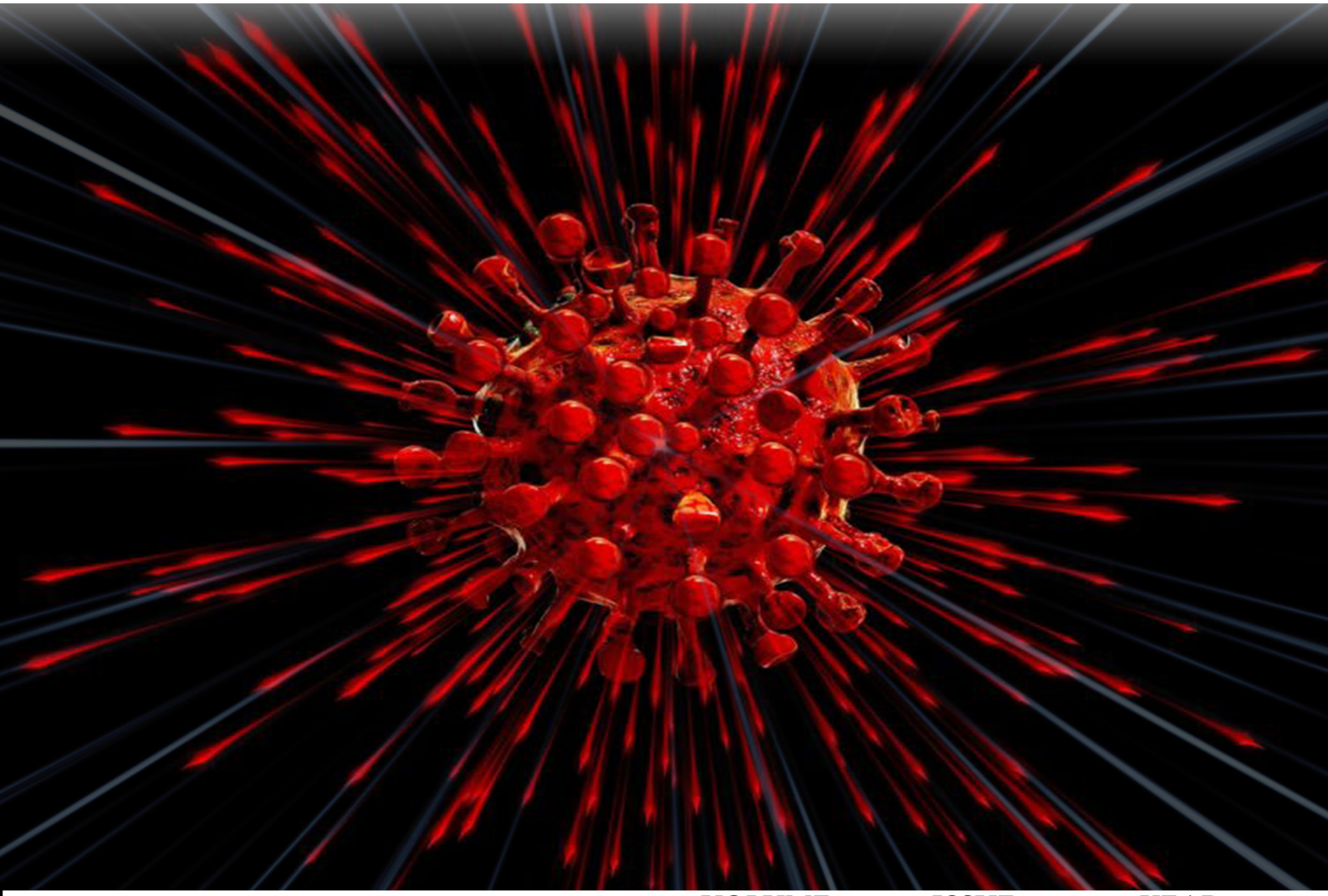
e-ISSN: 2718-0115

ACMJ

Anatolian Current Medical Journal



VOLUME: 6 ISSUE: 2 YEAR: 2024

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ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1410973

Anatolian Curr Med J. 2024;6(2):108-115

A biomarker for estimating no-reflow phenomenon in PCI-treated non-ST-segment elevation myocardial infarction patients: serum Cystatin C

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Cite this article as: Genç Ö, Yıldırım A, Erdoğan A. A biomarker for estimating no-reflow phenomenon in PCI-treated non-ST-segment elevation myocardial infarction patients: serum Cystatin C. *Anatolian Curr Med J.* 2024;6(2):108-115.

Received: 29.12.2023 ◆ **Accepted**: 18.01.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Cystatin C (Cys-C) is a biochemical marker associated not only with renal function but also with inflammatory processes. We aimed to investigate the relationship between the post-percutaneous coronary intervention (PCI) no-reflow phenomenon (NRP) and Cys-C in patients with non-ST-segment elevation acute coronary syndrome (NST-ACS).

Methods: This retrospective, single-center observational study consecutively enrolled patients who were hospitalized with a diagnosis of NST-ACS and underwent PCI between October 2021 and February 2022. Baseline characteristics, medications, admission laboratory parameters, and angiographic features were recorded. Logistic regression and sensitivity analyses were performed to identify parameters associated with NRP.

Results: Out of 199 patients (mean age: 62.0±10.3, 59.8% male), 36 (18.1%) developed NRP. Patients who developed NRP had a lower ejection fraction (49.7±10.3% vs. 53.5±7.1%, p=0.046) and were less likely to be male (36.1% vs. 65.0%, p=0.001). Additionally, individuals with NRP exhibited higher blood urea and C-reactive protein levels than those without NRP (p<0.05 for both). Similarly, serum Cys-C levels were elevated in the former group (1.44±0.57 vs. 1.07±0.40 mg/L, p=0.001). Multivariable logistic regression analysis demonstrated that Cys-C [odds ratio (OR)=4.793, p=0.014] and culprit lesion [OR=8.112, p=0.043 for LCx, OR=27.025, p=0.001 for RCA] were independently associated with NRP. Receiver operating characteristic curve analysis showed a cut-off point >1.1 mg/L for Cys-C determined NRP with 72.2% sensitivity and 66.9% specificity (area under the curve=0.711, p<0.001).

Conclusion: We have demonstrated a potential association between the serum Cys-C level at admission and the occurrence of NRP among NST-ACS patients undergoing PCI.

Keywords: Cystatin C, no-reflow phenomenon, non-ST-segment elevation acute coronary syndrome, percutaneous coronary intervention

INTRODUCTION

Despite the widespread use of percutaneous coronary intervention (PCI) and improvements in in-hospital care policies, as well as evidence-based antiplatelet regimens in recent years, acute coronary syndrome (ACS) remains a significant cause of morbidity and mortality worldwide.[1] While the term ACS has been classified into ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation acute coronary syndrome (NST-ACS) based on electrocardiographic features due to its diverse range of pathophysiological foundations, there is a demand for additional early risk stratification strategies to predict prognosis in both subgroups.[2] Hence, traditional scores such as Thrombolysis In Myocardial Infarction (TIMI) and The Global Registry of Acute Coronary Events (GRACE) risk scores, as endorsed by the European Society of Cardiology, along with electrocardiographic findings, clinical variables such as high-sensitivity cardiac troponin I, and more recently, biochemical markers indicating inflammation, have been employed to ascertain the optimal timing for implementing an invasive strategy in patients with NST-ACS.[3-5]

The no-reflow phenomenon (NRP) is a complex condition characterized by inadequate distal flow in the epicardial coronary artery during the procedure, despite the absence of angiographic evidence of mechanical obstruction, dissection, or spasm.[6] The incidence of NRP varies between 2.0% and 18.8% in

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patients undergoing primary or elective PCI, as reported in various studies and meta-analyses.[7,8] While the pathophysiology remains unclear, distal embolization, high thrombus burden, ischemic injury, and reperfusion injury are commonly implicated factors in ACS. The cause is typically multifactorial and individual.[9-11] The occurrence of NRP after PCI is closely linked to major adverse cardiovascular events, including heart failure (HF), stroke, myocardial injury, ventricular arrhythmias, and in-hospital mortality when compared to cases with normal coronary flow.[12,13]

In contemporary research, low molecular-mass plasma proteins have emerged as crucial contributors to both physiological and pathological conditions. Cys-C, a member of the type 2 cystatin superfamily, serves as an endogenous inhibitor of cysteine proteinases. The kidney acts as the primary catabolic pathway for Cys-C, which is found in nearly all body fluids. This proteinase undergoes nearly complete elimination from circulation through glomerular ultrafiltration, with minimal peritubular reuptake.[14] Consequently, it stands out as a superior marker for estimated glomerular filtration rate (e-GFR) compared to creatinine.[15] The advent of fast, accurate, and widely available immunoassay methods now enables the routine use of Cys-C as a marker for various clinical conditions beyond renal function. Further substantiating this, certain studies have indicated the utility of Cys-C in post-PCI risk stratification and the prediction of adverse cardiac events in ACS.[16,17] In our study, the primary objective was to clarify the role of Cys-C in determining NRP in patients presenting with NST-ACS and undergoing PCI.

METHODS

Ethical Statement

The design of the present study received approval from the Adana City Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.12.2023, Decision No: 3051), and the research was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Study Design and Population

We included a total of 199 consecutive patients who were admitted to Adana City Training and Research Hospital between October 2021 and February 2022 with symptoms related to ischemia in this single-center, retrospectively designed study. These patients were diagnosed with NST-ACS and subsequently underwent PCI. Diagnosis and treatment protocols followed the current guidelines of the European Society of Cardiology.[18] Various parameters, including epidemiological, demographic, clinical, laboratory, echocardiographic, and procedural data, were systematically recorded. Patients meeting any of the following criteria were excluded from the study: age <18 years, chronic lung and liver disease, hereditary coagulation disorders, history of malignancy, previous fibrinolytic therapy, active infection, autoimmune connective tissue disease, medical decision after coronary angiography or inability to undergo PCI, e-GFR <60 mL/min/1.73 m², and cases with missing files and records (**Figure 1**). Echocardiographic examinations were conducted during hospitalization by independent cardiologists who were blinded to the study data, following the guidelines of the European Association of Cardiovascular Imaging.[19]

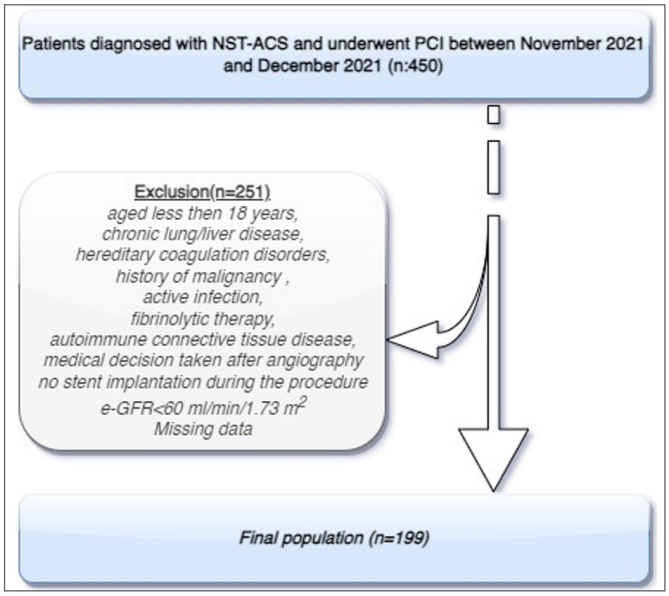

Figure 1. Flow chart

The medical histories of patients were retrieved from both the hospital records and the national health registry systems. Hypertension was defined as a systolic blood pressure greater than 140 mmHg and/or a diastolic blood pressure greater than 90 mmHg, or the use of antihypertensive medication. In addition to the presence of signs and symptoms, diabetes mellitus (DM) was diagnosed in individuals who met at least one of the following criteria: fasting blood glucose of ≥126 mg/dl, 2-hour post-load plasma glucose of ≥200 mg/dl, HbA1c ≥6.5%, or random blood glucose of ≥200 mg/dl.

Laboratory Analysis

The blood samples were collected in EDTA tubes at the emergency triage unit upon the patient's admission. Biochemical samples were promptly analyzed using a fully automated systems analyzer (Roche Diagnostics, Indianapolis, USA) without delay. Plasma high-sensitivity cardiac troponin I was examined using Beckman Coulter automatic analyser (Beckman

Coulter, Brea, CA). Cys-C samples were obtained from the antecubital vein within the first 6 hours of hospital admission and before the PCI procedure. These samples were analyzed using fully Siemens nephelometric analyzer (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The range of the kit used for analysis is 0.56-0.99 mg/L.

Coronary Angiography

Coronary angiographies for all patients were conducted using SIEMENS AXIOM Artis zee 2011 equipment (Siemens Healthcare, Erlangen, Germany) through the femoral or radial route with a 6-7 French catheter, adhering to current recommendations. Prior to the procedure, all patients received an intravenous bolus of unfractionated heparin at a dose of 70-100 IU/kg. Throughout the procedure, pre- and post-dilatation, the use of glycoprotein IIb/IIIa inhibitors, and thrombus aspiration were carried out at the discretion of the operator. The stents implanted were new-generation drug-eluting stents, with their diameter and length left to the operator's choice. Loading and maintenance doses of oral P2Y12 inhibitors after the procedure [prasugrel (60 mg loading-10 mg daily maintenance), ticagrelor (180 mg loading-180 mg daily maintenance), and clopidogrel (600 mg loading-75 mg daily maintenance)] were administered in accordance with current guidelines. Acetylsalicylic acid loading (300 mg) took place before the procedure, and a low dose (81-100 mg) was maintained post-procedure. Subsequently, angiographic images were independently evaluated by two cardiologists. The flow status in the infarct-related artery after PCI was assessed using the TIMI flow grading system.[20] The NRP was defined as a TIMI score ≤2 without significant residual obstruction and/or flow-limiting conditions, while normal flow was designated as a TIMI score of 3.[21]

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 20; Armonk, NY: IBM Corp., SPSS Inc., Chicago, Illinois, USA). Continuous variables were assessed for distribution, and normality was determined using histogram graphs, skewness, and kurtosis measures, and the Kolmogorov-Smirnov test. Normally distributed variables were presented as mean ± standard deviation, while non-normally distributed variables were reported as median (interquartile range: 25th-75th percentile). Categorical variables were expressed as frequency and percentage. The χ^2 (Chi-square) test was employed for comparing categorical parameters between groups. For normally distributed groups, the independent two-sample t-test was used, whereas the Mann-Whitney U test was applied for the comparison of non-normally distributed numerical parameters when comparing two groups. Variables with a significant p-value of <0.05 in hypothesis testing were subjected to univariable logistic regression analysis. Multivariable analysis for regression was then conducted with parameters having p<0.05 in univariable logistic regression analysis. Receiver Operating Characteristic (ROC) analysis was performed to assess the discriminative ability of Cys-C in determining NRP. The cut-off point for Cys-C was established based on the Youden index. Statistical significance was defined as p<0.05.

RESULTS

The mean age of the 199 NST-ACS patients included in the study was 62.0±10.3 years, and 59.8% were male. NRP developed in 36 (18.1%) patients who underwent PCI. Patients who developed NRP had a lower left ventricular ejection fraction (EF) (49.7±10.3% vs. 53.5±7.1%, p=0.046) and were less likely to be male (36.1% vs. 65.0%, p=0.001). Risk factors were similar between the groups with and without post-PCI NRP. Similarly, medications used at admission were also comparable. When laboratory parameters were evaluated, urea (43.5±16.9 vs. 35.2±13.6 mg/dl, p=0.008) and C-reactive protein (CRP) (7.1 vs. 3.8 mg/dl, p=0.002) were higher in the NRP group. Additionally, Cys-C was higher in those with NRP than those without NRP (1.44±0.57 vs. 1.07±0.40 mg/L, p=0.001) (**Figure 2**). Angiographic characteristics were similar between the two groups except for the culprit lesion. Detailed baseline characteristics, risk factors, medications, and angiographic features of the study population are shown in **Table 1**.

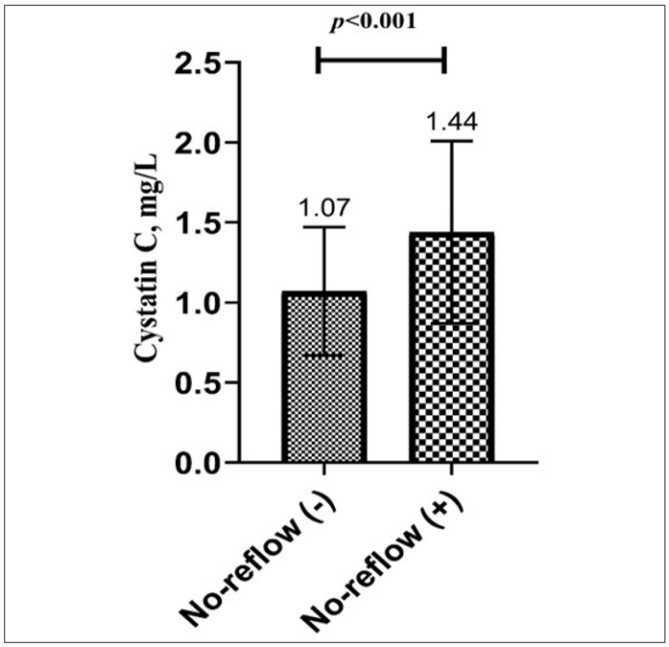

Figure 2. Categorizing mean serum Cystatin C levels based on coronary no-reflow status.

