital before their procedures were completed, the final sample consisted of 2,120 patients.

The patients were divided into two groups according to whether they had an in-vehicle or out-of-vehicle accident. The patients' age and gender, the type of vehicle that caused the traffic accident, whether the injured was the driver or passenger, the Glasgow Coma Scale (GCS) scores, the date and time of the accidents, the diagnoses made, injury sites, and outcomes were recorded. The types of vehicles were evaluated as automobiles, tractors, motorcycles, pedestrians, trucks, and other vehicles. The injured were evaluated as drivers, passengers, and pedestrians. Motorcycle accidents were considered out-of-vehicle traffic accidents. The diagnoses of the patients were divided into head-brain injuries, upper extremity, clavicular pathologies, lower extremity pathologies, abdominal pathologies, rib fractures, lung pathologies, vertebral fractures, three or more organ injuries (multi-trauma), facial bone pathologies, and ecchymosis-laceration pathologies. Injury sites were grouped as head-neck, lower extremity, upper extremity, abdomen, thorax, face region, pelvis, multi-injury region (multi-trauma), and back-scapula region. The diagnoses made as a result of head injuries were evaluated as cephalohaematoma, subarachnoid hemorrhage, epidural-subdural hemorrhage, intracranial hemorrhage, contusion cerebri, and diffuse axonal injury. The diagnoses of abdominal injury were evaluated as liver laceration, spleen laceration, multi-organ injury, and perforation. The clinics to which the patients were admitted were recorded as discharge from the emergency department, emergency department intensive care unit, anesthesia intensive care unit, and other intensive care units. The outcomes of the patients were evaluated as discharge from the emergency department, death in the emergency department, and admission to inpatient wards. Finally, mortality during hospitalization was recorded.

2.1. Statistical Analysis

Data analysis was calculated using the IBM SPSS v. 24 statistical program. Normal variables were expressed as mean, standard deviation, percentage, and numbers, and continuous variables as median and minimum-maximum values. Whether the data followed a normal distribution was checked using the Kolmogorov-Smirnov test. The independent-samples t-test was used for the pairwise group comparisons of normally distributed data, and the Mann-Whitney U test was used for non-normally distributed data. The Pearson chi-square test was used for categorical variables. A logistic regression model for mortality was created using significant variables. Logistic regression was performed for each variable. Then, the final results were specified for significant parameters using the backward stepwise (likelihood ratio) model. The statistical significance level was taken as $p<0.05$.

3. Results

The study included a total of 2.120 patients, of whom 1.716 (80.9%) had an in-vehicle traffic accident and 404 (19.1%) had an out-of-vehicle traffic accident.

Male patients constituted 1.134 (66.1%) of the patients in the in-vehicle traffic accident group and 279 (69.1%) of those in the out-of-vehicle traffic accident group. When the whole sample was considered, 1.413 (66.7%) of the patients were male. There was no statistically significant difference between the in-vehicle and out-of-vehicle traffic accident groups in terms of gender ($p=0.265$). The mean age was 34.00±17.76 years for the in-vehicle traffic accident group and 29.97±22.17 years for the out-of-vehicle traffic accident group, indicating a statistically significant difference ($p=0.000$) (Table 1).

In the in-vehicle traffic accident group, the GCS score of 1,662 (96.9%) patients was 15, and that of the remaining 20 (1.2%) patients was 3. In the out-of-vehicle traffic accident group, the GCS score was 15 in 369 (91.3%) patients and 3 in the remaining 13 (3.2%). There was a statistically significant difference between the two groups in relation to the GCS scores ($p=0.000$) (Table 1).

Of the patients involved in an in-vehicle traffic accident, 1.629 (94.9%) had a collision with a car, while 59 (14.6%) patients in the out-of-vehicle traffic accident group had a motorcycle accident. The difference between the two groups was statistically

significant (p=0.000). While 828 (48.3%) patients who had an in-vehicle traffic accident were drivers, 336 (83.2%) of those who had an out-of-vehicle traffic accident were pedestrians. When the characteristics of the people involved in a traffic accident were examined, a statistically significant difference was found between the two groups (p=0.000) (Table 1).

According to the time of the accident, 191 (47.3%) patients had an out-of-vehicle traffic accident between 12:01 and 18:00, and this rate was statistically significant (p=0.000). When the patients were evaluated according to injury sites, 119 (29.5%) of the patients who had an out-of-vehicle traffic accident had injuries in the lower extremity region, 20 (5.0%) in the pelvis region, and 69 (17.1%) in more than one region. Injury sites statistically significantly differed between the in-vehicle and out-of-vehicle traffic accident groups (p=0.000). The detailed demographic and clinical characteristics of the patients who had an in-vehicle or out-of-vehicle traffic accident are detailed in Table 1.

Table 1: Comparison of demographic and clinical data between the in-vehicle and out-of-vehicle traffic accident groups.

Variables		In-vehicle traffic accident (n=1.716, 80.9%)	Out-of-vehicle traffic accident (n=404, 19.1%)	Total (n=2.120, 100%)	P (0.000)
Age (Mean±SD)		34.00±17.76	29.97±22.17	33.23±18.74	0.000
Gender	Male	1,134 (66.1%)	279 (69.1%)	1,413 (66.7%)	0.265
	Female	582 (33.9%)	125 (30.9%)	707 (33.3%)	
GCS score	15	1,662 (96.9%)	369 (91.3%)	2,031 (95.8%)	0.000
	11-14	10 (0.6%)	8 (2.0%)	18 (0.8%)	
	7-10	11 (0.6%)	7 (1.7%)	18 (0.8%)	
	4-6	13 (0.8%)	7 (1.7%)	20 (0.9%)	
	3	20 (1.2%)	13 (3.2%)	33 (1.6%)	
Type of vehicle	Car	1,629 (94.9%)	289 (71.5%)	1,918 (90.5%)	0.000
	Tractor	39 (2.3%)	9 (2.2%)	48 (2.3%)	
	Motorcycle	0 (0%)	59 (14.6%)	59 (2.8%)	
	Truck	13 (0.8%)	3 (0.7%)	16 (0.8%)	
	Other	35 (2.0%)	44 (10.9%)	78 (3.7%)	
Injured Person	Driver	828 (48.3%)	57 (14.1%)	885 (41.7%)	0.000
	Passenger	888 (51.7%)	1 (2.7%)	899 (42.4%)	
	Pedestrian	0 (0%)	336 (83.2%)	336 (15.8%)	
Time of accident	00:01-06:00	165 (9.6%)	21 (5.2%)	186 (8.8%)	0.000
	06:01-12:00	377 (22.0%)	51 (12.6%)	428 (20.2%)	
	12:01-18:00	609 (35.5%)	191 (47.3%)	800 (37.7%)	
	18:01-00:00	565 (32.9%)	141 (34.9%)	706 (33.3%)	
Injury site	Head-neck	451 (26.3%)	74 (18.3%)	525 (24.8%)	0.000
	Lower extremity	210 (12.2%)	119 (29.5%)	329 (15.5%)	
	Upper extremity	370 (21.6%)	54 (13.4%)	424 (20%)	
	Abdomen	66 (3.8%)	6 (1.5%)	72 (3.4%)	
	Thorax	123 (7.2%)	23 (5.7%)	146 (6.9%)	
	Face	177 (10.3%)	24 (5.9%)	201 (9.5%)	
	Pelvis	40 (2.3%)	20 (5.0%)	60 (2.8%)	
	Multi-trauma	151 (9.0%)	69 (17.1%)	223 (10.5	
	Back-scapula	125 (7.3%)	15 (3.7%)	140 (6.6%)	
Patient outcome	Discharge	986 (57.5%)	187 (46.3%)	1,173 (55.3%)	0.001
	Ward admission	721 (42.0%)	210 (52.0%)	931 (43.9%)	
	Mortality in ED	9 (0.5%)	7 (1.7%)	16 (0.8%)	
Mortality status	Discharged	1,701 (99.1%)	391 (96.8%)	2,092 (98.7%)	0.001
	Died	15 (0.9%)	13 (3.2%)	28 (1.3%)	

SD: standard deviation, GCS: Glasgow Coma Scale, ED: emergency department.

While 1,701 (99.1%) patients in the in-vehicle traffic accident group were discharged and 15 (0.9%) patients died. While 391 (96.8%) patients in the out-of-vehicle traffic accident group were discharged and 13 (3.2%) patients died, indicating a statistically significant difference between the two groups (p=0.001). When the patients who died were compared in terms of clinical characteristics and etiology, 13 (46.4%) had an out-of-vehicle traffic accident (p=0.001), 19 (67.9%) had a GCS score of 3 (p=0.001), three (10.7%) had a motorcycle accident (p =0.025), and 11 (39.3%) had a pedestrian accident (p=0.002). Furthermore, in the mortality group, 17 (60.7%) patients had head-brain injuries, and three (10.7%) patients had three or more

organ injuries (p=0.001). When evaluated according to injury sites, 18 (64.3%) patients who died had multi-trauma (p=0.001). The remaining characteristics of the discharged and deceased traffic accident patients are detailed in Table 2.

Table 2: Comparison of mortality according to the etiology and clinical characteristics of the patients.

Variables		Discharged (n=2.092, 98.7%)	Died (n=28, 1.3%)	**P**
Injury mechanism	In-vehicle	1.701 (81.3%)	15 (53.6%)	0.001
	Out-of-vehicle	391 (18.7%)	13 (46.4%)	
GCS score	15	2.027 (96.9%)	4 (14.3%)	0.001
	11-14	16 (0.8%)	2 (7.1%)	
	7-10	17 (0.8%)	1 (3.6%)	
	4-6	18 (0.9%)	2 (7.1%)	
	3	14 (0.7%)	19 (67.9%)	
Type of vehicle	Car	1.894 (90.5%)	24 (85.7%)	0.025
	Tractor	48 (2.3%)	0 (0%)	
	Motorcycle	56 (2.7%)	3 (10.7%)	
	Truck	15 (0.7%)	1 (3.6%)	
	Other	79 (3.8%)	0 (0%)	
	Pedestrian	36 (1.7%)	0 (0%)	
Gender	Female	702 (33.6%)	5 (17.9%)	0.056
	Male	1.390 (66.4%)	23 (82.1%)	
Injured Person	Driver	874 (41.8%)	11 (39.3%)	0.002
	Passenger	893 (42.7%)	6 (21.4%)	
	Pedestrian	325 (15.5%)	11 (39.3%)	
Diagnoses	Head-neck injuries	136 (6.5%)	17 (60.7%)	0.001
	Upper extremity and clavicula injuries	120 (5.7%)	0 (0%)	
	Lower extremity injuries	201 (9.6%)	2 (7.1%)	
	Abdominal injury	62 (3.0%)	2 (7.1%)	
	Thorax injuries	133 (6.4%)	3 (10.7%)	
	Vertebral fracture	102 (4.9%)	1 (3.6%)	
	Multiple organ injuries	61 (2.9%)	3 (10.7%)	
	Facial bone injuries	99 (4.7%)	0 (0%)	
	Soft tissue injuries	1178 (56.3%)	0 (0%)	
Injury site	Head-neck	517 (24.7%)	8 (28.6%)	0.001
	Lower extremity	329 (15.7%)	0 (0%)	
	Upper extremity	424 (20.3%)	0 (0%)	
	Abdomen	70 (3.3%)	2 (7.1%)	
	Thorax	146 (7.0%)	0 (0%)	
	Face	201 (9.6%)	0 (0%)	
	Pelvis	60 (2.9%)	0 (0%)	
	Multi-trauma	205 (9.8%)	18 (64.3%)	
	Back-scapula	140 (6.7%)	0 (0%)	

GCS: Glasgow Coma Scale.

Table 3 shows the logistic regression model for predicting mortality in patients involved in traffic accidents. In this model, the GCS score was associated with increased mortality risk following a traffic accident (p=0.017), i.e. as the GCS decreased, mortality increased.

4. Discussion

Traffic accidents constitute a part of patient visits to the emergency department. There are variables that affect mortality in traffic accidents. In our study, when traffic accident patients were evaluated, those who had been involved in an out-of-vehicle traffic accident had a higher rate of mortality than those involved in an in-vehicle traffic accident. Mortality was also higher among the patients with head-brain injuries and multi-organ injuries. Traffic accidents rank first among all accidents around the world [4]. Fatalities due to traffic accidents rank 11th among all deaths and constitute 2.1% of all deaths [5]. In our study, the mortality rate due to traffic accidents was 1.3%. We consider that our rate differs from the global mortality rate associated with traffic accidents due to the many independent variables that have an effect on mortality.

Table 3: Results of logistic regression analysis for the prediction of mortality in traffic accident patients.

Variables		Exp (B)	95% CI for Exp(B)		P
			Lower	Upper	
Step 1	Type of vehicle	1.694	0.420	6.825	0.545
	Mechanism of injury	16.197	0.024	1003.186	0.095
	GCS score	0.003	0.101	3.822	0.000
	Person injured	0.008	0.000	6.175	0.177
	Diagnosis	0.035	0.001	2.509	0.094
	Injury site	2.195	0.091	52.753	0.004
Step 2	GCS score	0.018	0.002	0.199	0.017
	Injury site	0.361	0.011	6.374	0.387

GCS: Glasgow Coma Scale, CI: confidence interval, Exp(B): exponentiation of the B coefficient.

In the literature, it has been reported that the majority of deaths due to traffic accidents (63.4%) occur in male patients [6-8]. In addition, the age range of patients who die after a traffic accident has been reported to be 21-30 years and 30-49 years in previous studies [4, 6, 9-10]. Similar to the literature, in our study, mortality was more common among the male patients, regardless of whether the accident occurred inside or outside a vehicle, and the ages of the patients ranged from 30 to 49 years.

There are some scoring systems used for evaluating trauma systems and assessing the outcomes of major trauma. These include the Injury Severity Score, the GCS score, the Revised Trauma Score, and the Abbreviated Injury Scale. Many studies have shown that a low GCS score is associated with high mortality [11, 12]. Similarly, in the current study, it was determined that a low GCS score was a factor affecting mortality in patients who had an in-vehicle or out-of-vehicle traffic accident.