Table 1. Baseline features, risk factors, medications, and angiographic data for the study population

Variables	All (n=199, 100%)	No-reflow phenomenon		p*
		No (n=163, 81.9%)	Yes (n=36, 18.1%)	
Baseline Characteristics and Risk Factors				
Age (years)	62.0±10.3	61.6±10.2	63.8±10.6	0.243
Male gender, n (%)	119 (59.8)	106 (65.0)	13 (36.1)	0.001
Ejection fraction, %	52.8±7.9	53.5±7.1	49.7±10.3	0.046
Diabetes mellitus, n (%)	93 (46.7)	77 (47.2)	16 (44.4)	0.761
Hypertension, n (%)	62 (31.2)	49 (30.1)	13 (36.1)	0.478
Hyperlipidemia, n (%)	43 (21.6)	36 (22.1)	7 (19.4)	0.727
Family history of CVD, n (%)	18 (9.0)	17 (10.4)	1 (2.8)	0.206
Current smoker, n (%)	36 (18.1)	32 (19.6)	4 (11.1)	0.229
Medications taken by patients upon admission, n (%)				
ACEi /ARB	31 (15.6)	27 (16.6)	4 (11.1)	0.414
Beta-blockers	20 (10.1)	19 (11.7)	1 (2.8)	0.134
Calcium channel blockers	7 (3.5)	6 (3.7)	1 (2.8)	0.629
Diuretics	5 (2.5)	4 (2.5)	1 (2.8)	0.635
Statins	7 (3.5)	7 (4.3)	0 (0)	0.355
Laboratory findings				
WBC, 10^3/µl	8.4±2.5	8.3±2.5	8.4±2.5	0.988
Hemoglobin, mg/dl	13.2±2.0	13.4±1.9	12.4±2.6	0.032
Platelet count, 10^3/µl	238±73	237±71	243±83	0.662
Urea, g/dl	36.7±14.6	35.2±13.6	43.5±16.9	0.008
Creatinine, mg/dl	0.83±0.21	0.82±0.20	0.87±0.23	0.213
LDL-C, mg/dl	125±47	127±49	117±37	0.236
HDL-C, mg/dl	44±12	44±11	44±17	0.991
Total cholesterol, mg/dl	193±65	195±67	182±56	0.278
Triglycerides, mg/dl	149 (110-208)	153 (112-223)	134 (95-168)	0.056
C-reactive protein, mg/dl	4.3 (2.0-9.8)	3.8 (1.8-8.3)	7.1 (3.2-30.9)	0.002
BNP, pg/ml	1153 (282-3576)	1075 (225-3306)	1668 (437-6280)	0.041
hs-cTnI, pg/ml	3800 (717-6850)	3666 (658-6850)	4400 (1175-7164)	0.411
Angiographic features				
Culprit lesion, n (%)				<0.001
LAD	69 (34.7)	67 (41.1)	2 (5.6)	
LCx	32 (16.1)	27 (16.6)	5 (13.9)	
RCA	98 (49.2)	69 (42.3)	29 (29.6)	
Stent diameter, mm	3.0 (2.25-3.0)	3.0 (2.25-3.5)	2.5 (2.25-3.0)	0.375
Stent length, mm	27 (8-36)	21 (12-54)	28 (12-36)	0.528
Pre-dilatation, n (%)	126 (63.3)	101 (62.0)	25 (69.4)	0.399
Post-dilatation, n (%)	67 (33.7)	55 (33.7)	12 (33.3)	0.963
Thrombus aspiration, n (%)	18 (9.0)	16 (9.8)	2 (5.6)	0.537
Gp IIb/IIIa receptor inhibitors, n (%)	21 (10.6)	18 (11.0)	3 (8.3)	0.632

Values are n (%), median (interquartile range [IQR]), or mean± standard deviation. P value was calculated using an independent samples t-test or the Mann-Whitney U-test for continuous variables and a chi-squared test or the Fisher's exact test for categorical variables, as appropriate. Abbreviations: ACEi, Angiotensin-Converting enzyme inhibitors; ARB, angiotensin receptor blockers; BNP, Brain natriuretic peptide; CVD, cardiovascular disease; Gp, Glycoprotein; hs-cTnI, high-sensitivity cardiac troponin I; HDL-C, High-density lipoprotein cholesterol; LAD, left anterior descending; LCx, left circumflex; LDL-C, low-density lipoprotein cholesterol; RCA, right coronary artery; WBC, white blood cell. *p<0.05 was considered significant.

The univariable logistic regression analysis revealed that sex [Odds Ratio (OR)=0.304, 95% Confidence Interval (CI):0.143-0.645, p=0.002, for male], left ventricular EF [OR=0.946, 95% CI:0.906-0.988, p=0.012], urea [OR=1.035, 95% CI:1.011-1.059, p=0.003], CRP [OR=1.018, 95% CI:1.003-1.034, p=0.019], brain natriuretic peptide [OR=1.010, 95% CI:1.003-1.017, p=0.007], Cys-C [OR=4.483, 95% CI:2.097-9.581, p<0.001], and culprit lesion [OR=6.204, 95% CI:1.134-33.945, p=0.035 for LCx, OR=14.080, 95% CI:3.231-61.346, p<0.001 for RCA] are associated with NRP. When these parameters were entered into multivariable logistic regression analysis, serum Cys-C [OR=4.793, 95% CI:1.371-16.763, p=0.014] and culprit lesion [OR=8.112, 95% CI:1.067-61.685, p=0.043 for LCx, OR=27.025, 95% CI:4.174-174.967, p=0.001 for RCA] were independent predictors of NRP (**Table 2**). ROC curve analysis showed that a cut-off point >1.1 mg/L for Cys-C determined NRP with 72.2% sensitivity and 66.9% specificity (Area Under the Curve=0.711, 95% CI:0.613-0.809, p<0.001) (**Figure 3**).

Table 2. Univariable and multivariable logistic regression analyses of no-reflow phenomenon

Variable	Univariable			Multivariable+		
	OR	95% CI	p*	OR	95% CI	p*
Gender, male	0.304	0.143-0.645	0.002	0.471	0.199-1.114	0.087
Ejection fraction	0.946	0.906-0.988	0.012	1.010	0.945-1.080	0.771
Urea	1.035	1.011-1.059	0.003	0.999	0.964-1.035	0.956
C-reactive protein	1.018	1.003-1.034	0.019	1.001	0.985-1.018	0.880
BNP	1.010	1.003-1.017	0.007	1.008	0.998-1.019	0.133
Cystatin C	4.483	2.097-9.581	<0.001	4.793	1.371-16.763	0.014
Culprit lesion			0.001			0.001
LAD	1 (ref)	1 (ref)	-	1 (ref)	1 (ref)	-
LCx	6.204	1.134-33.945	0.035	8.112	1.067-61.685	0.043
RCA	14.080	3.231-61.346	<0.001	27.025	4.174-174.967	0.001

Abbreviations: BNP, brain natriuretic peptide; CI, confidence interval; LAD, left anterior descending; LCx, left circumflex; OR, odds ratio; RCA, right coronary artery. +Model performance parameters: Omnibus tests of model coefficients p<0.001, Model chi-square=51.2, -2 Log likelihood=136.9, Hosmer-Lemeshow test p=0.968. *p<0.05 was considered significant.

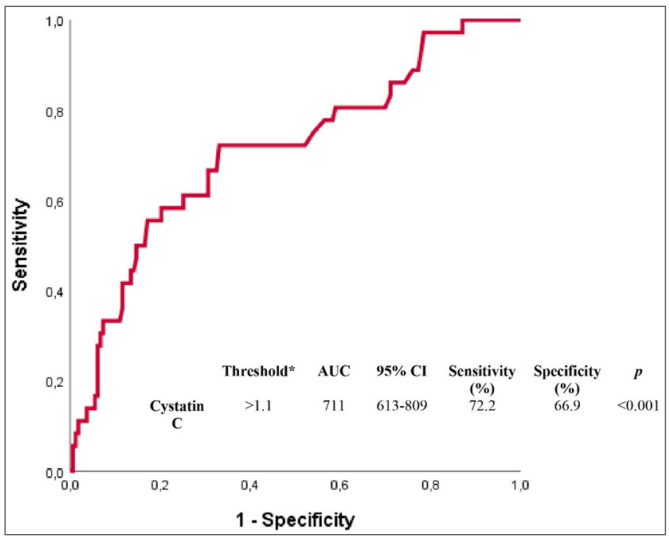

Figure 3. Receiver operating characteristic curve analysis for Cystatin C in the detection of the no-reflow phenomenon among NST-ACS patients undergoing PCI. Abbreviations: AUC, Area Under the Curve; CI, Confidence Interval. * The cut-off point was established based on the Youden index.

DISCUSSION

Our study, investigating the role of Cys-C in detecting NRP, can be summarized with the following key findings: (i) NRP was detected in 36 out of 199 NST-ACS patients undergoing PCI, constituting 18.1% of the cohort. (ii) Cys-C and the culprit lesion were found to be associated with NRP. (iii) A cut-off point >1.1 mg/L for Cys-C determined NRP with 72.2% sensitivity and 66.9% specificity.

In current guidelines, the recommended treatment option for NST-ACS patients involves triage based on risk stratification, with revascularization of eligible patients through PCI.[22] A successful invasive strategy typically results in the improvement of coronary microcirculation within 24 hours post-PCI. However, NRP, a significant complication associated with PCI of the infarct-related artery, can lead to microcirculatory disturbances, suboptimal ventricular remodeling, enlargement of the infarct, early signs of HF, and impaired cardiac function due to inadequate blood flow.[23] Despite advancements in PCI techniques, the utilization of new-generation stents, improved intracoronary imaging modalities, and the implementation of new-generation antiplatelet therapy regimens, the incidence of NRP remains high. In our study, we observed an NRP incidence of 18.1%, consistent with a wide range of 5-60% reported in other studies.[24,25] This variability may be partly attributed to clinicopathological conditions such as demographic factors (e.g., gender), medical history (hypertension, diabetes, and hypercholesterolemia), clinical presentation (long total ischemic time, presentation with shock), laboratory parameters (poor renal function, increased inflammatory markers), and per-procedural factors (high thrombus burden).[23,26-28] NRP is more frequently observed, particularly in degenerated saphenous veins and following rotational atherectomy. Despite the availability of alternative treatments such as intracoronary vasodilators, glycoprotein IIb/IIIa platelet receptor antagonists, and intracoronary epinephrine, there is no universally accepted standard treatment.[23,29] Therefore, identifying factors related to NRP may represent the most effective approach for preventing adverse outcomes.30

Cystatin C, a cysteine protease inhibitor, has been previously investigated in patients presenting with ACS. Previous studies and meta-analyses have reported that Cys-C plays a prognostic role, is a predictor of mortality, and is useful in risk stratification. However, data regarding its association with PCI-related adverse events is limited. Cheng et al.[30] identified an independent association between admission Cys-C levels and NRP among STEMI patients treated with primary PCI. Another study found an independent association between Cys-C and NRP in 68 STEMI patients.[31] In our study, we observed similar results in patients with NST-ACS. Several mechanisms may elucidate the potential role of Cys-C in patients with ACS and its association with NRP. The first and most well-established mechanism is the robust correlation

between Cys-C and renal function. In a study involving 726 NST-ACS patients, Jernberg et al.[2] demonstrated that Cys-C was an independent predictor of mortality and adverse outcomes. Upon categorizing patients based on serum Cys-C levels, they observed a 12-fold higher mortality rate in the 4th quartile compared to the 1st quartile. This association was attributed to Cys-C being a more reliable indicator of renal function and the robust connection between renal function and mortality in NST-ACS patients. The well-established association between renal function and mortality has been attributed to various mechanisms, including atherosclerosis and vascular damage.[32] Renal dysfunction can accelerate atherosclerosis by inducing alterations in pathways such as glucose metabolism, blood pressure, lipids, lipoproteins, homocysteine, and inflammatory processes.[33]

Although our study did not include a mortality analysis, it affirmed the association between NRP and Cys-C, a significant indicator of adverse events. The role of Cys-C in interpreting our findings is crucial due to the established link between poor prognosis and renal function in ACS patients. However, it's noteworthy that our study comprised patients with creatinine-based e-GFR >60 ml/min/1.73 m², which might be a limitation in predicting renal failure. Hence, a more accurate e-GFR estimation would enhance the predictive ability of outcomes in NST-ACS patients. Nonetheless, the association of elevated Cys-C with NRP may not be entirely attributable to renal dysfunction; it could also be explained by the association between Cys-C and HF. For instance, in a large cohort study involving 5956 ACS patients, Lou et al.[34] observed correlations between Cys-C and brain natriuretic peptide, left ventricular EF, and ventricular diameters. Furthermore, a close association was identified between Cys-C and adverse cardiovascular events as well as all-cause mortality in ACS. This association may suggest that Cys-C could serve as a biomarker reflecting systemic dysregulation and pathophysiological pathways in early HF. In a separate study, Suthahar et al.[35] demonstrated the association between Cys-C and HF in both sexes. It has been observed that the incidence of NRP after PCI may be higher in patients with a prolonged duration of ischemia, a large area of myocardial involvement, a diagnosis of HF, or a history of myocardial infarction. Pantea-Roşan et al.[36] found that NRP was associated with lower EF and short-term complications in 942 STEMI patients. In a meta-analysis of 27 studies, NRP was linked to Killip class ≥2, elevated creatinine, and lower EF.[8]

Another potentially significant role of Cys-C is its potential contribution to the link between inflammatory mechanisms and NRP. In a large cohort study, Grubb et al.[37] reported that elevated serum Cys-C and CRP levels were associated with increased cardiovascular events and death in elderly patients. Urbonaviciene et al.[38] identified a significant correlation between Cys-C and high-sensitivity CRP. These studies suggest that Cys-C may serve as a marker of inflammation. Consequently, inflammation-related atherosclerotic changes, Cys-C, and cardiovascular diseases may be implicated in the mechanisms associated with NRP. However, there are also studies with conflicting results on this issue. Some argue that the relationship between Cys-C and CRP may reflect the early stages of chronic kidney disease rather than the inflammatory process.[39] Grubb et al.[37] demonstrated that Cys-C was not associated with CRP when adjusted for renal function. Niccoli et al.[40] proposed that Cys-C is linked to atherosclerotic burden in a CRP-independent manner. In this context, we recognize that Cys-C is a better indicator of renal function. In summary, the role of Cys-C in predicting cardiovascular events may be more relevant to renal function.

Limitations

This study has certain important limitations. Foremost among these is its nature as a single-center retrospective investigation, including a limited patient cohort, which may potentially constrain the generalizability of our findings. Additionally, the study lacks control for temporal changes in Cys-C levels and does not delve into the prognostic implications of these observed outcomes. Lastly, due to its retrospective design, various confounding factors associated with NRP could not be comprehensively addressed in the analysis. To enhance the robustness of our conclusions, it is imperative to conduct prospective, large-scale studies that systematically explore the prognostic implications of NRP in NST-ACS patients with a focus on Cys-C levels. These future investigations may facilitate a more comprehensive understanding of the prognostic impact of NRP in the context of Cys-C and enable more nuanced and widely applicable insights into its clinical significance.

CONCLUSION

This study has revealed the role of Cys-C in determining NRP among NST-ACS patients undergoing PCI. The clarification of these processes contributes to a comprehensive understanding of the nature of NRP and, consequently, may aid in its prevention and improved treatment. Enhancing the identification and treatment of no-reflow to ensure adequate blood flow in infarct-related arteries could yield significant benefits, including the reduction of infarct area expansion, subsequent ventricular remodeling, and ultimately a decrease in the rates of congestive HF and mortality.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was initiated with the approval of the University of Health Sciences, Adana City Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.12.2023, Decision No: 3051).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1409450

Anatolian Curr Med J. 2024;6(2):116-120

Relationship between coronary artery disease and high-density lipoprotein cholesterol-monocyte ratio

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Cite this article as: Özkan E, Erdoğan A. Relationship between coronary artery disease and high-density lipoprotein cholesterol-monocyte ratio. *Anatolian Curr Med J.* 2024;6(2):116-120.

Received: 25.12.2023 ◆ **Accepted**: 23.01.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: To investigate the association between high-density lipoprotein cholesterol (HDL-C)-to- monocyte ratio(HMR) and coronary artery disease (CAD) proven by coronary angiography.

Methods: A total of 311 patients were enrolled in the retrospective study. The grouping was based on whether the stenosis of one coronary artery exceeds 50%. Gensini Score (GS) to evaluate the severity of coronary artery stenosis. Spearman correlation analysis, progressive logistic regression, and receiver operating characteristic (ROC) curve analysis were used.

Results: The HMR difference between the CAD and non-CAD groups was statistically significant (P<0.001). Spearman correlation analysis showed that the HMR was slightly and moderately correlated with other independent risk factors of CAD (r<0.5). HMR was a new independent risk factor by progressive logistic regression analysis (odds ratio=0.784. 95% CI, 0.682-0.901). HMR was related to the GS of CAD patients (r=-0.244, P<0.001). Moreover, the HMR was better than HDL-C and HDL-C-to-cholesterol by comparing AUC (AUC HDL-C-to-monocyte=0.642, AUC HDL-C=0.603, AUC HDL-C-to-cholesterol=0.584).

Conclusion: HMR was an independent factor of CAD and can be used as a biomarker to evaluate the severity of CAD.

Keywords: Coronary artery disease, HDL-C-to-monocyte ratio, coronary angiography

INTRODUCTION

Despite the rapid advancements in diagnosis and treatment, coronary artery disease (CAD) continues to be the primary cause of mortality in developed nations. The most prevalent etiology is atherosclerosis, which is classified as a systemic inflammatory immune disorder.[1] Inflammation, endothelial dysfunction and oxidative stress are essential mechanisms of the atherosclerotic process.[2]

Recently, high density lipoprotein cholesterol (HDL-C) and monocyte ratio (HMR) research has been gradually found and wildly applied to heart disease, diabetes, and other aspects. HMR is a recently emerged inflammation-based marker. The marker, circulating monocytes give rise to inflammatory and pro-thrombotic through releasing various molecules and cytokines to activate platelets and endothelial cells.[3] On the contrary, HDL-C hinders low-density lipoprotein (LDL) oxidation and macrophage migration and rapid cholesterol efflux from these cells leading to anti-inflammation and antioxidation.[4,5] Research on inflammatory markers associated with atherosclerosis has been a top topic in recent years, including HDL-C and monocytes. This study aimed to investigate the independence between HMR and multiple factors of coronary artery disease and the relevance of HMR in coronary artery severity.

METHODS

The Research Ethics Institute of the in our hospital of reviewed this study involving human subjects. It is approved by Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 13.12.2023, Decision No: KAEK/2023.12.714). This study was conducted per the guidelines outlined in the Declaration of Helsinki.