Traffic accidents usually occur between 18:00 and 00:00 [8, 9, 13, 14]. In a study by Meral et al. it was reported that while the rate of traffic accidents was 27.4% between 12:00 and 17:59 hours, this rate increased to 36.3% between 18:00 and 00:00 [15]. In our study, the rate of traffic accidents that occurred between 12:01 and 18:00 was determined to be 47.3% for in-vehicle traffic accidents and 34.9% for out-of-vehicle traffic accidents. In other studies, the reason why traffic accidents mostly occurred between these hours was attributed to the drivers' fatigue, carelessness, and lack of visibility in the evening [15]. In our study, we consider that the higher incidence of out-of-vehicle traffic accidents between 12:00 and 18:00 could be related to the traffic being busier during the day than at night and previous studies not including a separate category for out-of-vehicle traffic accidents.

The majority of injuries in traffic accidents are caused by the driver or the passenger sitting next to the driver [15-17]. Although this only applies to in-vehicle traffic accidents, in our study, we also determined that pedestrians were the most injured individuals in out-of-vehicle traffic accidents. Mortality due to traffic accidents was seen in 39.3% of pedestrians.

In traffic accidents, the most common injury site is reported to be the head-neck region, followed by the lower extremity [8, 18-20]. Upper extremity injuries have been detected in 16.5% of traffic accident victims [15]. In our study, similar to the literature, the head and neck region was the most frequently injured site in all accidents, and this was followed by lower extremity injuries. However, the most common injury site in out-of-vehicle traffic accidents was the lower extremity. This may be because vehicles hit pedestrians at the lower extremity level, according to the trauma mechanism. Facial injuries were observed in 10.3% of the patients involved in an in-vehicle traffic accident, which can be attributed to the trauma caused by airbags in vehicles.

5. Conclusions

Mortality was found to be higher in out-of-vehicle traffic accidents than in in-vehicle traffic accidents. Furthermore, mortality was higher among the patients with a low GCS score, regardless of whether they had been involved in an accident inside or outside a vehicle. We consider that mortality due to traffic accidents can be reduced if both drivers and pedestrians comply with traffic rules and take the necessary precautions.

Limitations of the Study

Our study has certain limitations. First concerns the single-center design, as a result of which the data of traffic accident patients who were referred to other centers could not be reached. Second, although we investigated mortality due to traffic accidents, we were not able to evaluate the data of patients who died at the accident scene before referral to our hospital. And lastly, patients with simple injuries may have been excluded from our study since they would not have applied to the hospital.

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Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

AG contributed to the writing-original draft preparation of the manuscript, writing-review & editing, methodology, visualization, and investigation. FC contributed to conceptualization, writing-review &

editing, and data curation. BKC contributed to formal analysis, resources, and visualization. FC contributed to investigation, software, and resources. Final approval was given by AG, FC, BKC.

Ethical Approval

The study was approved by the Ataturk University Ethics Committee (B.30.2.ATA.0.01.00/66).

Data sharing statement

None.

Consent to participate

Informed consent was obtained from the patients.

Informed Statement

The study complies with the principles of the Declaration of Helsinki. The consent of all the patients was obtained before commencing the study.

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Endothelin-1 Gene Polymorphism in Chronic Obstructive Pulmonary Disease: A Case-Control Study

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Abstract: In the current studies there being a high ratio of Endotelin-1 (ET-1) in BAL liquid of Chronic Obstructive Pulmonary Disease (COPD) cases made researchers think ET-1 may have an important role in pathogenesis of COPD. Our study group does research on the relation of COPD with single nucleotide gene polymorphism at ET-1 gene. This prospective case-control study included 87 smokers with COPD and 89 smokers but not COPD. We investigated the density of single nucleotide gene polymorphism (+134 insA/delA) in the ET-1 gene in cases. Allele ratio and genotype distribution, distribution amongst three genotype (3A3A, 3A4A, 4A4A) in the COPD patient and control group was analyzed. In this study, for endothelin gene -3A/-4A (-138 insertion/deletion) polymorphism analysis, polymerase chain reaction-restriction fragment length polymorphism method was used. In comparison with the control group, the COPD group has higher ratio of ET-1 gene (+134 insA/delA) polymorphism ($p<0.001$). Endotelin-1 gene polymorphism (+134 insA/delA) significantly increased in the smokers with COPD than control group ($p<0.013$). Endothelin-1 gene polymorphism may be a predictive genetic factor of COPD. ©2023 NTMS.

Keywords: Endothelin-1; Chronic Obstructive Pulmonary Disease; Gene Polymorphism.

1. Introduction

With population aging, tobacco use and other risks, chronic respiratory diseases have become an increasingly important cause of morbidity and mortality. Chronic obstructive pulmonary disease (COPD) is an increasingly important cause of death worldwide [1, 2]. In 2002, COPD was the eleventh cause of disability adjusted life years (DALYs); It is expected to be the seventh leading cause by 2030 [3].

Significant differences in onset, progression, and lung function between populations at different life stages limit understanding of the causes that predispose to COPD [2, 4]. However, tobacco use and air pollution and occupational pollutants have been reported as primary risk factors [5].

Gene polymorphisms are point mutations or allelic variations in the DNA, including single nucleotide polymorphisms [6]. A gene may be polymorphic if it occupies more than one allele gene locus in a population. Most of the studies on gene polymorphisms have been performed in cancer little is understood

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about the genetic components that may contribute to the development of COPD [7].

Smoke exposure causes harmful vascular effects by increasing the expression of vasoconstrictor and mitogenic factors such as endothelin-1 (ET-1) [8]. The autocrine effects (proinflammatory, vasoconstrictor, and mitogenic) of ET-1 may increase disease severity by being involved at the early stage of smoking-induced lung remodeling [8, 9].

In addition to many pathogenic mechanisms in inflammatory cycle cases, there being high ratio of Endotelin-1 (ET-1) in BAL liquids of COPD give rise to thought of this may have an important role in pathogenesis of COPD in the latest studies [10,11]. ET-1 gene, composed of five short exons and four introns, is on the p branch of the 6. chromosome. ET-1 gene codes preproendotelin which is a primary molecule and later on turns into amino acid ET-1 peptide [12,13]. When compared to other organs, ET-1 production and activity is on the highest level in the lungs. Because primary or active ET-1 peptide is not stored in the cell, this level is kept up by the activation of gene transcription. ET-1 take charge in locally effective ET-A and ET-B receptors in the lungs by producing autocrine-paracrine signal [12, 13]. The single nucleoid gen polymorphism in ET-1 is associated with ET-1 level [8, 14]. The single nucleoid gene polymorphism created by adding one adenine (+134 insA/delA), is seen as low as 138 bp in the transcription start region at 5'UTR of exon 1. Transinfection studies show that rather than translation effect of this polymorphism, it is responsible for the high ET-1 level provided by increased mRNA stability [12, 14]. And this is provided by unengaged energy affecting transcriptional stability by changing its second structure and amount in the stem loop, by creating different stem loops at 5'UTR transcripts of preproET-1 mRNA and adding adenine [14]. In a study, it is showed that 3A4A and 4A4A genotypes where +134 insA/delA polymorphism is, increases the risk of COPD [12]. COPD takes place with the interaction of genetic and environmental factors and ET-1 polymorphism which is about genetic load is worthy of attention [10, 11]. It is possible that ET-1 polymorphisms modulate the risk of developing COPD because of their effect on the maintenance of inflammation in the lungs of COPD patients and has not been adequately studied in the literature.

The aim of the present study was to research on the relation of COPD with single nucleotide gene polymorphism at ET-1 gene in a Turkish population.