311 patients who were discharged from the Cardiology department after coronary angiography (CAG) in our hospital between January 1, 2020 and July 12, 2023 were included in the study. 165 patients with CAD were selected as the CAD group, 146 patients including coronary arteriosclerosis with 1%-50% stenosis and no coronary atherosclerosis as the non-CAD group.

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Exclusion criteria record previous coronary artery bypass grafting, previous coronary artery stenting, incomplete or unobtainable documented monocyte count, HDL-C, or other essential data, continuous use of lipid-lowering drugs for more than three months, malignant tumors, severe liver and kidney dysfunction, blood system diseases, immune system diseases, undergoing immunosuppressive therapy, major surgery and severe trauma within three months.

Coronary Angiography Methods

The Judkins technique was utilized to perform CAG via the right or left femoral approach with a French (F) catheter of 6 or 7. As an opaque agent, either Iopromide (Ultravist-370®) or Iohexol (Omnipaque® 350 mg/ml) was utilized. Coronary artery imaging was performed in the left and right oblique planes, with cranial and caudal orientation, on all patients. CAG image evaluations of all patients were performed by two experienced cardiologists. The intra- and interobserver variability for assessing CCC was 2% and 3%, respectively.

Severe CAD was defined as >50% stenosis in a major coronary artery. The Gensini scoring (GS) system was used to determine the severity of CAD.[6] This method scores the prevalence and severity of coronary artery stenosis as well as its classification. Firstly, in this system, stenosis between 1% and 25% is scored as 1 point, between 26% and 50% as 2 points, between 51% and 75% as 4 points, between 76% and 90% as 8 points, between 91% and 99% as 16 points, and total occlusion as 32 points. This score is then multiplied by a factor indicating the importance of the localization of the lesion in the coronary artery system. Scores for localization are multiplied by 5 for the left main coronary, 2.5 for the proximal left anterior descending (LAD) and left circumflex (LCX), 1.5 for the mid-segment LAD and LCX, 1 for the distal segment of the LAD and LCX, 0.5 for the first diagonal branch, first broadside branch, right coronary artery, posterior descending artery and intermediate arteries, and second diagonal and second broadside branches. After obtaining GS in CAD patients, receiver operating characteristic (ROC) analysis was performed to define cut-off values. CAD patients were divided into two groups: below the cut-off value and above the cut-off value.

The first group was composed of patients with mild atherosclerosis (GS<25 points [mild CAD group]) and the second group was composed of patients with severe atherosclerosis (GS≥25 points [severe CAD group]), and this grouping was performed as follows: consistent with the literature.[7]

Laboratory Measurements

In all patients, blood samples were collected between 08 and 10 am following a 12-hour fasting period before CAG for laboratory analysis. Antecubital venous blood samples were collected in tripotassium EDTA-based anticoagulated tubes. Venous blood samples measuring basic blood variables (such as comprehensive metabolic panel and complete blood count) and thiol levels were obtained. All routine biochemical tests were performed on an autoanalyzer (Roche Diagnostic Modular Systems, Tokyo, Japan). Haematological parameters were stored at 4°C and evaluated with a Sysmex K-1000 autoanalyzer within 30 min after sampling. HMR was found by dividing the HDL-C level by the number of monocytes.

Transthoracic Echocardiography

Transthoracic echocardiography was conducted on every participant in both the patient and control groups. The measurements were conducted with a Vivid 5 machine, (GE Medical System in Horten, Norway), equipped with a 3.5 MHz transducer. We conducted 2D echocardiographic measures to evaluate the left ventricular ejection fraction (LVEF) and identify any valvular diseases. We employed Simpson's technique and color Doppler echocardiography to evaluate the ejection fraction and valvular diseases, respectively, in the apical 4-chamber view.

Statistical Analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc, Armonk, NY, USA). Baseline characteristics were divided into two groups according to coronary angiographic findings and were presented as mean (SD) or IQR for continuous data and frequency (percent) for categorical data. We compared two groups by the independent-sample t-test for continuous variables and the $\chi 2$ test for categorical ones. A two-tailed P value <0.05 was deemed statistically significant.

Spearman correlation coefficient was calculated to describe the correlation of HMR and other CAD risk factors included in the study, of HMR and GS. The forward, conditional progressive logistic regression analysis within a 95%confidence interval and at a two-ailed P value < 0.05 was performed to identify that HMR was an independent risk factor. Obtain AUC by drawing a ROC curve to compare the performance of HMR, HDL-C, and HDL-C-to-cholesterol models.

RESULTS

After excluding patients who met the exclusion criteria, 311 patients were included. In the light of the results of coronary angiography,165 patients entered the non-CAD group, and 146 patients entered the CAD group. Compared with the non-CAD group, the CAD group had higher levels of age, smoking, hypertension, diabetes mellitus, LVEF, Neutrophils count, and monocyte count

and had lower levels of HDL-C and higher involvement rate of the left main coronary artery, left anterior descending, left circumflex artery, right coronary artery, and their main branches (**Table 1**).

Table 1. Baseline clinical and angiographic characteristics of the study population

	Non-CAD group (n=165)	CAD group (n=146)	P value
Male, n(%)	75 (45.4)	98 (67.1)	<0.001
Age (years)	65.17±7.51	68.16±8.26	0.03
Smoking, n (%)	35 (21.2)	61 (41.7)	<0.001
HTN, n (%)	80 (48.4)	74 (50.6)	0.475
DM, n (%)	35 (21.2)	46 (31.5)	0.006
LVEF (%)	59.00 (54.00-67.00)	62.00 (56.00-68.00)	0.04
TC (mmol/L)	4.67±1.19	4.58±1.23	0.415
TG (mmol/L)	1.78±1.12	1.85±1.28	0.434
C-LDL (mmol/L)	2.97±1.01	2.96±1.13	0.921
HDL-C (mmol/L)	1.21±0.36	1.09±0.37	<0.001
NEUP (10^9/L)	4.74 (2.60-7.56)	5.63 (3.82-7.47)	<0.001
LYMPH (10^9/L)	1.92±0.58	2.03±0.47	0.934
MONO (10^9/L)	0.46±0.29	0.54±0.24	<0.001
Target coronary artery			
LMS, n (%)	2 (1)	12 (8.9)	<0.001
LAD, n (%)	42 (25.4)	120 (82.1)	<0.001
LCx, n (%)	11 (6.6)	130 (89.0)	<0.001
RCA, n (%)	52 (17.6)	91 (62.3)	<0.001
HDL-C/monocyte	2.91 (1.90-3.90)	2.07 (1.55-2.86)	<0.001

HTN, Hypertension; DM, Diabetes mellitus; TC, Cholesterol; TG, Triglycerides; C-LDL, low density lipoprotein cholesterol; HDL-C, High density liptein cholesterol; LVEF, Left Ventricular Ejection Fractions; NEUP, Neutrophils count; LYMPH, Lymphocyte count; MONO, monocyte count; LMS, left main coronary artery; LAD, left main coronary artery; LCx, Left circumflex artery; RCA, Right coronary artery

The CAD risk factors involved in the study were analyzed for correlation. Due to that the variables were Dichotomous and continuous variables, and Kolmogorov-Smirnov test at two-tailed for continuous variables showed that all variables did not conform to the normal distribution (P<0.001), Spearman correlation analysis was used. The results revealed that HMR was slightly and moderately correlated with other CAD risk factors (r<0.5) and LDL was strongly correlated with cholesterol (r=0.816).

To determine the independent risk factors of CAD. Multivariable logistic regression analysis was conducted for the variables included in the study, and the progressive logistic regression analysis at forwarding conditional was conducted. Age, Smoking, Neutrophils count, and HMR be used as independent risk factors for CAD (**Table 2**).

Table 2. Multivariable analysis of independent factors for coronary artery disease

Variables	OR	95% CI	P value
Age	1.028	1.019- 1.047	<0.001
Smoking	2.347	1.554- 3.367	<0.001
Neutrophils count (10^9/L)	1.204	1.072- 1.346	<0.001
HDL-C-to-monocyte ratio	0.781	0.678- 0.920	<0.001

HDL-C, High density liptein cholesterol

ROC curve analysis was exploited to verify further the potential value of HMR in the screening of CAD patients. As shown in Figure 1, the AUC of HMR was more remarkable than HDL-C and HDL-C-to-cholesterol models (0.642 vs. 0.603, 0.584) (**Figure 1**).

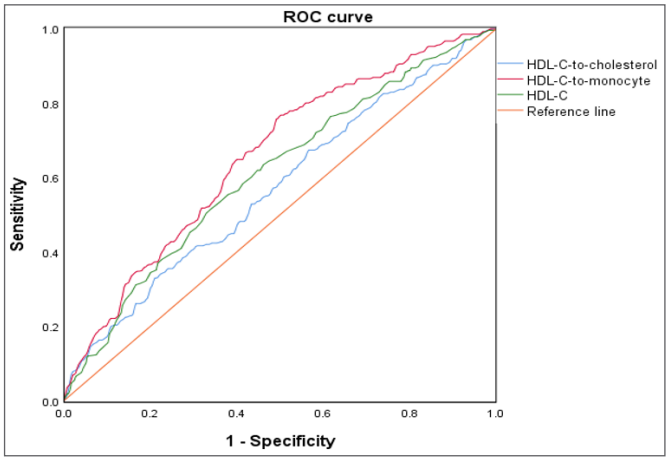

Figure 1. ROC curve for HDL-C-to-monocyte ratio and HDL-C and HDL-C-to-cholesterol

The scatter plot was based on the association between HMR's and the GS of CAD patients. The results showed that HMR was negative correlated with the GS of CAD r2=0.017). The correlation coefficient between the GS of CAD and HMR was -0.219 (P<0.001), which was better than that of monocyte count and HDL-C alone by Spearman correlation analysis (r monocyte count=-0.139, P monocyte count=0.021; r HDL-C=0.129, P HDL-C<0.001) (**Figure 2**).

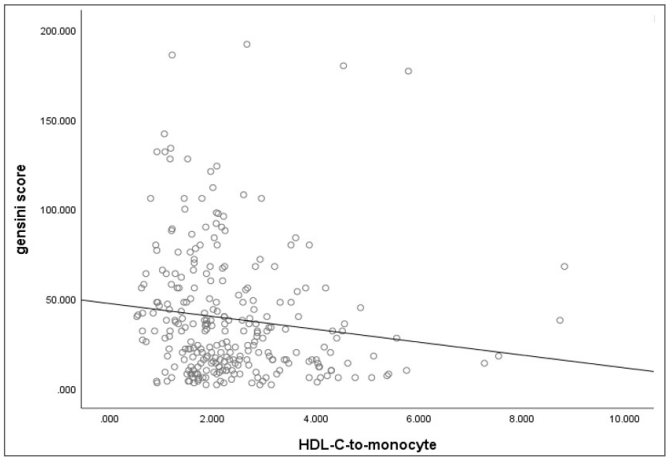

Figure 2. Distribution among of HDL-C-to-monocyte ratio and Gensini Score

DISCUSSION

In the light of the results of our study, it could be summarized as follows. First, the HMR was statistically different in patients with or without CAD, and this difference still existed after propensity score matching (PSM). Secondly, there is no strong correlation between HMR and other risk factors of coronary heart disease. Third, HMR could be used as an independent risk factor

of CAD. Forth, the HMR was superior to HDL-C and HDL-C-to-cholesterol in reflecting CAD and was related to the GS of CAD severity.

Inflammation plays significant role pathogenesis of atherosclerosis and cardiovascular disease.[2,8,9] Increasing concentration of mediators or markers of inflammation indicates that CAD might occur.[10] For example, C-reactive protein, neutrophil-to-lymphocyte ratio, IL-6, and platelet-to-lymphocyte ratio have been independent risk factors for atherosclerosis.[11-14] Monocytes are the source of macrophages and foam cells. Monocytes contribute significantly to the initiation and development of the atherosclerotic plaque by migrating to and attaching themselves to the location of the plaque. Within the subendothelial region, these cells undergo differentiation into macrophages and actively uptake oxidized LDL particles through scavenger receptors, resulting in the development of foam cells. Additionally, they are involved in the early inflammatory stage that follows the destabilization and rupture of the atherosclerotic plaque, as well as the development of an acute blood clot. Monocyte-platelet interactions induce the synthesis of enzymes that cause the degradation of the fibrous cap. During the healing process, particularly in the hypoxia phase, they have a role in both facilitating beneficial and detrimental inflammatory processes in the cardiac tissue. The resultant reactive oxygen species augment inflammation.[15-18] HDL-C primarily exerts its antiatherosclerotic action through its impact on reverse cholesterol transport. HDL-C induces vasodilation through the stimulation of nitric oxide synthesis in endothelial cells.[19] HDL-C resists monocyte macrophages by directly counteracting the migration of macrophages and removing cholesterol from macrophages, and ApoA1 inhibits monocyte activation by inhibiting the activation of CD11b.[19,20] Based on the characterization of monocytes and HDL-C in atherosclerosis, it is not unreasonable to speculate that HMR is closely associated with coronary artery severity in patients with CAD. Literature data also support this thesis.[21-25]

Our study has several advantages. We analyzed and verified our results by analyzing and adjusting potential confounding factors that might affect the relationship between HMR's and CAD incidence by PSM. We excluded the close relationship between HMR and other risk factors related to coronary heart disease included in this study.

Limitations

Our research still had some limitations. First, because we conducted a single-center retrospective study, our research had a potential bias. Further prospective research is needed to solve this problem. Secondly, many risk factors affect CAD, we did not cover all aspects in our research, which also affects the tightness of HMR and CAD. Third, our study indicated that HDL is an independent risk factor for CAD, but the predictive value of CAD patients and the prognostic value of CAD patients, especially those receiving the percutaneous coronary intervention, must be further verified. Fourth, the use of drugs affected the relationship between CAD and HMR's. Besides lipid-lowering drugs, there are antiplatelet drugs, antihypertensive drugs, B receptor blockers, which would cause significant bias in the results. In conclusion, further studies are needed to verify this result.

CONCLUSION

HMR is an independent risk factor for CAD. ROC analysis showed that the HMR is superior to monocyte and HDL-C alone in reflecting coronary artery disease. However, our results need to be verified by a large number of prospective studies.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 13.12.2023, Decision No: KAEK/2023.12.714).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1415215

Anatolian Curr Med J. 2024;6(2):121-126

Pioglitazone/Exenatide/SGLT-2 inhibitor combination therapy versus insulin therapy in patients with poorly controlled type 2 diabetes

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Cite this article as: Düğer H, Uçan B. Pioglitazone/Exenatide/SGLT-2 inhibitor combination therapy versus insulin therapy in patients with poorly controlled type 2 diabetes. *Anatolian Curr Med J.* 2024;6(2):121-126.

Received: 05.01.2024 ◆ **Accepted**: 01.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: We aimed to investigate the changes in glycemic status and beta cell function in type 2 diabetes mellitus (T2DM) patients with poor glycemic control despite receiving basal/bolus insulin therapy when switched from insulin therapy to combination therapy [exenatide/pioglitazone/sodium glucose cotransporter 2 inhibitor (SGLT-2i)].

Methods: A retrospective examination was made of the data of 64 patients, aged >18 years, diagnosed with T2DM, who were being followed up in the endocrinology outpatient clinic and were switched from basal/bolus insulin therapy to triple combination therapy. At the time of the patients changing to combination therapy, the glycosylated hemoglobin (HbA1c) value was ≥8.5% and fasting c peptide value was within the normal reference range. The anthropometric data of the patients, and glycemic and biochemistry values with modified homeostastis model assessment β (HOMA-β) levels were compared before the combination therapy and at 6 months after.

Results: Compared to the baseline values, a decrease was seen after 6 months in the values of body weight (89.6±5.8 vs. 83.8±3.6, p=0.015), body mass index (BMI) (38.3±2.7 vs. 33.5±1.9, p=0.011), and waist circumference (105.6±8.8 vs. 99.7±6, p=0.027). A decrease was determined in fasting blood glucose (FBG) (197±27.3 vs. 129±13.1, p<0.01) and HbA1c (9.8±1.6 vs. 8.1±1.1, p<0.01) values, and an increase in the HOMA-β value [233 (187.5, 282.3) vs. 318 (272.1, 365.2), p<0.001].

Conclusion: T2DM is a complex metabolic disease with more than one disorder in the pathogenesis, so it is difficult to control the disease in the long term with a single drug class. The use of drugs in a combined form, which will allow weight loss, have a positive effect on insulin resistance and improve beta cell function, without causing hypoglycemia, can achieve a better and sustainable glycemic and metabolic status.

Keywords: Diabetes mellitus, exenatide, piogitazone, SGLT-2

INTRODUCTION

Insulin resistance and beta cell dysfunction are the main defects in Type 2 diabetes mellitus (T2DM).[1] Hyperglycemia-induced glucotoxicity exacerbates these two major defects.[2,3] Therefore, by improving beta cell function, good glycemic control prevents disease progression.[4]

In T2DM patients with poor glycemic control, it is recommended to start insulin treatment when more than one oral agent has not been successful.[5] Insulin therapy is extremely effective in lowering blood glucose levels but there are disadvantages such as the treatment being parenteral, it requires glucose follow-up at home, causes hypoglycemia, and leads to weight gain.[5] In particular, the continuation of beta cell loss together with hypoglycemia and weight gain are the most important obstacles to strict glycemic control.[6]

Pioglitazone is a potent insulin sensitizer and improves beta cell function, does not cause hypoglycemia and achieves sustained A1c reduction.[7-9] Exenatide is a glucagon-like peptide-1 receptor agonist (GLP-1 RA). GLP RAs are drugs that reduce insulin resistance by obtaining weight loss, improving beta cell function and not causing hypoglycemia.[10] Sodium glucose co-transporter 2 inhibitors (SGLT-2i) are drugs that reduce plasma glucose by forming glucosuria.[11] These anti-diabetic drugs have the properties of improving glycemic control, correcting glucotoxicity, and providing weight loss, while not causing hypoglycemia. Therefore,

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combination therapies with drugs that reduce insulin resistance and improve beta cell function would seem to be rational.[11,12]

The aim of this study was to examine the changes in metabolic parameters, glycemic status and beta cell function with transition to exenatide/pioglitazone/SGLT-2i combination therapy in T2DM patients who could not obtain sufficient glycemic control despite basal/bolus insulin treatment.

METHODS

This retrospective study was conducted in Kilis Prof. Dr. Alaeddin Yavaşca State Hospital with the written permission of the Non-interventional Clinical Research Ethics Committee of Gaziantep Islam Science and Technology University (Date: 19.12.2023, Decision No: 339.33.05). All the study procedures were in compliance with the Helsinki Declaration.

The study included 64 patients who presented at the Endocrinology Outpatient Clinic between June 2022 and April 2023, were diagnosed with T2DM, received basal/bolus insulin treatment, and were determined with glycosylated hemoglobin (HbA1c) value ≥8.5% and fasting C-peptide value within the normal reference range. Patients who had taken any of the drugs in the combination therapy (pioglitazone, exenatide, SGLT-2i) within the last 3 months were excluded from the study. All the patients met the diabetes diagnostic criteria defined by the World Health Organization (WHO) in 1999.[10,11] The information of patients age, gender, body mass index (BMI), waist circumference, duration of diabetes and diabetes-associated microvascular and macrovascular complications was recorded from the medical records.

From a blood sample taken in the morning after overnight fasting, the blood glucose, HbA1c, serum lipids, c-peptide, and estimated glomerular filtration rate (eGFR) values were measured and recorded. Pancreatic beta cell function was evaluated with the modified homeostasis model assessment-β (HOMA-β). The modified HOMA-β was calculated using the formula of 270×fasting c-peptide (ng/ml)/fasting blood glucose (mmol/l)−3.5)×0.333.[13] The basal (glargine or detemir) and bolus (aspart) insulin treatment of the patients was stopped and the switch was made to the triple combination therapy of pioglitazone 30 mg/day, exenatide (first month 2x5 mg/day, and in the following months 2x10 mg/day) and dapagliflozine 10 mg/day or empagliflozine 10 mg/day.

In Türkiye, exenatide treatment can be started for patients with BMI ≥35 kg/m², and therefore, all the patients in the study met this condition. The blood glucose, HbA1c, modified HOMA-β values, and other biochemical variables of the patients before treatment and after 6 months of treatment were compared.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS 28.0 software. Quantitative data were stated as mean±standard deviation values and categorical data as number and percentage. Conformity of the data to normal distribution was assessed with the Kolmogorov-Smirnov test. In the analysis of categorical variables, the Chi-square test and Fisher's Exact test were used. Normally distributed continuous variables were analyzed with the Student's t-test whereas the Mann-Whitney U Test was applied to non-normally distributed variables. The variables measured before and 6 months after treatment were compared by Paired Samples t-test when the distribution of the data was normal, and by Wilcoxon test when the data were not normally distributed. The effect of basal-bolus insulin-only and basal-bolus plus oral anti-diabetic (OAD) therapy groups, as well as the effect of baseline total daily insulin doses on outcomes at 6 months of combination therapy was evaluated by one-way analysis of variance (ANOVA). A value of $P<0.05$ was considered statistically significant.

RESULTS

Evaluation was made of 64 patients with T2DM, comprising 67% females, with a mean age of 48.7±9.9 years. The mean duration of diabetes was 9.3±5.1 years, fasting blood glucose (FBG) was determined as 188±27.3 mg/dl, and HbA1c as 9.8%±1.6%. Of the 64 patients, 43 received basal/bolus insulin treatment alone, 12 received basal/bolus insulin + dipeptidyl peptidase 4 inhibitor (DPP-4i) + metformin treatment, and 9 received basal/bolus insulin + metformin treatment. The BMI value of the patients was mean 38.3±2.7 kg/m², and waist circumference was measured as 105.6±8.8 cm.

Microvascular complications were recorded as microalbuminuria at the rate of 28.1%, retinopathy at 20.3%, and neuropathy at 42.1%. Hypertension was present in 62.5% of the patients and ischaemic heart disease in 21.8%. The lipid profile results of the study cohort are also shown in **Table 1**. The comparisons of the glycemic values, modified HOMA-β, and other metabolic parameters of the patients who switched from basal/bolus insulin treatment to triple combination treatment measured before the change and at 6 months after are shown in **Table 2**.

Table 1. Baseline characteristics of participants (n=64)

Age, years	48.7±9.9
Sex: female, n, (%)	43 (67)
BMI, kg/m²	37.3±2.7
WC, cm	105.6±8.8
Diabetes duration, years	9.3±5.1
Diabetes therapy	
Basal/bolus insulin only, n, (%)	43 (67)
Basal/bolus insulin + metformin, n, (%)	9 (14)
Basal/bolus insulin + metformin + DPP4i, n, (%)	12 (19)
Total daily dose of insulin	
<0.5 units/kg/day, n, (%)	13 (20)
0.5-0.8 units/kg/day, n, (%)	40 (63)
>0.8 units/kg/day, n, (%)	11 (17)
FBG, mg/dl	188±27.3
HbA1c, %	9.8±1.6
Triglycerides, mg/dl	276 (248, 305)
Total cholesterol, mg/dl	231 (213, 245)
LDL cholesterol, mg/dl	143 (121, 159)
HDL cholesterol, mg/dl	41 (35, 48)
eGFR, ml/min/1.73 m²	78.8±16.1
Microalbuminuria, n, (%)	18 (28.1)
Diabetic retinopathy, n, (%)	13 (20.3)
Diabetic neuropathy, n, (%)	27 (42.1)
Hypertension, n, (%)	40 (62.5)
Ischemic heart disease, n, (%)	14 (21.8)

Data are presented as the mean±SD or prevalence (%). Measurement data for skewed distribution are expressed as median (interquartile range)

Table 2. Comparison of patients switched from basal/bolus insulin therapy to combination therapy (n=64)

	Basal/bolus insulin therapy	Combination therapy (6th month)	P value
Weight, kg	89.6±5.8	83.8±3.6	0.015
BMI, kg/m²	37.3±2.7	33.8±1.9	0.011
WC, cm	105.6±8.8	99.7±6	0.027
SBP, mmHg	136 ±4.5	130.9±4.3	<0.01
DBP, mmHg	92±3.1	85±2.8	<0.01
FBG, mg/dl	197±27.3	129±13.1	<0.01
HbA1c, %	9.8±1.6	8.1±1.1	<0.01
Modifiye HOMA-β	233 (187.5, 282.3)	318 (272.1, 365.2)	<0.01
Triglycerides, mg/dl	276 (248, 305)	249 (235, 266)	0.034
Total cholesterol, mg/dl	231 (213, 245)	219 (209, 231)	0.061
LDL cholesterol, mg/dl	143 (121, 159)	133 (116, 148)	0.066
HDL cholesterol, mg/dl	41 (35, 48)	48 (43, 50)	0.029

Counting data were expressed as number (percentages, %). Measurement data for normal distribution were expressed as (mean±SD). Measurement data for skewed distribution are expressed as median (interquartile range)
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment

Compared to the baseline values, a decrease was seen after 6 months in the values of body weight (89.6±5.8 vs. 83.8±3.6, p=0.015), BMI (38.3±2.7 vs. 33.5±1.9, p=0.011), and waist circumference (105.6±8.8 vs. 99.7±6, p=0.027). A decrease was observed in systolic blood pressure (SBP) (136±4.5 vs. 130.9±4.3, p<0.001) and diastolic blood pressure (DBP) (92±3.1 vs. 85±2.8, p<0.001). A decrease was determined in FBG (197±27.3 vs. 129±13.1, p<0.01) and HbA1c (9.8±1.6 vs. 8.1±1.1, p<0.01) values, and an increase in the HOMA-β value [233 (187.5, 282.3) vs. 318 (272.1, 365.2), p<0.001]. At 6 months after the change in treatment, in the lipid profile there was seen to be a decrease in triglycerides (TG) value [276 (248, 305) vs. 249 (235, 266), p=0.034], and an increase in the HDL cholesterol level [41 (35, 48) vs. 48 (43, 50), p=0.029]. In addition, **Table 3** shows the comparison of the values at 6 months of combination therapy between patients receiving insulin alone at baseline and those receiving insulin plus OAD combination. Accordingly, although both groups showed improvement in glycemic values and metabolic parameters at 6 months of combination therapy, there was no significant difference between the two groups. Total daily insulin doses administered at baseline were also not associated with glycemic status, weight, BMI and WC values 6 months after switching to combination therapy (**Table 4**).

Table 4. Comparison of variables according to baseline insulin doses at 6 months of combination therapy

	Baseline insulin doses			P
	<0.5 units/kg/day (n=13)	0.5-0.8 units/kg/day (n=40)	>0.8 units/kg/day (n=11)	
ΔWeight, kg	4.7±1.1	4.7±1.2	4.9±1.2	0.071
ΔBMI, kg/m²	3.5±9	3.5±1	3.6±1.1	0.12
ΔWC, cm	5.6±1.4	5.8±1.5	5.9±1.5	0.065
ΔFBG, mg/dl	64±6.8	65±7.2	67±7.5	0.092
ΔHbA1c, %	1.7±0.4	1.7±0.3	1.7±0.4	0.234

Δ: refers to the change from baseline at the 6th month of treatment; all changes are in the negative direction
BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin

Table 3. Comparison of patients in the 6th month of combination therapy according to their initial treatment patterns

	Only insulin therapy (n=43)		P	Insulin + OAD therapy (n=21)		P	ANOVA
	Baseline	6th month		Baseline	6th month		
Weight, kg	90.7±5.6	84.1±3.4	0.011	87.7±4.9	82.9±3.3	0.02	0.277
BMI, kg/m²	37.8±2.8	34.6±1.9	0.009	36.7±2.7	33.1±1.8	0.015	0.31
WC, cm	106.4±8.5	100.8±5.9	0.022	104.1±8.2	98.9±5.5	0.031	0.11
SBP, mmHg	137±4.6	131.7±4.2	<0.01	135.3±4.4	130±4.3	<0.01	0.078
DBP, mmHg	92±3.1	86.1±2.7	<0.01	92±2.7	84.6±2.7	<0.01	0.092
FBG, mg/dl	201±31.3	132.6±13.1	<0.01	193±28.9	127.5±13.1	<0.01	0.51
HbA1c, %	9.9±1.7	8.2±0.9	<0.01	9.6±1.7	8±1	<0.01	0.23
Modifiye HOMA-β	224 (181.5, 273.4)	311 (268, 361.1)	<0.01	239 (181.5, 273.4)	324 (258.1, 360)	<0.01	0.088

Counting data were expressed as number (percentages, %). Measurement data for normal distribution were expressed as (mean±SD). Measurement data for skewed distribution are expressed as median (interquartile range)
OAD, oral anti-diabetic; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment; ANOVA, one-way analysis of variance

DISCUSSION

In this study, which evaluated patients who could not achieve good glycemic control with basal/bolus insulin treatment and therefore changed to triple combination (exenatide/pioglitazone/SGLT-2i) therapy, the results showed that there were improvements in glycemic control and beta cell function.

The majority of patients who start insulin treatment for T2DM continue to express residul insulin.[14] A precondition of the transition from basal/bolus insulin treatment to combination treatment in the current study was that the patients had sufficient beta cell function.

As T2DM is generally a progressive disease, there is a continuing decrease in beta cell capacity because of glucotoxicity developing, especially when glycemic control cannot be achieved.[15] Therefore, good glycemic control protects beta cell function. With the combination therapy in the current study, there was seen to be a significant decrease in fasting blood glucose and HbA1c values. Many experimental studies have shown that thiazolidine and incretin effective drugs protect beta cell function and mass.[16,17] This effect is not only the result of the improvement in glycemic status but can also be explained by other mechanisms. Pioglitazone has been shown to protect beta cells against proinflammatory cytokines and prevent the formation of islet amyloid.[18,19] Of SGLT-2 inhibitors, dapagliflozine and empagliflozine have been reported to be effective in protecting beta cell survival and improving beta cell function.[20,21] However, the majority of these studies have shown the results of examining diabetes in the early stages. Kimura et al.[22] reported that pioglitazone and liraglutide increased beta cell function and mass in the early stage of diabetes but these effects weakened as diabetes progressed. Even though patients with a relatively longer duration of diabetes were included in the current study, an increase was seen in the HOMA-β values with the combination therapy. Therefore, it can be said that the efficacy of pioglitazone and GLP-1 analogs continued when there was a beta cell reserve in the pancreas, irrespective of the duration of diabetes.

In another meta-analysis that compared the glycemic efficacy of insulin with GLP-1 analogs, there was reported to be a minimal difference in terms of glycemic efficacy and this difference was in favour of GLP-1.[23] In this respect, for patients who are planned to transition to injectable treatment, it would seen to be reasonable to start GLP-1 analogs, which increase the incretin effect and provide weight control without causing hypoglycemia, before insulin treatment. There is also greater patient compliance to GLP-1 analogs compared to insulin treatment. This is because in treatment with GLP-1 analogs, standard increasing dose titration is made at the beginning, whereas in insulin use, dose titration is made more personalised and in a wider range.[24]

One of the reasons that good glycemic control cannot be achieved in patients receiving insulin treatment is hypoglycemia. Reactive and exaggerated hyperglycemia developing after hypoglycemia causes glucotoxicity. In addition, the feeling of hunger caused by hypoglycemia leads to weight gain. "Confirmed hypoglycemia" was not seen in any of the patients in the current study after transition to combination therapy, and a significant decrease was seen in the weight, BMI, and waist circumference values of the patients. This change is due to the positive effects on weight of exenatide and SGLT-2 inhibitors in addition to eliminating the hypoglycemic effect of insulin. Moreover, the negative effects of pioglitazone such as oedema and weight gain seem to be neutralised when it is included in combination therapy.

Another important result of the current study was that a decrease in TG level and an increase in HDL cholesterol level were seen with combination therapy. When the insulin resistance status and metabolic disorders of patients with T2DM are taken into consideration, dyslipidemia is a common comorbidity.[25] Many patients with T2DM have an abnormal lipid profile characterised by increased TG and decreased HDL cholesterol.[26] In studies of many different monotherapies and combination therapies in patients with T2DM, the use or addition of pioglitazone has been shown to improve fasting and satiated TG metabolism, increase HDL cholesterol levels, and have a positive effect on lipoprotein particle size.[26] In another meta-analysis, GLP-1 RAs were shown to be associated with moderate decreases in LDL cholesterol, total cholesterol, and TG, but no significant improvement was seen in HDL cholesterol.[27] In a meta-analysis that examined the effect on lipids of SGLT-2 inhibitors, it was reported that SGLT-2 inhibitors significantly increased total cholesterol, LDL cholesterol, and HDL cholesterol, and decreased the TG level.[28]

After 6 months of combination therapy in the current study, there was seen to be a significant decrease in systolic and diastolic blood pressure values. Dapagliflozine is known to obtain a decrease of approximately 4 mmHg in systolic blood pressure.[29] This effect can be explained by a decrease in volume contraction and sympathetic nerve system activation.[30] The decrease in blood pressure provided by GLP-1 agonists can be explained by the decrease in vascular resistance, natriuresis, and weight loss.[31] Van Ruiten et al.[32] reported that combination therapy of dapagliflozine and exenatide provided a greater decrease in blood pressure than the effect of either drug alone.

Limitations

To the best of our knowledge, this is the first study in literature to have evaluated the results of exenatide/pioglitazone/SGLT-2i triple combination therapy in T2DM, and this can be emphasised as a strong aspect of the study. However, there were also some limitations, primarily the retrospective design and relatively low number of patients. In addition, the study was conducted in a second-level hospital and the results only represent a single centre.

CONCLUSION

T2DM is a progressive disease, in which several factors play a role in the pathogenesis. It is difficult to achieve sustainable glycemic control with a single drug class. The findings of this study demonstrated that the combined use of drugs with different effect mechanisms resulted in better glycemic control and beta cell function. In patients with beta cell reserve, rather than treatments which patients find difficult to adhere to such as insulin therapy, the combined use of drugs that do not cause hypoglycemia, provide weight loss, and improve cell function can provide a better and sustainable glycemic and metabolic status.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Non-interventional Clinical Researches Ethics Committee of Gaziantep Islam Science and Technology University (Date: 19.12.2023, Decision No: 339.33.05).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1421102

Anatolian Curr Med J. 2024;6(2):127-132

Analgesic effects of thoracic fascial plane blocks in postoperative pain management following cardiac surgery with sternotomy: a retrospective study

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Cite this article as: Zengin EN, Salman N. Analgesic effects of thoracic fascial plane blocks in postoperative pain management following cardiac surgery with sternotomy: a retrospective study. *Anatolian Curr Med J.* 2024;6(2):127-132.

Received: 16.01.2024 ◆ **Accepted**: 03.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Acute poststernotomy pain is very severe and causes adverse hemodynamic disturbances. Various thoracic fascial plane blocks are used in the management of this pain. This study aimed to compare the analgesic effects of conventional analgesic methods and thoracic fascial plane blocks in the treatment of post-sternotomy pain.

Methods: Patients aged over than 18 years and with American Society of Anesthesiologists (ASA) physical status I-II-III who underwent elective cardiac surgery with sternotomy in 2022-2023 were included in this retrospective study. Patient records were categorized into groups based on the regional analgesia preferences applied. The groups are as follows: Group I: Patients without any blocks. Group II: Patients who received parasternal block (PSB). Group III: Patients who received serratus anterior plane block (SAPB). Group IV: Patients who received erector spinae plane block (ESPB). Then, the patients' demographic data, laboratory data, Behavioral Pain Score (BPS) values, Visual Analog Scale (VAS) values, and additional analgesia needs were recorded and compared.

Results: The files of 128 patients were included in the study. The patients are statistically similar in terms of demographic data and surgical characteristics. Remifentanil consumption, BPS values, VAS values, and the need for additional analgesia were statistically lower in the groups in which thoracic fascial plane blocks were applied compared to the group in which conventional analgesia was applied.