2. Material and Methods

The study protocol was reviewed and approved by the Medipol Universty ethics committee. All patients signed a consent form. The study conforms to the relevant ethical guidelines for human and animal research.

2.1. Subjects

For the study 176 people, 87 of which has COPD and 89 of which is control group, were obtained. All participants for the study resided in Türkiye. To the study, people above 40 with at least 20 p/y smoking history were obtained.

Of the both patient group and control group anamnesis was taken and physical examinations were carried out. Epidemiologic characteristics like age, sex, kilo, height and smoking of patients and control group were recorded. After the assessment of spirometry results and cases, people with additional diseases, people who do not abide by the criteria of acceptance to the study, and people who has the exclusion criteria are not accepted for the research. Approximately 10 cc peripheral venous blood was taken from the participants who provide acceptance criteria and who signed informed consent form; these blood kept in hemogram tube with EDTA and at +4 degrees was studied on the condition of protocol transfer in Genetic Study and Diagnosis Laboratory in the rest of the day.

Study group: People whose FEV1/FVC ratio is below 70% at spirometry, males and females above 40 age, who has smoking history at least 20 p/y, who read and sign informed consent document. Control group: Male and females above 40 age, who has at least 20 p/y smoking history, whose FEV1/FVC ratio is above 70% at spirometry, who read and sign informed consent document. The patient who has lung diseases creating dyspnea symptoms like asthma, bronchiectasis, tuberculosis, sarcoidosis, interstitial lung disease (with x-ray, spirometry and history), comorbidities (malignancy, diabetes, congestive heart failure, liver and renal diseases) and the cases who did not sign informed consent document were excluded from the study.

During the pulmonary function tests, all tests were performed by the same investigator and a pulmonologist was the observer. All procedures were carried out according to the guidelines of the American Thoracic Society and the European Respiratory Society [15, 16].

2.2. Blood Collection and Genotyping

DNA Isolation

From the case participants constituting the study group, 1cc 0.5 M ethylenediaminetetraacetic acid (EDTA) (Sigma, ABD) and 9 cc blood sample was taken into tube. Isolated DNA was stored at +4 ºC.

Endothelin Gene -3A/-4A (-138 insertion/deletion) Polymorphism Scan. In this study, for endothelin gene -3A/-4A (-138 insertion/deletion) polymorphism analysis, PCR-RFLP method was used. To determine endothelin gene -3A/-4A (-138 insertion/deletion) the part containing the gene transformation was multiplied with the use of 5'GCTGCTTTTCTCCCCGTTAA3' and 5'CAAGCCACAAACAGCAGAGA3' primers

and PCR products of 195 bd were gained. Temperature conditions in PCR; at 95 °C for 5 mins denaturation, as 35 cycle at 95 °C for 1 min denaturation, at 58 °C for 1 min hybridization, at 72 °C elongation and at 72 °C for 7 mins last elongation was carried out (Biometra, USA). After PCR, the products were checked by putting 5µl into agarose gel of 2% on agarose gel electrophoresis; if seen any amplification of correct gene region, cutting operation was carried out with restriction endonuclease.

Cutting PCR Products with Restriction Endonuclease. So as to scan endothelin gene -3A/-4A (-138 insertion/deletion) polymorphism, BsiYI (Fermentas, Lithuania) enzyme was used. PCR products, in volume of 12.5 µl, were treated with 33mM Tris-acetate, 10mM Magnesium acetate, 66 mM Potassium acetate and RE buffer, including 0.1 mg/ml BSA (37 ºC, pH:7.9) and for each individual buffer-enzyme mixture of 10units/µl BsiYI. PCR product-enzyme-buffer mixture was left for rest at 55 ºC which is optimum working temperature of the enzyme for 14-16 hours for incubation.

Electrophoresis of Agarose Gel Restriction enzyme cut results were evaluated in agarose gel of 3%. Products which were cut by BsiYI enzyme were loaded on the gel by treating Bromene-phenol blue (Merck, Germany). It was carried out with 90-100V current for 30-50 mins (Biogen, USA). It was investigated under ultraviolet light (Spectroline, USA).

When the examples, which were carried out in agarose gel of 3%, were evaluated under ultraviolet light; individuals with -4A/4A genotype were not cut by enzyme and 195 base pair band was observed. Individuals with -3A/3A there was 195,176,19 base double pairs, individuals with -3A/3A there was 176,19 base pair bands observed.

2.3. Statistical Analysis

Data was evaluated with the help of SPSS v.26 (SPSS Inc., ABD) program. Allele ratio and genotype distribution, distribution amongst three genotype (3A3A, 3A4A, 4A4A) in the COPD patient and control group was analyzed by chi-square test. P value of comparison numbers including two alleles and two separate locus was confirmed with the help of Bonferroni method (Pc). Fisher exact test was applied to compare the small groups below the expected value of 5. Hardy-Weinberg equilibrium test was carried out with chi-square test. pulmonary function data analysis was carried out with t test. $p<0.05$ was accepted as statistical significance.

3. Results

Demographical characteristics of 176 subjects included in the study were summarized (Table 1). While the average age of COPD group was determined to be 60.4±8.9, the average age of control group was determined to be 50.2±8.3. When compared to control group, the average age of COPD group was significantly higher in the ($p<0.001$), (Table 1).

Table 1: Demographic characteristics of the subjects involved in the study.

	COPD patients (n:87)	Control group (n:89)	*P value*
Age; on average±SD	60.4±8.9	50.2±8.3	<0.001
Cigarette box/year	46.4±18.6	32.6±13	<0.001
Smoker/exsmoker	56/31	62/27	NS
BMI	24±4	27±7	0,06
Male/female	80/7	73/16	NS
FEV1(% predicted)	44.3±18	89±15	<0.001
FEV1/FVC (% predicted)	56±8	81±5	<0.001

BMI: Body mass index, NS: Not statistically significant, SD: Standard deviation.

Table 2: Endothelin 1 +134 insA/delA gene allele and genotype frequency of subjects.

Polymorphism	COPD patients (n= 87)	Control group (n=89)	P value (X^2)	Odss ratio	(95% CI)
134insA/delA					
Genotype					
3A3A	45 (51%)	63 (71%)		1,52	1.08-2.1
3A4A	41 (47%)	23 (2%5)	0.013 (6.7)	0.67	0.5-0.9
4A4A	1 (2%)	3 (4%)			
Allele frequency					
3A	131 (76%)	149 (84%)	0.044 (3.8)	0.7	0.62-0.98
4A	43 (24%)	29 (16%)			

CI: confidence interval, Odss ratio: risk ratio.

Whereas smoking amount in COPD group as box/year was 46.4±18.6 box/year on average, smoking amount in control group was 32.6±13 box/year. Smoking duration of COPD group was significantly higher than control group ($p<0.001$).

Genotype distribution and allele frequency is shown on table 2 for Endotelin-1 gene polymorphism (+134 insA/delA). As a result of statistical analysis, it is determined that +134insA/delA single nucleoid gene polymorphism is significantly higher in the patients than control group ($p<0.013$). There was no significant relationship between the respiratory function parameters, demographic characteristics and 134 insA/delA gene polymorphisms of the subjects (Table 3).

Table 3: The relation between subjects' respiratory function parameters, demographic characteristics and 134 insA/delA gene polymorphism.

	3A3A (n:45)	3A4A (n: 41)	*P value*
Age on average ± SD	59.9±7.9	61±9.7	NS
Cigarette box/year	45±16	48±21	NS
Smoker/ex-smoker	28/17	27/14	NS
BMI	25±3	24±4	NS
Male/Female	41/4	38/3	NS
FEV1(% predicted)	44±16	57±8	NS
FEV1/FVC (% predicted)	55±8	81±5	NS

BMI: Body mass index, NS: Not statistically significant, SD: Standard deviation.

4. Discussion

In our study, we researched Endotelin-1 gene (+134insA/delA) polymorphism, which plays an important role in COPD pathogenesis and we detected that ET-1 gene (+134insA/delA) is significantly higher in COPD cases than control group in Turkey ($p<0.001$). Endothelium is the source of factors with vasodilator and vasoconstrictor activities. Endothelin family, which is a powerful vasoconstrictor substance, was isolated in aorta endothelial cells at first [12, 17]. Endothelin isoforms have 3 types which are endothelin-1 and two small peptide ET-2 and ET-3. Each of them are products of different genes ET-1 is principally synthesized and excreted by endothelial cells [17]. ET-1 affects vascular smooth muscle cells, cardiac myocytes, fibroblasts and mesangial cells in the renal glomerulus in a mitogenic way. Furthermore, it also shows several effects on central and peripheral nervous system, gastrointestinal system, liver, urinary tract, male and female reproductive systems, eyes, skeletal system and the skin [17].

ET-1 participates in pulmonary hypertension as a mediator of changes in pulmonary vasculature. Its overexpression in the lung suggests that it has an important role in the initiation and progression of pulmonary hypertension (PH). In a study carried out by Kwon and his friends, they found out the ET-1 level of COPDs with pulmonary hypertension was significantly higher than the level of COPDs without pulmonary hypertension [18]. Beneficial effects of chronical treatment were stated with the help of specific ET receptor antagonists at experimental PH [19, 20].

ET-1 is a strong inotropic agent of tracheal and bronchial smooth muscle in the inflammatory system. It is related to various inflammatory diseases including COPD. ET-1, which is produced by vascular endothelin, bronchial epithelia, monocytes and fibroblasts in the lungs, is an important regulator in inflammatory area. Without having any starting irritator in inflammatory system mucosa, it is shown that ET-1 plays role in COPD pathogenesis by causing inflammatory cycle [21]. Many reasons causing COPD attacks rise in phlegm and is related to ET-1 concentration [22]. All these data indicate that ET-1 can play role in COPD ethiopathogenesis.

ET-1 shows local effects by signaling autocrine-paracrine in ET-A and ET-B receptors. At the same time, lungs are chief organs as to eliminate ET-1 from the cycle. In the latest studies, it is stressed that single gene mutation (SNP) in ET-1 gene probably rises ET-1 level by increasing ET-1-mRNA stabilization [12]. Hence, genetic polymorphism carriers, as their ET-1 level which is produced as a response to environmental stimulators, may have higher risk of COPD development comparing to non-carriers.

It can be said that ET-1 gene polymorphisms play role in regulating pathogenic mechanisms in COPD development, keeping inflammation at COPD patients in mind [10,11]. In a study carried out by Sampsonas and his friends [12], they drew attention to biologically and clinically probable significant polymorphic regions of ET-1 gene. SNP, which goes with adenine insertion, is found to be related to COPD. In the same study, in addition to ET-1 +134insA/delA allele polymorphism, G198T allele polymorphism having a relation with increased COPD risk was stated. Kaparianos et al. found that the +138 3A/4A and G198T SNPs of the ET-1 gene are probably not only involved in the pathogenesis of COPD, but also modulate the phenotypic expression of this disease, namely emphysema and chronic bronchitis [9]. In our study, only ET-1 +134insA/delA allele polymorphism was inspected and was found related to increased COPD risk.

Autocrine effects of ET-1 (pro-inflammatory, vasoconstrictor, mitogenic) can contribute to early remodeling in lungs related to smoking, and this can be related to disease severity for COPD and pulmonary hypertension. In a study Carratu and his friends found ET-1 level to be higher in COPDs with pulmonary hypertension than control group and related this result with disease severity [23]. In this situation, correlation between disease severity and polymorphism is expected. In another study, it was shown that in COPD cases G198T polymorphism correlates with disease phase and spirometry which shows disease severity [12]. In study Kaparianos and his friends made on COPDs, they related ET-1 gene polymorphism with annual FEV1 decrease and concluded the evaluation that COPD development and severity may be linked to this reason [9].

Contrarily, no relation between other ET-1 gene (+134insA/delA) polymorphism and spirometry and disease phase was found. In our study, there is not any detected relation between ET-1 gene (+134insA/delA) polymorphism and parameters showing disease severity. Besides, due to not doing ECHO, we couldn't comment on the relation between ET-1 gene polymorphism and pulmonary hypertension.

The relation between SNP which goes with adenine insertion in ET-1 polymorphism at COPD and COPD pathogenesis was found [12]. Patients, who probably carrying at least one 4A allele, produce mRNA creating PreproET-1, and this leads to 50-UTR transcription, and changes the second structure and amount of stem-loop and adenine insertion free energy [12]. In order to enlighten the underlying mechanisms beneath these changes, further studies are needed. If need any speculation about this topic, polymorphism may be developing as a response to ET-1 level increasing after smoking and this continues the inflammation and at last develops COPD. In our study, it was thought that, for detecting ET-1 gene polymorphism in COPD patients higher than control group, this polymorphism forms a basis for COPD development by playing an important role in COPD pathogenesis.

5. Conclusions

Our study is the first in our country relating ET-1(+134insA/delA) single nucleotide gene polymorphism and COPD phenotype according to the latest data. In consequence of our study, we think ET-1(+134insA/delA) gene polymorphism can distinctively take part in COPD pathogenesis and thus it can be a beneficial marker for identifying people with increased COPD risk. We think, in Türkiye single nucleoid gene polymorphism (+134insA/delA) in ET-1 gene increases the sensitivity to COPD development.

Limitations of the Study

When it comes to limitations of our study, the age and sex distribution was not homogenous between patients and control group, age in COPD patients was higher and in both groups sex distribution was on behalf of males. As a result of this, we observed smoking and consequently COPD shows male predominance and the hesitance of females comparing to males to involve in the study.

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None.

Conflict of Interests

All authors declare there is no conflict of interest**.**

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Author Contributions

MD designed the research. GK and ECS participated in data collection and data analysis. MD and GK wrote the manuscript, GK and MB read and approved the final script.

Ethical Approval

No ethical approval was needed for this study.

Data sharing statement

Not applicable for this study.

Consent to participate

Consent was obtained from the patient and control groups participating in the study.

Informed Statement

All patients signed a consent form.