Conclusion: As a result, thoracic fascial plane blocks, which have been used increasingly frequently in recent years, can provide more effective analgesia than conventional analgesia methods in cardiac surgery. Additionally, considering enhanced recovery after surgery protocols, these blocks may reduce undesirable side effects by limiting the need for opioids in the perioperative period. Since PSB and SAPB can be applied in the supine position, they may be more advantageous than ESPB in terms of ease of application.

Keywords: Acute pain, cardiac surgery, erector spinae plane block, parasternal block, serratus anterior plane block, thoracic fascial plane blocks

INTRODUCTION

Acute postoperative pain following sternotomy in cardiac surgery should be appropriately treated to prevent adverse hemodynamic outcomes and pulmonary complications. In the era of fast-track management, an adequate and effective postoperative analgesic technique facilitates early extubation, mobilization, and discharge from the intensive care unit.[1] Additionally, if postoperative acute pain is not adequately addressed, chronic pain may develop after sternotomy, hindering patients from recovering their normal activities for an extended period.[2]

After sternotomy, various thoracic fascial plane blocks can be employed in the treatment of acute pain. One of these blocks, Erector Spinae Plane Block (ESPB), was described by Forero et al.[3] in 2016. ESPB involves the administration of a local anesthetic solution, which not only blocks the dorsal branches of the spinal nerve but also disperses into the paravertebral and epidural spaces.[4] Moreover, the targeted point is distant from the pleura and neuroaxial plane, reducing the risk of complications.[5] Another thoracic fascial plane block used in the treatment of acute pain after sternotomy is the Serratus Anterior

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Plane Block (SAPB).[6] Although there is no randomized controlled study on SAPB application in adults due to the lack of innervation of the sternum, SAPB can have an analgesic effect on surrounding tissues related to drain pain and sternal retraction.[7] As a result, it may reduce patients' pain levels and limit morphine consumption.[7] SAPB can be performed below, above, or both below and above the serratus anterior muscle.[8,9] Another block used in the treatment of acute pain after sternotomy is the parasternal block application.[10] For PSB application, a local anesthetic solution is administered into the interfascial space between the pectoralis major and intercostal muscles at the second and fourth intercostal space levels.[11] Despite all these regional methods, in some cases (such as patient consent or infection in the block area), patients are treated solely with intravenous analgesics.

The hypothesis of this study is that effective postoperative analgesia can be provided with thoracic fascial plane blocks applied before cardiac surgery. This study aims to compare the postoperative analgesic effects of different analgesic preferences applied to patients undergoing cardiac surgery at our clinic.

METHODS

This study was retrospectively designed and received approval from the Bilkent City Hospital Ethics Committee (Date: 13.12.2023, Decision No: E.Kurul-E1-23-4389). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Patients aged 18 and over, with American Society of Anesthesiologists (ASA) physical status of I-II-III, who underwent elective cardiac surgery with sternotomy in 2022 and 2023, were included in the study. Patients under 18 years of age, those with ASA scores of IV and above, those who did not undergo sternotomy, those who underwent emergency interventions, and those with missing data in their medical records were excluded from the study.

Patient records were categorized into groups based on the regional analgesia preferences applied. The groups are as follows:

- Group I: Patients without any blocks.
- Group II: Patients who received PSB
- Group III: Patients who received SAPB.
- Group IV: Patients who received ESPB.

The age, body mass index (BMI), sex, ASA physical status, type of surgery performed, smoking status, preoperative White blood cells (WBC), Neutrophil-Lymphocyte ratio (N/L) values, anesthesia duration, intraoperative remifentanil consumption, preoperative arterial lactate value, postoperative arterial lactate value, intraoperative maximum glucose values, extubation durations, postoperative side effect incidents of the patients, and first and second postoperative day Behavioral Pain Score (BPS) values, Visual Analog Scale (VAS) values, and additional analgesia needs, were recorded and compared.

According to the protocol implemented in our department, anesthesia induction involved propofol (1.5-2 mg kg^{-1}), fentanyl (1-2 µg kg^{-1}), and rocuronium (0.5-1 mg kg^{-1}) after preoxygenation. Anesthesia was maintained with sevoflurane and a remifentanil infusion (0.05-0.25 µg kg^{-1} min^{-1}) while monitoring the bispectral index to maintain it within the range of 40-50. At the end of the surgery, all groups received 1 gram of acetaminophen and 1 mg kg^{-1} of intravenous tramadol before being transferred to the intensive care unit. It was observed that a standard anesthesia technique and postoperative care protocol were applied to all groups. Then, analgesics were administered based on pain scores reported in the intensive care unit. In all groups, if the VAS score 4 and exceeded 4, the initial treatment involved administering acetaminophen (10 mg kg^{-1}) and tramadol (1 mg kg^{-1}). Following this, either morphine (0.5 mg kg^{-1}) or fentanyl (1 µg kg^{-1}) was added as needed.

Additionally, based on the patient's consent and the anesthetist's preference, various thoracic fascial plane blocks were administered to patients using a linear ultrasound probe and a 21-G Pajunk needle, following this protocol.

The PSB procedure was performed under general anesthesia, in the supine position, following the induction of anesthesia. For the PSB, a local anesthetic solution of 10 mL of 0.25% bupivacaine (for each level) was administered into the interfascial space between the pectoralis major and intercostal muscles, approximately 2 cm lateral to the midline, at the levels of the 2^{nd} and 4^{th} intercostal spaces. The block was performed bilaterally with 40 mL of 0.25% bupivacaine.

The SAPB procedure was also performed under general anesthesia, in the supine position, following the induction of anesthesia. For the SAPB, a local anesthetic solution of 20 mL of 0.25% bupivacaine (for each side) was administered along the midaxillary line, at the level of the 4^{th} or 5^{th} rib, above the serratus anterior muscle and below the latissimus dorsi muscle. The block was performed bilaterally with 40 mL of 0.25% bupivacaine.

The ESPB procedure was performed in a seated position during the preoperative period, prior to general anesthesia. For the ESPB, a local anesthetic solution of 20 mL of 0.25% bupivacaine (for each side) was administered below the erector spinae muscle, at the level of the transverse process of thoracic 5. The block was performed bilaterally with 40 mL of 0.25% bupivacaine.

Statistical Analysis

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables was normal or not was determined by the Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean±SD for normal distributions, and median (Q_1;25. Persentile-Q_3;75. Persentile) for skewed distributions. Categorical data were described as a number of cases (%). Statistical analysis differences in normally distributed variables between the independent groups were compared by One Way Anova Test, Kruskal Wallis Test was applied for comparisons of the not normally distributed data. The Conover-Iman test was used for binary comparisons among the groups. Categorical variables were compared using Pearson's Chi-Square Test or Fisher's Exact Test. İt was evaluated degrees of relation between variables with spearman correlation analysis. It was accepted p-value <0.05 as a significant level on all statistical analysis.

RESULTS

The files of 128 patients who underwent cardiac surgery with sternotomy under elective conditions at Ankara Bilkent City Hospital in 2022-2023 and whose data were complete were included in the study. The patients are statistically similar in terms of demographic data and surgical characteristics (**Table 1**).

When the groups were compared in terms of preoperative lactate, postoperative lactate and lactate difference, they were found to be statistically similar (p>0.05). Similarly, no statistical difference was found between the groups in terms of glucose level, WBC, Neutrophil, lymphocyte and N/L (p>0.05). Finally, extubation times were found the shortest in the ESPB group and the longest in the control group, but no statistical difference was found (p>0.05) (**Table 2**).

When the groups were compared in terms of remifentanil consumption in the intraoperative period, a statistically significant difference was found. Remifentanil consumption was found to be lower in

Table 1. Demographic and surgical characteristics of the patients

	Group I (n:54)	Group II (n:21)	Group III (n:20)	Group IV (n:34)	p
Sex					0.261 Φ
Female	14 (25.9%)	10 (47.6%)	5 (25.0%)	12 (36.4%)	
Male	40 (74.1%)	11 (52.4%)	15 (75.0%)	21 (63.6%)	
Age (year)	59.5 (53.0-66.0)	62.0 (45.0-66.0)	56.5 (1.5-64.0)	61.0 (59.0-67.0)	0.272 β
BMI kg/m²	26.74±4.77	27.84±5.38	27.84±3.87	27.04±3.70	0.700*
ASA	3.0 (2.0-3.0)	3.0 (2.0-3.0)	2.0 (2.0-3.0)	3.0 (2.0-3.0)	0.603 β
Smoking	21 (38.9%)	10 (47.6%)	12 (60.0%)	12 (36.4%)	0.320 Φ
Surgery type					0.077 Φ
CABG	23 (42.6%)	4 (19.0%)	11 (55.0%)	17 (51.5%)	
Valve replacement	26 (48.1%)	16 (76.2%)	8 (40.0%)	11 (33.3%)	
CABG+ valve replacement	5 (9.3%)	1 (4.8%)	1 (5.0%)	5 (15.2%)	
Anesthesia duration (min)	320 (285-375)	320 (295-395)	315 (257.5-355)	310 (270-435)	0.826 β

Continuous variables are expressed as either the mean±standard deviation (SD) or median (Q1-Q3) and categorical variables are expressed as either frequency (percentage).
One way anova Test *. Kruskal wallis Test Φ. Chi square Test β
p=Level of Significance. p<0.05. ASA: American Society of Anesthesiologists, BMI: Body Mass Index, CABG: Coronary artery bypass graft

Table 2. Comparison of groups in terms of lactate, glucose, extubation time, WBC, neutrophil, lymphocyte, N/L ratio

	Group I (n:54)	Group II (n:21)	Group III (n:20)	Group IV (n:34)	p
Lactate-pre (mmol/L)	1.16 (0.89-1.43)	1.26 (0.70-1.39)	1.16 (1.03-1.69)	1.17 (0.89-1.37)	0.827 Φ
Lactate-post (mmol/L)	2.56 (1.79-3.65)	2.13 (1.71-3.45)	2.57 (1.69-3.39)	2.33 (1.60-3.03)	0.353 Φ
Lactate (delta)	1.40 (0.80-2.48)	1.02 (0.63-2.29)	1.08 (0.47-2.12)	1.13 (0.58-1.73)	0.324 Φ
Glucose (mg/dL) (max)	182.2±50.3	177.7±40.5	191.4±64.3	171.4±38.5	0.518*
Extubation time (min)	480 (405-580)	450 (360-490)	427 (387-562)	390 (330-495)	0.106 Φ
WBC ($\times10^9$/L)	7.53 (6.16-9.02)	7.25 (5.96-8.47)	7.65 (6.84-9.37)	6.70 (5.94-8.91)	0.793 Φ
Neutrophil ($\times10^9$/L)	4.92 (3.58-6.10)	4.50 (3.48-5.53)	4.52 (3.63-6.13)	4.39 (3.34-5.70)	0.830 Φ
Lymphocyte ($\times10^9$/L)	1.90 (1.40-2.30)	1.78 (1.53-2.29)	2.14 (1.67-2.81)	1.87 (1.36-2.31)	0.387 Φ
N/L ratio	2.74 (1.96-3.42)	2.27 (1.86-3.42)	1.99 (1.52-2.99)	2.58 (1.94-2.90)	0.379 Φ

Continuous variables are expressed as either the mean±standard deviation (SD) or median (Q1-Q3).
One Way Anova Test *. Kruskal Wallis Test Φ. p=Level of Significance. p<0.05
Conover-Inman Test was performed for the binary comparisons among the groups and the p value was set at 0.05. Significant differences were found between; a: Group I vs Group II, b: Group I vs Group III, c: Group I vs Group IV. N/L: Neutrophil/Lymphocyte. WBC: White blood cells

all block groups than in the control group (p<0.001). When the groups were compared in terms of BPS on the zero and first postoperative days, a statistically significant difference was found. BPS levels were found to be lower in all block groups than in the control group (p<0.001). When the groups were compared in terms of VAS scores on the zero and first postoperative days, a statistically significant difference was found. VAS scores were found to be lower in all block groups than in the control group (p<0.001). Similarly, A statistically significant difference was found when the groups were compared in terms of additional analgesia needs on the zero and first postoperative days. Additional analgesia needs were found to be lower in all block groups than in the control group (p<0.001). When the groups were compared in terms of side effects, only nausea was observed, but no statistically significant difference was found (**Table 3**).

DISCUSSION

The study results have demonstrated that in patients undergoing CABG, valve replacement, and the combined procedures, ESPB, SAPB, and PSB provided effective analgesia in the early postoperative period compared to those without thoracic fascial plane block. Although not statistically significant, the reduction in intraoperative remifentanil consumption, especially in the ESPB group, suggests that ESPB may contribute to a more stable intraoperative anesthesia management. On the other hand, the lack of a need for additional patient positioning and the ability to be applied after anesthesia induction make PSB and SAPB more advantageous, especially in this patient group highly sensitive to stress, compared to ESPB.

In addition to the stress factors arising from the compromised clinical condition present in patients undergoing cardiac surgery, the severe pain resulting from the surgical procedure itself can further complicate this stress response. Therefore, comprehensive perioperative pain management is crucial in preventing these stress factors and subsequently avoiding complications. In recent years, protocols for enhancing postoperative recovery, known as Enhanced Recovery After Surgery (ERAS), have become essential practices in various disciplines and have also gained significant importance in cardiac surgery.[12,13] The perioperative analgesic approach, a crucial parameter in ERAS protocols, not only suppresses the stress response but also facilitates conditions that accelerate postoperative recovery, such as the ability to breathe comfortably, sufficient coughing, and early mobilization. This approach is highly significant in limiting complications and ensuring early discharge by promoting a faster recovery in the postoperative period.[14,15] In our clinic, in accordance with these goals, consenting patients receive regional analgesia treatments in addition to conventional analgesic therapies as part of multimodal analgesia. The utilization of these regional analgesic treatments has resulted in lower intraoperative remifentanil consumption compared to patients receiving conventional analgesia.

Perioperative pain management aims to achieve both more effective analgesia with the concurrent use of multiple methods, involving drugs with different mechanisms, and the reduction of side effects by using lower doses. Particularly in cardiac surgery, limiting the consumption of systemic opioids, which provide effective analgesia, becomes even more critical through multimodal analgesia. Regional analgesia applications, as a significant component of multimodal analgesia, play a crucial role in significantly limiting opioid consumption and effectively minimizing opioid-related side effects.[16,17] In thoracic surgery, thoracic fascial plane blocks have been demonstrated to be applied either individually or in combination, thereby increasing their effectiveness.[8,9,18] In our clinic, although some patients receive combined blocks in this manner, the patients included in the study underwent a single thoracic fascial plane block.

Table 3. Comparison of groups in terms of pain scores, additional analgesia needs and side effects

	Group I (n:54)	Group II (n:21)	Group III (n:20)	Group IV (n:34)	p
Remifentanil consumption (mg)	4.5 (4.0-5.0)	2.5 (2.0-3.0)	3.0 (2.0-3.25)	2.5 (2.0-3.0)	<0.001 Φ a.b.c
BPS 1	5.0 (5.0-6.0)	3.0 (3.0-4.0)	3.5 (3.0-4.5)	3.00 (3.0-3.0)	<0.001 Φ a.b.c
BPS 2	4.0 (4.0-5.0)	3.0 (3.0-3.0)	3.0 (3.0-4.0)	3.00 (3.0-3.0)	<0.001 Φ a.b.c
VAS 1	5.0 (4.0-5.0)	2.0 (2.0-3.0)	3.0 (2.0-3.5)	1.00 (1.0-2.0)	<0.001 Φ a.b.c
VAS 2	4.0 (3.0-4.0)	2.0 (2.0-2.0)	1.5 (1.0-3.0)	1.00 (1.0-2.0)	<0.001 Φ a.b.c
Additional analgesic 1	43 (79.6%)	1 (4.8%)	5 (25.0%)	-	<0.001 β a.b.c
Additional analgesic 2	28 (51.9%)	1 (4.8%)	2 (10.0%)	-	<0.001 β a.b.c
Nausea	15 (27.8%)	4 (19.0%)	2 (10.0%)	5 (15.2%)	0.338 β

Continuous variables are expressed as either the median (Q1-Q3) and categorical variables are expressed as either frequency (percentage).
Kruskal Wallis Test Φ. Chi Square Test β p=Level of Significance. p<0.05
Conover-Inman Test was performed for the binary comparisons among the groups and the p value was set at 0.05. Significant differences were found between; a: Group I vs Group II, b: Group I vs Group III, c: Group I vs Group IV. 1: first day, 2: second day, BPS: Behavioral Pain Score, VAS: Visual Analog Scale.

For years, thoracic epidural applications, considered the gold standard, have been limited in cardiac surgery due to the potential catastrophic complication of epidural hematoma associated with intensive anticoagulant use. In recent years, the increasingly widespread use of thoracic fascial plane blocks aims to prevent these complications.[19] However, uncertainties still exist regarding their effectiveness and mechanisms. While studies have been conducted on various plane blocks in cardiac surgery, these studies often compare a control group with two different blocks.[7,13,20] In our clinic, while conventional analgesia methods are used for every patient, fascial blocks are increasingly being incorporated in addition to conventional analgesic treatments. As observed in the study results, all three plane blocks have provided a significant analgesic effect. Moreover, it is noteworthy that all three methods resulted in lower pain scores and less remifentanil consumption compared to conventional analgesia methods.

Application of thoracic fascial plane blocks before surgical incision may also limit opioid consumption during the intraoperative period.[21] As a result, it may also reduce opioid-related side effects. In this study, all blocks were performed before surgical incision. Unlike PSP and SAPB, ESPB was applied in a sitting position before anesthesia induction. Due to difficulties in positioning after anesthesia induction and at the end of surgery, ESPB was applied before anesthesia induction. Although ESPB was applied with sedo-analgesia in this patient group, the bilateral application of ESPB may not be practically feasible in clinical practice for cardiac surgery patients, where stress and its undesirable effects are quite common. In our study, intraoperative opioid consumption was found to be similar in the ESPB, PSB, and SAPB groups. Additionally, intraoperative opioid consumption in these three groups was lower compared to conventional analgesia. Despite showing similar analgesic efficacy, considering ease of application, SAPB and PSB may be more practical in clinical practice.