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Naringin is Protective in Paclitaxel-Induced Peripheral Neuropathy; A Multi-Biomarker Approach

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Abstract: Cancer is a disease that is on the rise worldwide. Paclitaxel (PTX) is one of the most common chemotherapeutic agents used in the treatment of many cancers. PTX causes toxic effects by increasing oxidative stress in tissues. Naringin (NRG) is a powerful antioxidant found naturally in many plants, especially citrus fruits. The aim of this study was to determine the protective effects of NRG in PTX-induced sciatic nerve injury. Thirty-five male rats were randomly divided into five groups: control, PTX, NRG, PTX+NRG-50, and PTX+NRG-100. PTX was administered intraperitoneally (i.p.) for the first five days, and NRG 50 or 100 mg/kg orally on days 6-14. Sciatic nerve tissues were harvested and analyzed for markers of oxidative stress, inflammation and apoptosis damage levels by biochemical methods. PTX caused oxidative stress damage by increasing lipid peroxidation (MDA) and decreasing antioxidant capacity (SOD, CAT, GPx, and GSH), inflammatory damage by increasing proinflammatory cytokine (NF-κB, TNF-α, IL-1β, SIRT1, TLR4, and NRF2) release, apoptotic damage by increasing apoptotic factor (Bax) and decreasing antiapoptotic factor (Bcl-2) in sciatic nerve tissue ($p<0.05$). NRG, on the other hand, reversed all these changes in sciatic nerve tissue and reduced PTX-induced oxidative stress damage, inflammatory damage and apoptotic damage ($p<0.05$). These effects were more effective at the 100 mg/kg dose of NRG than at the 50 mg/kg dose ($p <0.05$). In sciatic nerve tissue, PTX induced peripheral neuropathy with increased oxidative stress, inflammation and apoptotic damage. NRG showed a protective effect against PTX-induced peripheral neuropathy. ©2023 NTMS.

Keywords: Apoptosis; Naringin; Oxidative stress; Paclitaxel; Sciatic nerve.

1. Introduction

According to 2020 global data, 19.3 million new cases and 10 million deaths were reported to be associated with cancer [1]. Different treatment options for cancer have been developed over the years. Cancer treatment

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includes surgery and various specific therapies such as radiation therapy, chemotherapy, immunotherapy, hormonal therapy, radiotherapy and targeted therapy [2]. When using chemotherapy to treat cancer, unexpected side effects such as peripheral neuropathy (PN) occur. Chemotrapotic agents such as taxanes cause the development of PN [3]. PN leads to dose restriction and even treatment cessation in patients during treatment. Therefore, this leads to a decrease in patients' quality of life and survival rates [4].

Paclitaxel (PTX) is an effective chemotherapeutic agent used to treat many cancers, such as ovarian, bladder, and other solid tumor cancers [5]. In clinical trials (phase I, II and III) from 1977 to 1992, taxol (commercial form PTX) proved to have a therapeutic effect on ovarian cancer, breast cancer, uterine cancer and other cancers. Taxol was the US Food and Drug Administration (FDA)-approved in 1992 for the therapy of certain cancers [6]. It is the most widely used and effective natural remedy among existing anti-cancer drugs due to its action by stopping cell growth, cycle, and division [7]. PN is one of the most common side effects, occurring in approximately 60% of patients receiving chemotherapy treatment. Almost all (97%) of all gynecologic and urologic cancer patients receiving PTX therapy develop PN [8].

Compounds found naturally in plants and fruits can provide benefits against unwanted harmful effects in organs and tissues due to their antioxidant effects [9]. Flavonoids, which are important components in many medicinal plants, are highly effective in maintaining tissue and body health. These activities include anti-cancer, anti-mitotic, antiproliferation, anti-apoptotic, and anti-oxidation properties [10, 11]. Naringin (NRG) is a naturally occurring and clinically proven flavone glycoside found primarily in grapefruit and citrus fruits. The average amount of NRG in grapefruit is around 17 mg/100 g [12]. NRG inhibits cyclo-oxygenase and 5-lipo-oxygenase pathways, which play a significant role in arachidonic acid metabolism, thereby eliminating free radicals, reducing lipid peroxidation and showing anti-inflammatory effects [13]. The current study aimed to determine the effects of NRG on oxidative stress, inflammation, and apoptosis damage parameters in PTX-induced PN, an anticancer agent with known toxic side effects.

2. Material and Methods

2.1. Chemicals

PTX was purchased from Koçak Pharmaceuticals (Taksen 300 mg/50 ml, Istanbul/Türkiye). NRG and all other chemicals (analytical purities) were obtained from Sigma Chemical Co. (St. Louis, USA).

2.2. Experimental Procedure

Thirty-five male rats (Sprague dawley, 220-250 g, 10-12 weeks) obtained from Atatürk University Experimental Animal Center (Erzurum, Türkiye) were used in the experiments. Rats were housed under standard laboratory conditions (12-h light and dark cycle, ventilation, 23±2°C, standard cage). Unlimited access to food and drinking water was provided. Rats were randomly divided into 5 groups (n=7). PTX and NRG doses were determined from the literature [3, 10].

1-Control (CNT): Saline was administered intraperitoneally (i.p.) 0.2 ml for the first five days and then orally on days 6-14.

2-Naringin (NRG): After 0.2 ml saline was administered i.p. for the first five days, NRG 100 mg/kg was administered orally on days 6-14.

3-Paclitaxel (PTX): For the first five days, 0.2 ml PTX (2 mg/kg) solution was administered i.p. followed by oral administration of 0.5 ml saline on days 6-14.

4-Paclitaxel+Naringin 50 (PTX+NRG-50): PTX was administered i.p. for the first five days, followed by NRG 50 mg/kg orally on days 6-14.

5-Paclitaxel+Naringin 100 (PTX+NRG-100): PTX was administered i.p. for the first five days, followed by NRG 100 mg/kg on days 6-14.

2.3. Collection of Samples

Twenty-four hours after the last administration of NRG (day 15), sciatic nerve tissue was removed. Sciatic nerve tissue was washed in physiologic saline and stored.

2.4. Lipid Peroxidation Analysis

To determine the malondialdehyde (MDA) level in sciatic nerve tissues, 532 nm absorbance was measured after reaction with thiobarbituric acid. For MDA analysis of sciatic nerve tissues, homogenization was performed according to the previous method [3]. For the analysis of MDA levels, the method in the literature was used [14].

2.5. Antioxidant Analysis

Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities and glutathione (GSH) levels were analyzed to analyze the antioxidant status of sciatic nerve tissue. Homogenization of sciatic nerve tissues for analysis of antioxidant markers was performed according to the previous method [9]. SOD [15], CAT [16], GPx [17], and GSH [18] were determined using the literature. Lowry et al. method was used for protein analysis [19].

2.6. Analysis of Inflammatory Markers

Cytokine production in sciatic nerve tissue was determined by ELISA using commercial kits by the procedure. Supernatants obtained from homogenates prepared with phosphate buffer (pH 7.4, 0.1 M) were used in the analysis. Nuclear Factor kappa B (NF-κB), Tumor necrosis factor alpha (TNF-α), Interleukin-1β (IL-1β), Sirtuin 1 (SIRT1), Toll-Like Receptor 4 (TLR4), and Nuclear factor erythroid 2-related factor 2 (NRF2) levels were determined from sciatic nerve tissue using a rat ELISA kit (Sunred, China).

2.7. Analysis of Apoptotic Markers

Supernatants obtained from homogenates prepared with phosphate buffer (pH 7.4, 0.1 M) were used for apoptotic damage level analysis in sciatic nerve tissue. Bax and B-cell lymphoma 2 (Bcl-2) levels were determined from sciatic nerve tissue using a rat ELISA kit (YL Biont, China).

2.8. Statistical Analysis

Statistical analysis of the data obtained from sciatic nerve tissues was performed with SPSS 20.0 (IBM, NY) program. One-way ANOVA and Tukey's post hoc tests were used for comparison between groups. Data are presented as Mean±SEM. Statistical significance was accepted: $p<0.05$.

3. Results

3.1. Oxidant and Antioxidant Status Findings

It was found that SOD, CAT and GPx were inhibited and antioxidant activity decreased by decreasing GSH in PTX compared to the control ($p<0.05$). In addition, lipid peroxidation was manifested by an increase in MDA ($p<0.05$). Antioxidant enzyme activities and GSH increased and MDA decreased in PTX+NRG-50 and PTX+NRG-100 compared to PTX ($p< 0.05$). NRG showed more pronounced effects at a dose of 100mg/kg ($p< 0.05$). (Figure 1).

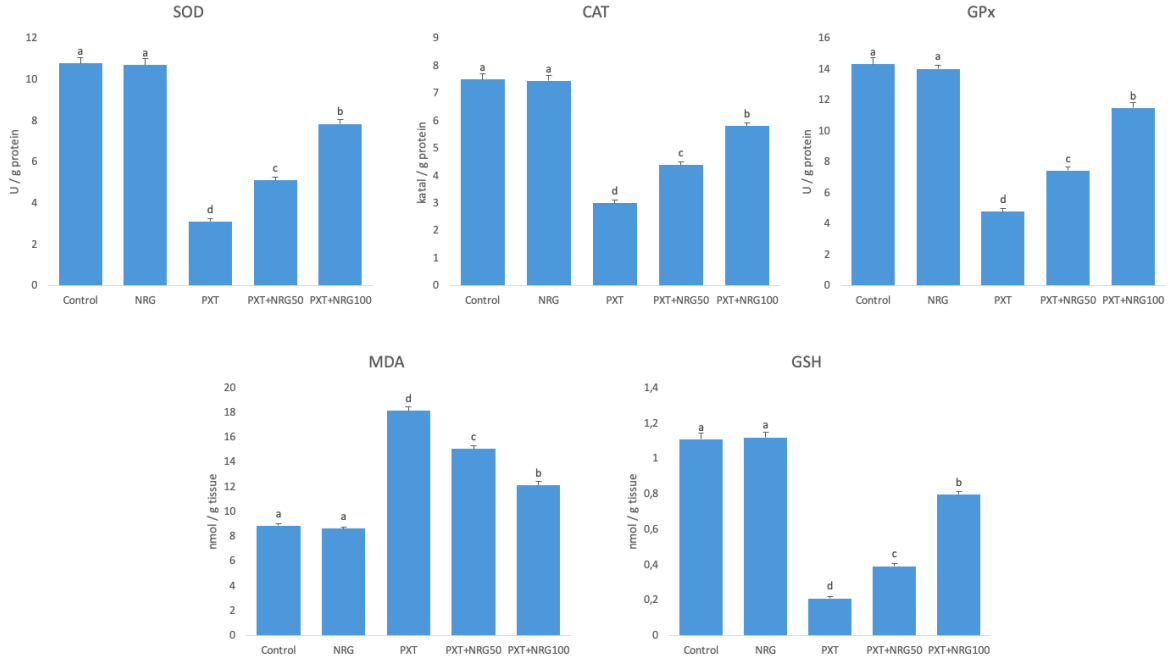

Figure 1: Effects of PTX and NRG administrations on oxidant and antioxidant markers in sciatic nerve tissues of rats. Values are given as Mean±SEM. Different letters indicate statistical difference: *$p<0.05$.

3.2. Inflammation Markers findings

Inflammation-related NF-κB, TNF-α, IL-1β, SIRT1, TLR4 and NRF2 in sciatic nerve tissues were analyzed by ELISA. There was an increase in NF-κB, TNF-α, IL-1β, SIRT1, TLR4 and NRF2 in the PTX compared to the control ($p<0.05$). PTX+NRG-50 and PTX+NRG-100 showed a decrease in all these parameters compared to PTX ($p<0.05$). When different doses of NRG were compared, 100 mg/kg was found to be more effective ($p< 0.05$) (Figure 2).

3.3. Apoptotic Markers Findings

In PTX, there was an increase in Bax ($p<0.05$) and a decrease in Bcl-2 ($p<0.05$). In PTX+NRG-50 and PTX+NRG-100, the changes in these parameters were reversed ($p<0.05$). NRG showed more pronounced effects at a dose of 100mg/kg (Figure 3).

4. Discussion

The taxane group of chemotherapeutics is a class of drugs commonly used in the treatment of many cancers. PTX is a taxane group chemotherapeutic that acts by inhibiting mitotic activity [20]. NRG is an active flavanone glycoside of grapefruit and various citrus plants with important biological effects such as antiulcer, antioxidant, anti-inflammatory, antiapoptotic and antihyperlipidemic [21, 22]. Therefore, in the current study, the effects of NRG on PTX-induced sciatic nerve tissue toxicity in rats were investigated.

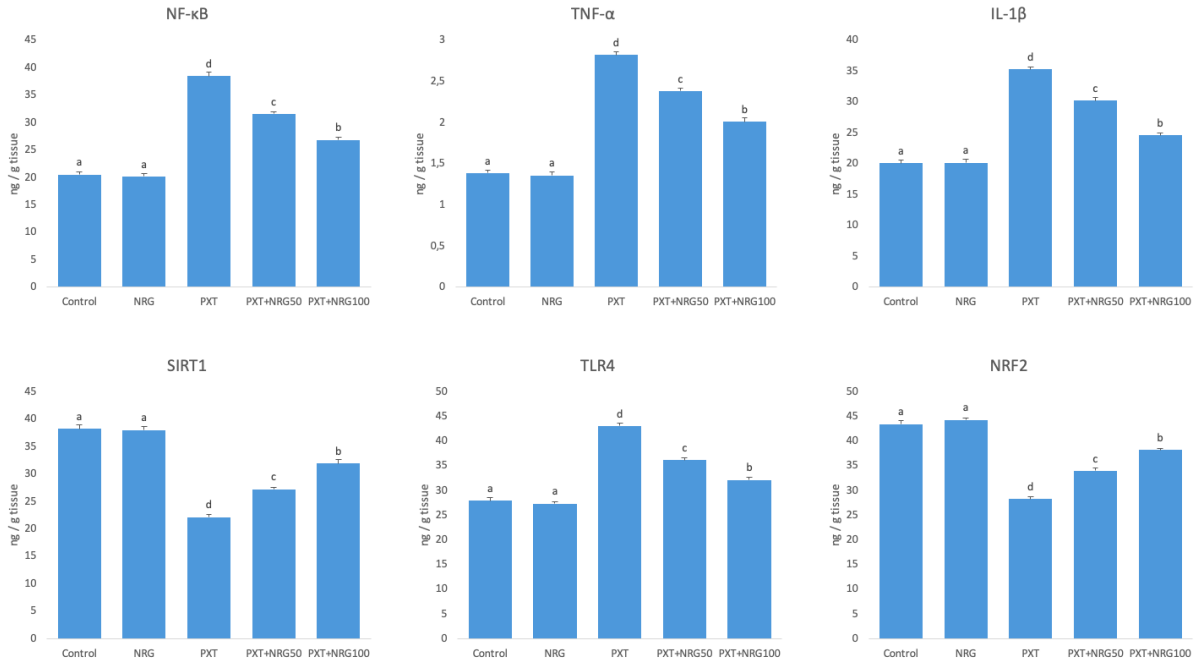

Figure 2: Effects of PTX and NRG administrations on NF-κB, TNF-α, IL-1β, SIRT1, TLR4 and NRF2 levels in sciatic nerve tissues of rats. Values are given as Mean±SEM. Different letters indicate statistical difference: *p<0.05.