Complex mechanisms are involved in lactate elevation during and after cardiac surgery. The increase in lactate is generally multifactorial, and these factors may vary from patient to patient and even change over time in individual patients.[22] Among these factors, anesthesia and analgesia preferences play a role. Surgical stress response and, consequently, lactate levels can be kept at lower levels based on anesthesia preferences.[23] In our study, despite different analgesia preferences, lactate levels were found to be similar in all groups. This situation may arise due to the influence of multiple factors on lactate levels.

In recent years, inflammatory parameters have been used for various predictive purposes. A high N/L ratio in the preoperative period has been noted to be associated with high postoperative pain scores and analgesic consumption.[24-26] The preoperative Neutrophil, Lymphocyte, and N/L ratios of the patients included in our study were similar among the groups. Therefore, it can be stated that these markers did not affect the postoperative pain status as the groups were similar in terms of inflammatory parameters in the preoperative period.

Limitations

This study has certain limitations. Firstly, it is a single-center and retrospective study. Secondly, due to the retrospective analysis of data, there is no homogeneous distribution. This lack of homogeneity limits a clearer assessment of the results. Lastly, the evaluation of basic laboratory parameters may not provide conclusive results in assessing surgical stress response. Comprehensive prospective randomized studies in this regard may yield better results regarding the effectiveness of thoracic fascial plane blocks.

CONCLUSION

Thoracic fascial plane blocks, increasingly utilized in recent years, may provide more effective analgesia compared to conventional analgesic methods. Furthermore, ERAS protocols, these blocks can limit opioid requirements during the perioperative period, reducing unwanted side effects. The advantages of PSB and SAPB, such as not requiring additional patient positioning and being applied after anesthesia induction, may make them more advantageous, especially in this patient group, which is highly sensitive to stress, compared to ESPB. More comprehensive prospective studies comparing these blocks could provide explanatory results.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study protocol was approved by the Ankara Bilkent City Hospital Ethics Committee (Date: 13.12.2023, Decision No: E.Kurul-E1-23-4389).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1412597

Anatolian Curr Med J. 2024;6(2):133-138

Relationship between T_{peak}-T_{end} (TPE), TPE/QT ratio and TPE dispersion in patients with subclinical hyperthyroidism

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Cite this article as: Bilen MN, Gözel N. Relationship between T_{peak}-T_{end} (TPE), TPE/QT ratio and TPE dispersion in patients with subclinical hyperthyroidism. *Anatolian Curr Med J.* 2024;6(2):133-138.

Received: 31.12.2023 ◆ **Accepted**: 08.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Subclinical hyperthyroidism has been associated with an increased risk of cardiovascular events, including atrial fibrillation, heart failure, and cardiovascular mortality. Tpeak - Tend interval (TPE), TPE/QT ratio, and TPE dispersion have been suggested as potential electrocardiographic markers of ventricular repolarization abnormalities, which may be associated with an increased risk of arrhythmias and sudden cardiac death. However, the relationship between subclinical hyperthyroidism and these parameters remains unclear.

Methods: We conducted a cross-sectional study to investigate the relationship between subclinical hyperthyroidism and TPE, TPE/QT ratio, and TPE dispersion. A total of 106 patients were included in the study, with 42 patients diagnosed with subclinical hyperthyroidism group and 64 control group. Conventional echocardiographic and electrocardiographic parameters were measured and compared between the two groups.

Results: There are no significant differences in age (p=0.707) or gender (p=0.552) between the two groups. Patients in the subclinical hyperthyroidism group had significantly higher TPE, TPE/QT ratio, and TPE dispersion compared to the control group (p<0.001). However, there were no significant differences between the two groups in terms of conventional echocardiographic parameters, including left ventricular (LV) ejection fraction, LV end-diastolic diameter, LV end-systolic diameter, and right ventricular fractional area change.

Conclusion: Our results suggest that subclinical hyperthyroidism is associated with increased ventricular repolarization abnormalities, as evidenced by higher TPE, TPE/QT ratio, and TPE dispersion. These findings may have clinical implications for the management of patients with subclinical hyperthyroidism, particularly those with cardiovascular risk factors.

Keywords: Arrhythmia, subclinical hyperthyroidism, ventricular repolarization

INTRODUCTION

Subclinical hyperthyroidism, defined as low thyroid-stimulating hormone (TSH) levels and normal thyroid hormone levels, is a common condition affecting up to 10% of the population. Although often asymptomatic, recent studies have suggested an association between subclinical hyperthyroidism and an increased risk of cardiovascular disease (CVD).[1,2]

One potential mechanism for this increased risk is an alteration in the electrocardiographic (ECG) parameters that reflect cardiac repolarization. Specifically, previous research has shown that subclinical hyperthyroidism is associated with changes in the Tpeak - Tend (TPE) interval, which is a measure of the duration of ventricular repolarization.[3,4] In addition to the TPE interval, the TPE/QT ratio and TPE dispersion are also ECG parameters believed to reflect cardiac repolarization abnormalities. Studies have suggested alterations in these parameters among patients with subclinical hyperthyroidism, yet the relationship between these parameters and the TPE interval remains unclear.[5,6]

A recent study by Aweimer et al.[7] aimed to explore the relationship between TPE, TPE /QT ratio, and TPE dispersion in patients with subclinical hyperthyroidism. The study found that TPE interval was significantly increased in patients with subclinical hyperthyroidism compared to controls, and TPE dispersion was also increased. Additionally, the TPE/QT ratio was significantly higher in patients with subclinical hyperthyroidism than controls. These findings suggest that subclinical hyperthyroidism may be associated with alterations in ECG parameters that reflect cardiac repolarization abnormalities. However, further studies

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are needed to confirm these findings and determine the clinical implications of these changes.

This study aims to investigate the relationship between the TPE interval, TPE/QT ratio, and TPE dispersion as potential arrhythmia markers in the electrocardiograms of patients with subclinical hyperthyroidism.

METHODS

Ethics

The study was carried out with the permission of Fırat University Non-invasive Researches Ethics Committee (Date: 08.12.2016, Decision No: 16). The study was conducted in accordance with the Declaration of Helsinki and obtained ethical approval from the institutional review board. All participants provided informed consent before data collection.

Study Population

Participants were recruited from outpatient clinics at tertiary hospitals and endocrinology centers. Participants' demographic characteristics (age, gender, body mass index), medical history, and medication use history were recorded. Those with systemic illnesses or atherosclerosis risk factors were excluded from the study. The sample comprised 42 cases of subclinical hyperthyroidism and 64 euthyroid participants, matched for age and gender, all aged between 18 and 55 years. The study included patients diagnosed with subclinical hyperthyroidism based on clinical criteria, characterized by low TSH levels and normal thyroid hormone levels. Patients who were taking various drugs that could suppress TSH (such as dopamine, dopamine agonists, glucocorticoids, somatostatin, aspirin, fenofibrate, furosemide), or who were taking antiarrhythmic agents or agents that could cause arrhythmia (such as propranolol, terfenadine, amiodarone, erythromycin, clarithromycin, antidepressant agents, antipsychotic agents), those with structural heart disease detected by echocardiography, those with electrolyte imbalances, those with a BMI>30, those with psychiatric disorders or pregnancy, those with left or right bundle branch block detected in the baseline ECG, and those with poor ECG quality were excluded from the study.

Laboratory Measurements

Serum samples (6 cc for biochemistry, 5 cc for complete blood count, 5 cc for hormones) were collected in vacuum tubes containing 15% K3 EDTA. Hemoglobin, hematocrit, platelet count, and white blood cell count and types (neutrophil, lymphocyte, eosinophil, and monocyte) were determined using an automated hematology analyzer (Beckman Coulter LH 780) by the electrical impedance method. Glucose, urea, creatinine, total cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were measured using a Cobas-601 (Roche) automated analyzer by the chemiluminescence method. The serum levels of free thyroid hormones, free T3 (FT3), and free T4 (FT4) were determined using the chemiluminescent immunoassay method, while thyroid-stimulating hormone (TSH) levels were measured using the two-site chemiluminescent immunometric assay method with the Immulite 2000 (DPC) kit. The reference ranges for FT3, FT4, and TSH levels were 1.8-4.2 pg/ml, 0.80-1.80 ng/dL, and 0.40-4.0 mIU/ml, respectively.

Electrocardiogram (ECG) Recording and Analysis

Standard 12-lead ECG recordings were taken using a digital ECG device with a sampling rate of 1,000 Hz (Nihon Kohden, Tokyo). ECG recordings were taken while participants were lying in a supine position after a 10-minute rest period. The recordings were analyzed offline by two independent observers who were unaware of the study objectives and participant group assignments. Any differences between observers were resolved by a third observer. After scanning all ECGs, QT interval, corrected QT interval (QTc), and TPE interval were calculated using MATLAB (MathWorks, Natick, Massachusetts, USA.) software. The QT interval was defined as the distance from the onset of the QRS complex to the point of isoelectric descent of the T wave. For QTc interval, Bazzet's formula was applied: QTc (ms)=QT interval /√RR interval.8 TPE interval was defined as the time between the peak and the end of the T wave and was measured from each precordial derivation, with the longest value taken.[9] TPE/QT and TPE/QTc ratios were calculated after these measurements. TPE dispersion (TPEd) was obtained by calculating the difference between the maximum and minimum TPE intervals measured from each precordial derivation (one beat for each derivation).

Transthoracic Echocardiography (TTE)

M-mode and 2D ECHO were performed in the left lateral decubitus position using a 3.25 probe from the Vivid 3 ECHO echocardiography device, according to the American Society of Echocardiography criteria.[10] Parasternal short-long axis images and apical 4 and 2 chamber views, which are standard echocardiography positions, were used for measurements. Left ventricular wall thickness, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD) were evaluated using M-mode method, and left ventricular ejection fraction (EF) was calculated using the Modified Simpson method.[11]

Data Analysis

ECG parameters (TPE interval, TPE/QT ratio, and TPE dispersion) were measured and analyzed for differences between the subclinical hyperthyroidism group and the control group.

Statistical Analysis

All statistical tests were conducted using the Statistical Package for the Social Sciences 25.0 for Windows (SPSS Inc., Chicago, IL, USA). The normality of the data was evaluated using the Kolmogorov-Smirnov test. Numerical data with a normal distribution were presented as mean ± SD, while parameters with a non-normal distribution were presented as median (minimum-maximum) percentile, while categorical data were expressed as percentage. Student's t-test or Mann Whitney U test was used to compare unpaired samples as needed. Chi-square test was used to assess differences in categorical variables between groups. Multiple linear regression analyses using the stepwise method were performed to assess the independent variables affecting the dependent variable QTc. All independent variables in the multiple linear regression were tested for multicollinearity. If the variance inflation factor (VIF) exceeded 3.0, the variable was considered to be collinear. All reported confidence interval (CI) values are calculated at the 95% level. Statistical significance was defined as $p<0.05$ for a two-sided test.

RESULTS

The demographic and clinical characteristics of 42 subclinical hyperthyroidism patients and 64 control subjects included in the study are given in **Table 1**. The statistical analysis revealed that the age and gender distributions of the two groups, comprising individuals with normal thyroid function and subclinical hyperthyroidism, did not significantly differ from each other, with p-values of 0.707 and 0.552, respectively. Glucose and creatinine levels were found to be significantly higher in the subclinical hyperthyroidism group than in the control group ($p<0.001$). Potassium and calcium levels were also found to be significantly higher in the subclinical hyperthyroidism group compared to the normal group ($p=0.022$ and $p=0.009$, respectively). There were no significant differences between the two groups in terms of FT3, FT4, LDL, HDL, triglyceride, urea, sodium, hemoglobin, hematocrit, and WBC levels. TSH levels were found to be significantly higher in the control group compared to the subclinical hyperthyroidism group ($p<0.001$) (**Table 1**).

Comparison of conventional echocardiographic and electrocardiographic parameters between cardiac patients, parameters such as LVEF (%), LVEDD (mm), LVESD (mm), PW, IWS, RV-FAC (%), HR (beats/min), TPE, QTmax (msn), QTc (msn), TPE/QT, TPE/QTc, and TPEd (msn) were examined between the control group (n=64) and subclinical hyperthyroidism group (n=42). There was no significant difference observed between the control and subclinical hyperthyroidism groups in LVEF, LVEDD, and LVESD parameters ($p>0.05$). There was also no significant difference found in PW and IWS parameters between the control and subclinical hyperthyroidism groups ($p>0.05$). RV-FAC parameter also showed no significant difference ($p>0.05$). However, the subclinical hyperthyroidism group had significantly higher values in HR (beats/min), TPE, QTc, TPE/QT, TPE/QTc, and TPEd compared to the control group ($p<0.05$) (**Table 2**, **Figure**).

Table 1. Baseline characteristics and laboratory parameters of groups

Variables	Normal (n=64)	Subclinical hyperthyroidism (n=42)	p
Age, y (mean ± SD)	41.1±12.1	42.1±13.4	0.707
Female, n(%)	30 (47%)	23 (55%)	0.552
Glucose (mg/dL)	90.2±11.8	110.2±23.7	<0.001
LDL (mg/dl)	109.9±32.0	116.4±28.8	0.503
HDL (mg/dl)	44.0±8.4	49.0±15.6	0.110
Triglyceride (mg/dl)	129.5±46.8	128.3±63.4	0.942
Ure (mg/dl)	35.2±13.1	29.2±9.2	0.059
Creatinine (mg/dl)	0.8±0.2	0.6±0.1	<0.001
Sodium (mmol/L)	139.1±3.4	140.0±2.6	0.356
Potassium (mmol/L)	4.2±0.5	4.5±0.4	0.022
Calcium (mmol/L)	9.0± 0.6	9.5±0.7	0.009
Hemoglobin (g/dl)	14.1±1.7	13.8±1.6	0.403
Hematocrit (%)	42.3±5.1	41.7±6.7	0.648
WBC (10^3/uL)	8.1±2.7	7.5±1.5	0.246
TSH (uIU/ml)	0.9(0.0-11.1)	0.05(0.01-0.28)	<0.001
FT4 (mcg/dl)	0.9±0.3	3.1±1.4	<0.001
FT3 (mcg/dl)	2.2±1.1	10.7±4.8	<0.001

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, WBC: White blood cell count, TSH: thyroid stimulating hormone, FT4: free T4, FT3: free T3

Table 2. Comparison of conventional echocardiographic and electrocardiographic parameters of patients

Variables	Normal (n=64)	Subclinic (n=42)	p
LVEF (%)	62.2±3.1	63.1±4.4	0.401
LVEDD (mm)	45.0±3.6	45.7±4.1	0.161
LVESD (mm)	29.8±3.8	30.3±4.0	0.231
PW	8.2±0.9	8.4±0.9	0.077
IWS	8.2±0.8	8.3±0.6	0.203
RV-FAC (%)	40.3±3.0	39.7±4.1	0.275
HR, beats/min	76.3±8.5	85.6±19.1	0.001
TpTe	65.2±4.3	82.3±12.9	<0.001
QTmax (msn)	344.9±16.8	340.6±36.6	0.412
QTc (msn)	388.0±20.5	399.4±19.9	0.006
TpTe/QT	0.18±0.01	0.24±0.03	<0.001
TpTe/QTc	0.16±0.01	0.20±0.03	<0.001
TpTed (msn)	13.0±5.0	24.4±7.6	<0.001

Abbreviations: LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; LVESV, left ventricular end systolic diameter; PW, posterior wall; IWS, interventricular septum; RV-FAC, right ventricular fractional area change; HR, heart rate;QTc, corrected QT.

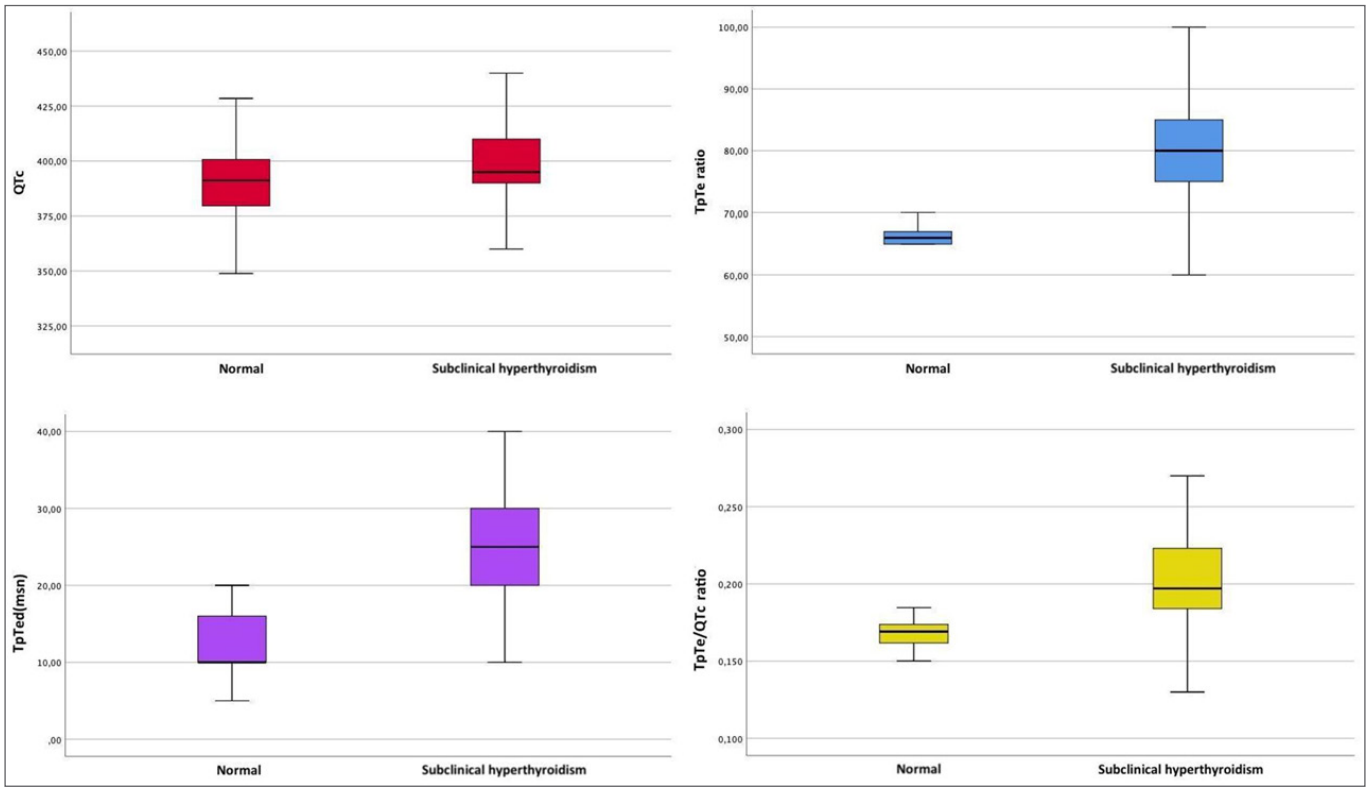

Figure. Comparison of QTc, Tpeak - Tend (TPE) interval, Tpeak - Tend dispersion, and TPE/QTc ratio in patients with subclinical hyperthyroidism and the control group

Factors affecting QTc were assessed using linear regression analysis, including both univariate and multivariate analyses. Primarily, statistically significant parameters and parameters likely to affect the QTc parameter were included in the model. Model fit R value was determined as 0.634 and R square was determined as 0.402. Heart rate, age, sodium and subclinical hyperthyroidism group were defined as an independent parameter of QTc in regression analysis (**Table 3**).

Table 3. Independent factors affecting QTc in subclinical hyperthyroidism patients in stepwise multiple linear regression analysis

Model	Unstandardized coefficients		Standardized coefficients	p value
	B	Std. Error	Beta	
(Constant)	293.081	81.455		0.001
Heart rate	-1.345	0.180	-0.665	<0.001
Age	-0.321	0.152	-0.188	0.038
Sodium	1.166	0.556	0.183	0.040
Subclinical hyperthyroidism group	10.650	5.091	0.187	0.040

DependentVariable: QTc
b. Correlates in the Model: (Constant), heart rate, age, sodium, subclinical hyperthyroidism group

DISCUSSION

The present study investigated the relationship between TPE, TPE/QT ratio, and TPEd in patients with subclinical hyperthyroidism. The findings indicate that patients with subclinical hyperthyroidism exhibit significantly higher TPE, TPE/QT ratio, and TPEd values compared to the control group.

Although subclinical hyperthyroidism was once considered a benign condition, recent studies have linked it to adverse cardiovascular outcomes.[4,12] The mechanisms underlying this relationship are not completely understood, but it has been suggested that subclinical hyperthyroidism may lead to various cardiovascular changes, including alterations in cardiac function and electrophysiology.[13] Several studies have investigated the association between subclinical hyperthyroidism and electrocardiographic (ECG) changes, and TPE interval and dispersion have been found to be increased in patients with subclinical hyperthyroidism.[14,15] TPE interval is a marker of ventricular repolarization, and an increase in this interval is associated with an increased risk of arrhythmia and sudden cardiac death.[16] TPE dispersion, which is the difference between the maximum and minimum TPE intervals across 12 leads, is also considered to be a marker of increased arrhythmic risk.[17]

Our findings align with previous studies that have shown an association between subclinical hyperthyroidism and elevated cardiovascular risk factors.[12,18] Several previous studies have demonstrated that TPE, TPE/QT ratio, and TPE dispersion are valuable markers of ventricular arrhythmias and sudden cardiac death.[14,16,19] Moreover, subclinical hyperthyroidism has been linked to an increased risk of cardiovascular events and mortality.[1,20] The TPE interval is a measure of the dispersion of repolarization in the heart,

which reflects the heterogeneity of ventricular recovery times and has been shown to be associated with increased risk of ventricular arrhythmias.[21] The TPE/QT ratio has been proposed as a marker of transmural dispersion of repolarization, which is an important determinant of the arrhythmogenic substrate.[22] In addition, TPE dispersion is an important parameter for assessing the heterogeneity of ventricular repolarization and has been shown to be a predictor of ventricular arrhythmias in various clinical conditions.[23,24] Our findings are consistent with these studies and provide further evidence that subclinical hyperthyroidism may increase the risk of ventricular arrhythmias.

The exact mechanisms linking subclinical hyperthyroidism and ventricular arrhythmias remain unclear. One proposed mechanism is the alteration of ion channels and intracellular calcium homeostasis, which can lead to an increase in early after depolarizations (EADs) and triggered activity.[25] In addition, subclinical hyperthyroidism may also increase sympathetic activity and induce hemodynamic changes that can further contribute to the development of arrhythmias.[26] The higher TPE, TPE/QT ratio, and TPE dispersion values observed in subclinical hyperthyroidism patients may be due to the effects of thyroid hormones on cardiac repolarization. Thyroid hormones have been shown to have direct effects on ion channels involved in cardiac repolarization, leading to changes in action potential duration and repolarization.[27] In addition, thyroid hormones can alter the expression of genes involved in cardiac ion channel regulation, which may also contribute to changes in cardiac repolarization.[28,29]

Limitations

The results of this study suggest that TPE, TPE/QT ratio, and TPE dispersion could serve as useful parameters for assessing cardiovascular risk in patients with subclinical hyperthyroidism. Although our study has several strengths, including the use of both conventional echocardiographic and electrocardiographic parameters and a well-defined study population, it also has some limitations. Firstly, the sample size was relatively small, which may limit the generalizability of our findings. Secondly, although we measured thyroid hormone levels, we did not investigate the specific mechanisms underlying the observed changes in electrocardiographic parameters.

CONCLUSION

Our study provides evidence that patients with subclinical hyperthyroidism have increased TPE, QTc, TPE/QT, TPE/QTc, and TPEd values, which may increase their risk of ventricular arrhythmias. Further studies with larger sample sizes and more comprehensive assessments of thyroid function are needed to confirm these findings and elucidate the underlying mechanisms. Clinicians should consider monitoring electrocardiographic parameters in patients with subclinical hyperthyroidism and implementing appropriate interventions to reduce the risk of adverse cardiovascular events.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Fırat University Non-invasive Researches Ethics Committee (Date: 08.12.2016, Decision No: 16).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1420858

Anatolian Curr Med J. 2024;6(2):139-143

The relationship between increased iron load and respiratory function tests in patients diagnosed with transfusion dependent thalassemia

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Cite this article as: Selim C, Çiftçiler R. The relationship between increased iron load and respiratory function tests in patients diagnosed with transfusion dependent thalassemia. *Anatolian Curr Med J.* 2024;6(2):139-143.

Received: 16.01.2024 ◆ **Accepted**: 11.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Thalassaemia syndromes are the most common single gene disorders affecting more than 200 million people worldwide. Beta thalassaemia (BT) is the most common cause of transfusion-dependent thalassaemia (TDT). It has been reported in studies that iron accumulation occurs in the lungs, especially in the alveolo-capillary membrane, and the frequency of parenchymal disease increases in patients receiving frequent blood transfusions. In our study, we aimed to investigate whether there is a correlation between iron overload and pulmonary function in patients with TDT.

Methods: The study included 61 patients aged between 18 and 45 years with a diagnosis of TDT who were followed up in the hematology clinic of our tertiary care center between 2018 and 2023. Based on spirometry measurements, the pattern of respiratory impairment was defined and correlated with serum ferritin levels.

Results: The mean age of the 61 patients included in the study was 24.83±6.02 years and 33 were female and 28 were male. The mean ferritin value was 3150.88±2553.51 ng/ml. The annual number of transfusions was 15.39±1.90. According to the PFT results, mean FVC % value was 81.59±9.28, mean FEV1 % value was 82.11±7.6, mean FEV1/FVC % value was 102.55±7.63. Mean ferritin values were found to be significantly higher in patients diagnosed with TDT with restrictive lung pattern (p=0.004).

Conclusion: Our study showed that high ferritin levels are related to increased restrictive lung disease in the adult age group.

Keywords: Thalassemia, respiration, iron load

INTRODUCTION

Thalassaemia syndromes are haemoglobinopathies in which globin chain biosynthesis is affected and can be classified as α, β, or β thalassaemia syndromes according to the affected globin chain or according to transfusion dependency.[1] Thalassaemia syndromes are the most common single gene disorders affecting more than 200 million people worldwide. Beta thalassaemia (BT) is the most common cause of transfusion-dependent thalassaemia (TDT).[2] BT is a disease that varies from asymptomatic anemia to severe chronic anemia that may be fatal if not approached correctly.[3] Depending on the degree of impairment in globin chain biosynthesis and clinical reflection, β-thalassemia is classified as minor, intermedia, and major. Cooley anemia or β-thalassaemia major (BTM) is the most severe form characterised by ineffective erythropoiesis, haemolytic anemia, and decreased tissue oxygenation capacity.[4] Patients with BTM are in need of regular blood transfusions. This leads to short- and long-term complications in patients. Both blood transfusion and ineffective erythropoiesis leading to increased iron absorption lead to parenchymal iron overload.[5] Histologically, the amount of iron increases in almost all organs, especially in the liver, heart, and pancreas, and to a minor extent in the endocrine glands.[5,6] In addition, it has been reported in studies that iron accumulation occurs in the lungs, especially in the alveolo-capillary membrane, and the frequency of parenchymal disease increases in patients receiving frequent blood transfusions.[6,7]

It has been reported that patients with TDT may have a high rate of pulmonary dysfunction, but restrictive or obstructive pulmonary lung disorders have been found separately in different studies.[8,9] Although the pathophysiology of pulmonary dysfunction has

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not been fully defined, autopsy data demonstrating pulmonary iron accumulation in patients with TDT who received more than one transfusion have suggested that iron accumulation resulting from repeated blood transfusions is a possible cause of pulmonary dysfunction.[10,11]

Markers including transferrin saturation and serum ferritin are used to measure iron toxicity and accumulation in patients diagnosed with TDT.[12] Although pulmonary function has been examined by pulmonary function test (PFT) in patients with TDT, the number of studies demonstrating the correlation between transferrin saturation and ferritin, which are indicators of disease iron load, and pulmonary function, especially in adult patients, is limited. In our study, we aimed to investigate whether there is a correlation between iron overload and pulmonary function in patients with TDT.

METHODS

The study included 61 patients aged between 18 and 45 years with a diagnosis of TDT who were followed up in the hematology clinic of our tertiary care center between 2018 and 2023. Patients with diagnosed lung diseases (asthma, pneumonia, COPD, tuberculosis, bronchiectasis, etc.), smokers, and patients with chronic diseases that may lead to secondary immunodeficiency were excluded from the study. Approval for the study was obtained from Harran University Clinical Researches Ethics Committee (Date: 05.06.2023, Decision No: HRÜ/23.10.02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients were instructed to avoid other drugs (except chelators) 24 hours before transfusion. Chest X-ray radiography was performed before PFT and PFT was performed in patients with normal radiographs. Immediately before transfusion, venous samples were obtained from all patients and serum ferritin levels were evaluated and pulmonary function tests were performed using standardized spirometry. Patients were advised to perform several normal breaths, followed by deep breathing, followed by momentary breath holding and forced and rapid expiration. For 6 seconds, expiration was made as hard and long as possible. The same process was repeated 3 times, and the best possible effort was made. Based on spirometry measurements, the pattern of respiratory impairment (obstructive or restrictive) was defined and correlated with serum ferritin levels. Patients with PFT results showing obstructive or restrictive patterns were referred to the pulmonology department.

Statistical Analysis

Statistical analyses were performed using "IBM SPSS Statistics for Windows. Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Descriptive statistics are presented as Mean±SD or Median (IQR) for continuous variables. The data were analysed by Kolmogorov Smirnov test in terms of normal distribution and since $p<0.05$, Mann Whitney U test, one of the nonparametric tests, was used for continuous variables and pair group comparisons. The correlation between continuous variables was analysed by Spearman Correlation test. $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the 61 patients included in the study was 24.8±6 years and 33 were female and 28 were male. The mean ferritin value was 3150.8±2553.5 ng/ml and transferrin saturations were 42.4±14.4. The annual number of transfusions was 15.3±1.9. According to the PFT results, mean FVC % value was 81.5±9.2, mean FEV1 % value was 82.1±7.6, mean FEV1/FVC % value was 102.5±7.6, mean PEF1 % value was 68.4±10.4, mean MEF 25-75% value was 72.2±14.3 (**Figure 1**).

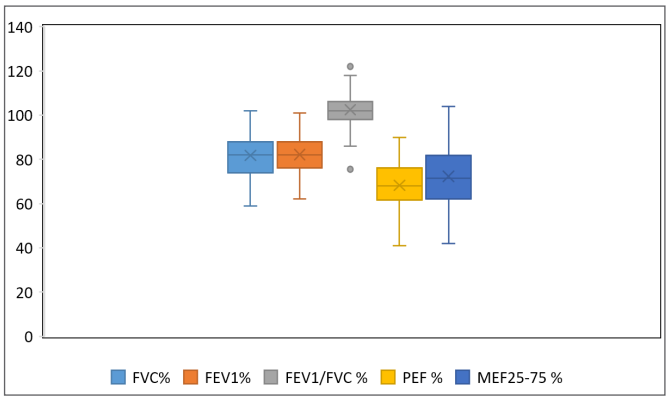

Figure 1. Pulmonary function test distribution summary

Accordingly, 35 (57.4%) of the patients evaluated by the pulmonology department were found to have normal PFTs, while 25 (41%) were found to have restrictive pattern lung disease and were followed up by the pulmonology department for further examination and treatment. Obstructive pulmonary disease was found in only 1 patient (1.6%) and this patient was also followed up in the pulmonary diseases department. Sociodemographic and Clinical Characteristics data of the patients are presented in **Table 1**.

Ferritin value was found to be significantly lower in patients diagnosed with TDT with normal PFT ($p=0.004$). However, transferrin saturation values did not show a statistically significant difference between the groups ($p=0.066$) (**Table 2**).

Table 1. Sociodemographic and clinical characteristics of the patients

Variables	n	%
Age		
Mean±SD	24.83±6.02	
Median (min-max)	23 (18-45)	
Gender		
Female	33	54.1
Male	28	45.9
Normal		
No	26	42.6
Yes	35	57.4
Obstructive		
No	60	98.4
Yes	1	1.6
Restrictive		
No	36	59.0
Yes	25	41.0
Parameters	**Mean±SD**	
Ferritin	3150.89±2553.52	
Annual number of transfusions	15.39±1.91	
Transferrin saturation	42.89±14.22	
FVC %	81.59±9.28	
FEV1 %	82.11±7.6	
FEV1/FVC %	102.55±7.63	
PEF %	68.44±10.45	
MEF25-75 %	72.26±14.33	

Table 2. Comparison of ferritin and transferrin saturation values in patients with normal Pulmonary function tests

Variables	Normal PFT		p
	No N=26 Median (IQR)	Yes N=35 Median (IQR)	
Ferritin	3384.0 (2602.5)	1750.0 (2015.0)	0.004
Transferrin saturation	44.5 (13.2)	39.0 (24.0)	0.066

Mann Whitney U test, p<0.05 is statistically significant

Mean ferritin values were found to be significantly higher in patients diagnosed with TDT with restrictive lung pattern (p=0.004). However, transferrin saturation values did not show a statistically significant difference between the groups (p=0.062) (**Table 3**).

Table 3. Comparison of ferritin and transferrin saturation values in patients with restrictive lung pattern

Variables	Restrictive		p
	No N=36 Median (IQR)	Yes N=25 Median (IQR)	
Ferritin	1807.0 (1928.2)	3614.0 (3068.0)	0.004
Transferrin saturation	39.5 (23.5)	45.0 (14.5)	0.062

Mann Whitney U test, p<0.05 is statistically significant

As seen in **Table 4**, a statistically significant negative correlation was found between ferritin values and FVC % (r=-0.469, p<0.001), FEV1% (r=-0.447 p<0.001), and MEF 25-75% (r=-0.281 p=0.028). No statistically significant correlation was found between transferrin saturation values and FVC %, FEV1 %, PEF % and MEF 25-75% (p>0.05).

Table 4. Correlation between various clinical parameters

		Ferritin	Transferrin saturation
FVC %	r	-.469**	-0.241
	p	<0.001	0.062
FEV1 %	r	-.447**	-0.238
	p	<0.001	0.065
FEV1/FVC %	r	0.152	0.141
	p	0.241	0.278
PEF %	r	-0.119	-0.191
	p	0.361	0.140
MEF25-75 %	r	-.281*	-0.218
	p	0.028	0.091

Correlation is significant at 0.05 level (Spearman correlation test), ** Correlation is significant at 0.01 level (Spearman correlation test)

DISCUSSION

In our study, PFT results were compatible with restrictive lung disease in 41% of patients with TDT and ferritin levels of these patients were found to be significantly higher than patients with TDT who were evaluated as normal lung findings according to PFT results. According to the results of our study, high ferritin levels may be an indicator of increased pulmonary damage and restrictive lung disease.

Pulmonary diseases in patients diagnosed with TDT have been investigated previously and different results were observed in the studies. In some studies, the risk of obstructive lung disease increased in patients diagnosed with TDT, whereas the frequency of lung disease in a restrictive pattern was found to be increased in patients with TDT.[13,14] The reason for this has been shown to be the accepted hypercoagulable state resulting in microembolisation of the pulmonary arteries and the chronic hypoxemic state that may cause an abnormal alveolar enlargement limiting the volume of the air cavities in patients diagnosed with TDT, as shown in studies involving autopsies.[15,16] In addition, it has been reported that thoracic developmental disorders may lead to restrictive lung disease in patients with a diagnosis of TDT due to growth retardation.[17]

As described by these results, the number of studies showing the relationship between ferritin level, which is an indicator of increased iron load due to increased ineffective erythropoiesis and transfusion therapy, and pulmonary function is limited and the results are contradictory.

In a study by Bourli et al.[14] pulmonary function, PFT, and carbon monoxide diffusion capacity tests were measured in 52 patients with TDT, and a restrictive lung pattern was found in 38% of the patients. Although this rate is similar to our study, no correlation was found between restrictive lung disease and ferritin levels. This difference in the results may be due to the fact that patients under

18 years of age were included in this study and ferritin levels were found to be much lower (1680 ng/ml) in the patients included in this study compared to our patients.