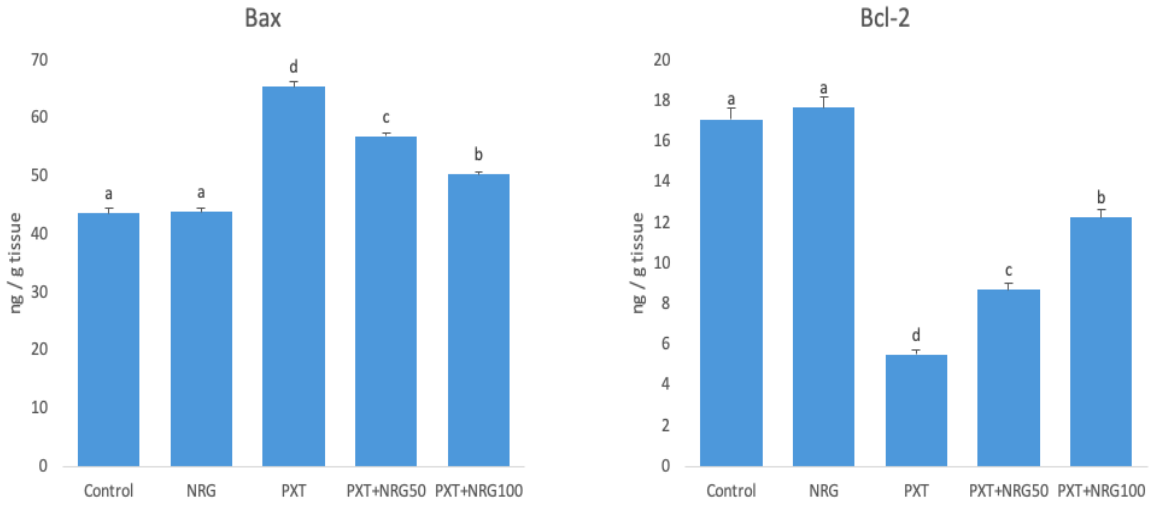

Figure 3: Effects of PTX and NRG administrations on Bax and Bcl-2 levels in sciatic nerve tissues of rats. Values are given as Mean±SEM. Different letters indicate statistical difference: *p<0.05.

ROS causes lipid peroxidation in cells and structural defects in proteins and nucleic acids [23]. Oxidative stress is an indicator of tissue damage characterized by an increase in ROS [24]. The end product of polyunsaturated fatty acid peroxidation is MDA [25]. GSH and GSH in tissues provide defense against ROS, highlighting their antioxidant and detoxification properties. GPx also contributes to antioxidant capacity by inhibiting lipid oxidation directly or indirectly together with GSH by reducing H_2O_2 [26]. Antioxidant enzymes contribute to cellular homeostasis by stabilizing ROS levels in healthy cells [27]. Antioxidant compounds are widely used to reduce the production and release of ROS [28]. In the current study, PTX increased oxidative stress by increasing MDA levels and decreasing antioxidant enzyme activities in sciatic nerve tissues. NRG administration, on the other hand, increased antioxidant activity by decreasing PTX-induced increased MDA levels and increasing antioxidant enzyme activities. This antioxidant activity was stronger, especially at 100 mg/kg.

Increasing evidence suggests that oxidative stress significantly triggers inflammation [29]. NF-κB is a transcription factor that is stimulated during inflammation in tissues and triggers the release of cytokines. With the activation of NF-kB, cytokines such as TNF-α and IL-1β are released and the inflammatory response is accelerated [30, 31]. In the current study, PTX caused inflammatory damage in sciatic nerve tissues by increasing NF-κB and related proinflammatory cytokines. NRG administration, on the other hand, reduced PTX-induced inflammatory damage by reducing NF-κB and related proinflammatory cytokines. NRG could emerge as an effective therapeutic agent in sciatic nerve tissue toxicity caused by inflammation due to PTX exposure.
Protective genes such as NRF2 are activated to protect against ROS-induced tissue damage. NRF2 is a redox-sensitive transcription factor that promotes transcription by binding to antioxidant response elements [32, 33]. NRF2 induction is a significant defense system in reducing damage against oxidative stress [34, 35]. There is growing evidence that TLR4 plays a pronociceptive role [36]. SIRT1, a histone deacetylase, is known to reduce neuropathic pain by activating NAD-dependent or NAD-independent pathways and inhibiting H4 acetylation. Activation of SIRT1 alleviates neuropathic pain [36]. In this study, PTX caused toxic damage to sciatic nerve tissues by increasing TLR4 and decreasing NRF2 and SIRT1. NRG administration reversed this situation and attenuated the damage.
Apoptosis (programmed cell death) is an essential physiological process that destroys and removes damaged or dangerous cells in the body [37, 38]. The increase of oxygen radicals negatively affects cellular activities related to intracellular signaling, such as the apoptotic pathway [39]. Bax and Bcl-2 play key roles in the mitochondrial pathway, an important pathway in apoptosis. As a result of the disrupted balance in the Bax/Bcl-2 ratio in favor of Bax, cytochrome c levels in the cytoplasm increase and enzymes that cause apoptosis such as Caspase-3 are activated [40]. In the current study, Bax levels, which is an apoptotic factor, increased with PTX exposure in sciatic nerve tissues, while Bcl-2, which is an antiapoptotic factor, decreased. When NRG was administered together with PTX, the opposite effect was observed and NRG exhibited antiapoptotic properties. Therefore, NRG may be an effective agent against apoptosis in PTX-induced sciatic nerve tissue toxicity.

5. Conclusions

In conclusion, PTX caused toxic effects by increasing inflammation, oxidative stress, and apoptosis damage levels in sciatic nerve tissue. On the other hand, NRG was found to reduce the toxic effect by decreasing all these damages. It can be concluded that administration of NRG, especially at 100mg/kg, would be much more effective in preventing sciatic nerve tissue damage in terms of all these pathways. We anticipate that NRG will increasingly come to the forefront of studies due to its powerful antioxidant properties and its inclusion in foods that are easily accessible in our daily diet. On the other hand, we anticipate that it will be effective in reducing the side effects of significant chemotherapeutic agents such as PTX used to treat cancer diseases, which are increasing worldwide, and in improving the quality of life of patients.

Limitations of the Study

The limitation of this study is that motor balance and coordination tests of rats could not be performed due to the lack of relevant devices.

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Conflict of Interests
There is no conflict of interest.
Financial Support
This study received no financial support.
Author Contributions
SY and FMK designed the research. SY, HŞ, SK, SA, EE and FMK participated in data collection and data analysis. SY and HŞ wrote the manuscript, read and approved the final script.
Ethical Approval
The study was approved by Erzurum Atatürk University Experimental Animal Ethics Committee (2023/07-113).
Data sharing statement
None.
Consent to participate
None applicable.
Informed Statement
None.

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