In the study by Li et al.[18] 29 patients with TDT were evaluated in terms of pulmonary diseases. The mean age of the patients included in this study was 14 years. The patients included in this study were evaluated by PFT and MRI, and restrictive lung disease was found in 34% of the patients. This rate is similar to the rate in our study. Although ferritin level was increased in patients with restrictive lung disease, this increase was not statistically significant. In contrast to our study, the small number of patients in this study and the fact that the patients were in the pediatric age group may have caused the results to be different.

In the study by Kanj et al.[19] 36 patients from pediatric and adult age groups were analyzed in terms of the correlation between pulmonary function and ferritin levels. Patients were also evaluated with cardiac T2 MR. In this study, patients from the pediatric age group were included in the study, which is different from our study. Restrictive lung pattern was observed in 47% of the patients in this study, which was slightly higher than in our study. Although the mean ferritin values of patients with normal lung function and patients with restrictive lung pattern were not given, similar to our study, ferritin levels were found to be significantly higher in patients with restrictive lung pattern.

In a study by Chan et al.[20] the relationship between pulmonary function, cardiac and liver MRIs, and iron load in 101 patients diagnosed with TDT with follow-up was evaluated. Both pediatric and adult patients were included in this study. Ferritin levels were found to be significantly higher in patients with iron accumulation in the liver and heart by MRI. The mean ferritin value of 38 patients with restrictive lung pattern on PFT was 4474 ng/ml and the mean ferritin value of 52 patients with normal lung pattern on PFT was 3625 ng/ml. Similar to our study, although ferritin values were found to be higher in patients with restrictive lung pattern on PFT, they were not statistically significant in this study.

CONCLUSION

Our study showed that high ferritin levels are related to increased restrictive lung disease in the adult age group. Regular monitoring of ferritin levels and regular use of iron chelation therapy in patients on TDT is essential. In patients with high ferritin levels, patient-drug compatibility should be evaluated, drug changes should be considered if necessary, and pulmonary complications related to this should be kept in mind.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Harran University Clinical Researches Ethics Committee (Date: 05.06.2023, Decision No: HRÜ/23.10.02).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1419846

Anatolian Curr Med J. 2024;6(2):144-149

The evaluation of gastrointestinal involvement and nutritional status in systemic sclerosis: identifying risk factors for malnutrition in a cross-sectional study

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Cite this article as: Avanoğlu Güler A, Tufan A. The evaluation of gastrointestinal involvement and nutritional status in systemic sclerosis: identifying risk factors for malnutrition in a cross-sectional study. *Anatolian Curr Med J.* 2024;6(2):144-149.

Received: 15.01.2024 ◆ **Accepted**: 13.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Gastrointestinal (GI) involvement is frequently observed in Systemic sclerosis (SSc). Considering the effect of GI involvement on SSc patients, the risk of malnutrition might be increased. The study aimed to evaluate GI involvement and the risk for malnutrition and to demonstrate the relationship between disease-related features and risk factors for malnutrition in SSc patients.

Methods: SSc-related clinical features and disease severity evaluated with Physician Global Assessment (PGA) were recorded. Detailed GI symptoms and the impact of GI involvement on patients were assessed with the UCLA SCTC GIT 2.0 questionnaire. Nutritional status was evaluated with Body Mass Index (BMI) and the Malnutritional Universal Screening Tool (MUST).

Results: 104 SSc patients were involved in the study. Mean age of patients with SSc was 52.24±12.82 years. GI involvement was found in 85.7% of patients. 76% of patients had GI symptoms. The median BMI of patients was 25.3 (9) kg/m² with 4.8% of patients categorized as underweight. The assessment of risk for malnutrition using MUST showed 74% of patients at low risk, 16% at moderate risk, and 9.6% at high risk. No important association was detected between risk groups for malnutrition and UCLA GIT 2.0 score. A significant association was found between moderate to high risk for malnutrition and dcSSc (OR 3.12, %95 CI:1.26-7.73; P=0.01), the presence of GI symptoms (OR 5.32, %95 CI:1.16-24.36; P=0.03), the decrease in oral aperture (OR 0.35, %95 CI:0.15-0.79; p:0.02), and severity of the disease investigated by PGA score (OR 1.52, %95 CI:1.09-2.13; p=0.01).

Conclusion: GI involvement is a common manifestation in SSc patients. Approximately 26% of patients were at moderate to high risk for malnutrition. Several SSc-specific clinical features, including disease severity, the presence of GI symptoms, dcSSc, and a decrease in oral aperture were related to a higher risk for malnutrition.

Keywords: Gastrointestinal involvement, malnutrition, MUST score, risk factors, systemic sclerosis

INTRODUCTION

Systemic sclerosis (SSc) is a chronic rheumatologic disease characterized by multisystem involvement with elevated morbidity and mortality rates. The primary pathogenetic mechanisms in SSc involve a dysregulation of the immune system, resulting in exaggerated inflammation, vasculopathy, and consequent augmented extracellular matrix synthesis, culminating in fibrosis.[1-3] In SSc, major organ involvements such as pulmonary, cardiac, or gastrointestinal (GI) systems are frequently observed, those play a pivotal role in contributing to disease-specific manifestations and serve as crucial determinants of both disease severity and progression of the disease.

The GI tract is the second most frequently affected site following the skin, with an incidence reported in 80%-90% of SSc patients.[4,5] SSc has the potential effect on any part of the GI tract, thereby contributing to a highly heterogeneous presentation of disease-related symptoms ranging from reflux symptoms such as regurgitation or heartburn sensation to diarrhea or fecal incontinence. Given that pulmonary and heart involvement stand as the primary cause of SSc-related mortality, therapeutic interventions, and clinical approaches predominantly prioritize these manifestations.[6] Therefore, GI involvement might be failed to notice leading to a lack of thorough assessment and proper treatments. Apart from its high incidence, GI involvement can cause a substantial decline in quality of life and functional capacity in SSc patients. GI involvement is reported as the major determinant of health quality in SSc patients.[7] Furthermore, severe GI disease, including malabsorption, need for hyperalimentation, pseudo-obstruction, and

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intestinal bacterial overgrowth, affects 8% of SSc patients. Moreover, severe GI disease is observed in the very early disease (disease duration<2 years) and is associated with higher mortality rate.[8,9]

The presence of GI involvement in SSc poses a risk for the development of malnutrition due to symptoms associated with the involvement, such as early satiety or distension, and dysmotility-related complications, particularly in intestinal bacterial over-growth leading to malabsorption.[10] Besides, the chronic course of SSc, coupled with its multisystem involvement and disease severity, might also contribute to the development of malnutrition. The objective of the study was to evaluate GI involvement and risk for malnutrition in SSc patients and to determine the impacts of SSc-related features on risk for malnutrition.

METHODS

This study was cross-sectional and conducted at the Department of Rheumatology, Gazi University Hospital. Patients who met the ACR/EULAR 2013 criteria of SSc were included.[11] Participants who supplied informed written consent by the principles delinated in the Declaration of Helsinki were incorporated into the study. The study received approval from Gazi University Hospital Ethics Committee (Date: 05.10.2020, Decision No: 664).

Sociodemographic information and clinical characteristics of SSc patients were derived from both medical records and interviews conducted with patients. Gastrointestinal (GI) involvement was evaluated with the presence of related symptoms, including reflux, dysphagia, early satiety, diarrhea, bloating, and constipation, based on self-reported information provided by patients and any evidence of esophageal involvement (esophageal dysmotility, as a reported by manometry).[12] Besides severe GI disease defined as the presence of hyperalimentation, small intestinal bacterial overgrowth or pseudo-obstruction was investigated.[9] The definition of microstomia was as an interincisal distance measuring less than 40 mm.[13]

The UCLA SCTC GIT 2.0 questionnaire is a measure to evaluate the impact of SSc-related GI symptoms on health-related quality of life and to assess the severity of GI involvement in SSc patients.[14] UCLA GIT 2.0 includes seven subscales related to GI manifestations and all subscales are scored from 0.0 to 3.0 except diarrhea (0.0-2.0) and constipation (0.0-2.5), and the total score is computed as the sum of all subscales, excluding constipation, divided by 6 yielding a range from 0.0 to 2.83 (higher scores reflect worse HRQOL). The Turkish-validated version of this measurement was utilized in the study 15. Moreover, participants underwent assessment using the Health Assessment Questionnaire (HAQ) and the Physician Global Assessment (PGA; scale: 0-10) to assess disease severity.[16]

Nutritional status and malnutrition were assessed by using body mass index (BMI) and the Malnutritional Universal Screening Tool (MUST). According to BMI value, patients were classified into different categories: underweight, normal weight, overweight, and obese.

The MUST which demonstrates the risk for malnutrition is calculated by adding all scores of BMI (>20=0, 18.5-20=1, <18.5=2), weight loss (unplanned weight loss in 3-6 months, <5%=0, 5-10%=1, <10%=2), and acute disease effect (no oral intake for more than five days=2). A score of 2 or higher means a high risk for malnutrition, necessitating intervention. A score of 1 signifies moderate risk for malnutrition, recommending observation and a score of 0 means low risk for malnutrition.[17]

Statistical Analysis

SPSS was used to analyse the data of study. In accordance with the distribution, numeric data were expressed as mean±standard deviation (SD) or median with interquartile range (IQR). Comparisons between groups were analyzed with One-Way ANOVA, Kruskal Wallis Test, The Student's T test, and Man Whitney U Test. The variables, which were found a statically meaningful difference ($p<0.05$) between malnutrition risk groups, were included in univariate regression analyses. Univariate regression analyses were employed to identify risk factors for malnutrition in SSc patients and results were exhibited as an odds ratio (OR) with 95% confidence intervals (95% CI). The Spearmen test was used to calculate correlation coefficients and assess their significance for the association between non-normally distributed variables.

RESULTS

One hundred four patients (92.3% female and 64.4% lcSSc) were enrolled. The mean age of patients was 52.24±12.82 years and the median disease duration of patients was 5 (8) years. The patients' characteristics were shown in **Table 1**. The assessment of disease severity indicated a mean PGA score of 4.70±1.52. The median score of HAQ was 0.625 (1.125) in patients.

GI involvement was observed in 87.5% of SSc patients, 76% of whom had GI symptoms. The predominant GI symptoms included reflux symptoms (heartburn or regurgitation) in 60.6% of patients, dysphagia in 51.9%, and early satiety in 47.1%. Other less frequent symptoms were bloating/distention (24%), constipation (12.7%), and diarrhea (8%). Approximately five percent

of patients exhibited severe GI symptoms. (**Table 2**). The median oral aperture among patients was measured at 36.5 (10) mm and microstomia was present in two-thirds of patients. The median UCLA GIT total score was 0.214 (0-2.11)

Table 1. Baseline patients' characteristics

Age, years, mean±SD	52.24±12.82
Gender, Female, n (%)	96 (92.3)
Smoking, ever, n (%)	29 (28)
Disease duration, years, median (IQR)	5 (8)
Disease subset, lcSSc/dcSSc, n (%)	67 (64.4)/37 (35.6)
mRSS, median (IQR)	13 (12)
Telangiectasia, n (%)	65 (62.5)
Digital ulcer history, n (%)	48 (46.1)
Musculoskeletal involvement, n (%)	57 (54.8)
Interstitial lung disease, n (%)	60 (57.7)
Pulmonary arterial hypertension, n (%)	10 (9.6)
Heart involvement, n (%)	26 (25)
Renal crisis, n (%)	7 (6.7)
Anti-topoisomerase I positivity, n (%)	60 (57.7)
Anti-centromere positivity, n (%)	21 (20.2)
HAQ score, median (IQR)	0.625 (1.125)
PGA score, mean±SD	4.70±1.52

dcSSc: diffuse cutaneous systemic sclerosis; HAQ: health assessment questionnaire; IQR: interquartile range; lcSSc: limited cutaneous systemic sclerosis; mRSS: modified Rodman skin score; PGA: physician global assessment.

Table 2. Features of gastrointestinal involvement and nutritional status in SSc patients

Oral aperture, mm, median (IQR)	36.5 (10)
Microstomia, n (%)	66 (63.5)
Gastrointestinal involvement, n (%)	91 (87.7)
Esophageal involvement, n (%)	83 (79.8)
Gastrointestinal symptoms, n (%)	79 (76)
Severe gastrointestinal symptoms, n (%)	5 (4.8)
UCLA GIT, total score, median (min-max)	0.214 (0-2.11)
UCLA GIT-reflux score, median (min-max)	0.375 (0-2.62)
UCLA GIT-distention score, median (min-max)	0.5 (0-3)
UCLA GIT-fecal soilage score, median (min-max)	0 (0-3)
UCLA GIT-diarrhea score, median (min-max)	0 (0-2)
UCLA GIT-social functioning score, median (min-max)	0.16 (0-1.83)
UCLA GIT-emotional well-being score, median (min-max)	0 (0-2.88)
UCLA GIT-constipation, median score (min-max)	0 (0-2.25)
Nutritional status	
BMI kg/m², median (IQR)	25.3 (9)
Underweight, n (%)	5 (4.8)
Normal, n (%)	45 (43.3)
Overweight, n (%)	26 (25)
Obese, n (%)	28 (26.8)
MUST Score, median (IQR)	0.39 (1)
Low risk, n (%)	77 (74)
Medium risk, n (%)	17 (16.3)
High risk, n (%)	10 (9.6)

BMI: body mass index; IQR: interquartile range; MUST: Malnutritional Universal Screening Tool; UCLA GIT: The University of California Los Angles Scleroderma Gastrointestinal tract questionnaire.

The examination of nutritional status in the study demonstrated that the median BMI of patients was 25.3 (9) kg/m². The assessment of risk for malnutrition using MUST showed 74% of patients at low risk, 16% at moderate risk, and 9.6% at high risk The evaluation of patients' characteristics in terms of risk for malnutrition was presented in **Table 3**. The comparison of disease subsets between risk groups for malnutrition displayed that the frequency of dcSSc in patients with moderate and high risk for malnutrition was %58 and 50%, respectively. The ratio of dcSSc was statistically lower in patients at low risk for malnutrition (28.6%) than patients at moderate risk for malnutrition (p=0.03). There were not any remarkable differences in the frequency of organ involvement, except renal crises, between risk groups for malnutrition (p>0.05). The renal crisis was frequently detected in patients with high risk in contrast to patients with low risk (p=0.02). Besides, patients at high risk for malnutrition had significantly increased HAQ scores meaning more disease-related disability, and more severe disease than in patients at low risk (p=0.03; p=0.004, respectively).

The median oral aperture of SSc patients in the moderate-risk group was 3.3 (0.9) mm which was prominently lower in comparison to patients with low risk for malnutrition (3.8 (0.7) mm; p=0.01). GI symptoms were more prevalent in patients at high risk for malnutrition in contrast to patients at low risk for malnutrition (p=0.04). The median UCLA GIT 2.0 score of patients was 0.18 (0-2.11) at low risk 0.28 (0-1.45) at moderate risk and 0.39 (0-1.1) at high risk for malnutrition. Despite the higher scores observed in the moderate and high-risk groups, there was no significant association to be found between risk groups and UCLA GIT 2.0 score.

The association between the MUST risk score and clinical variables was elucidated through regression analyses, with unadjusted crude OR being reported. An important association was displayed between moderate to high risk for malnutrition and dcSSc (OR=3.12, %95 CI:1.26-7.73; P=0.01), the presence of GI symptoms (OR=5.32, %95 CI:1.16-24.36; P=0.03), the decrease in oral aperture (OR=0.35, %95 CI:0.15-0.79; P=0.02), and disease severity investigated by PGA score (OR=1.52, %95 CI:1.09-2.13; p=0.01). There was not any important correlation between the MUST risk score and UCLA GIT 2.0 total and subscale scores (UCLA GIT total, r=0.019 p=0.85; UCLA GIT-reflux, r=0.035 p=0.73; UCLA GIT-distention, r=-0.012 p=0.90; UCLA GIT-fecal soilage, r=-0.027 p=0.78; UCLA GIT-social functioning, r=0.15 p=0.13; UCLA GIT-diarrhea, r=-0.013 p=0.89; UCLA GIT-emotional well-being, r=0.049 p=0.62; UCLA GIT-constipation, r=0.035 p=0.73).

Table 3. The assessment of clinical features in SSc patients in terms of risk for malnutrition

MUST	Low Risk n=77	Moderate Risk n=17	High Risk n=10	p	p1	p2	p3
Age, years, mean±SD	53.9±12.8	48.35±15.5	46±8	0.72	0.15	0.05	0.60
Disease duration, years, median (IQR)	5 (7)	5 (9)	2 (6)	0.26	0.62	0.14	0.13
Disease subset, dcSSc, n (%)	22 (28.6)	10 (58.7)	5 (50)	0.04	0.03	0.27	0.70
mRSS, median (IQR)	12 (11)	16 (16)	14.5 (22)	0.37	0.27	0.30	0.84
Telangiectasia, n (%)	48 (62.3)	12 (70.6)	5 (50)	0.55	0.81	0.49	0.41
Digital ulcer history, n (%)	37 (48.7)	7 (41.2)	6 (10)	0.77	0.77	0.74	1.00
Musculoskeletal involvement, n (%)	37 (48.7)	12 (70.6)	8 (80)	0.06	0.17	0.09	0.68
Interstitial lung disease, n (%)	44 (57.1)	10 (58.8)	6 (60)	0.99	1.00	1.00	1.00
Pulmonary arterial hypertension, n (%)	8 (11.4)	0	2 (20)	0.31	0.59	0.60	0.19
Heart involvement, n (%)	21 (29.2)	1 (6.3)	4 (40)	0.12	0.10	0.48	0.12
Renal crisis, n (%)	3 (4.1)	1 (6.3)	3 (30)	0.01	0.55	0.02	0.26
Anti-topoisomerase I positivity, n (%)	43 (55.8)	11 (64.7)	6 (60)	0.82	0.73	1.00	1.00
Anti-centromere positivity, n (%)	16 (20.8)	4 (23.5)	1 (10)	0.74	0.75	0.68	0.62
HAQ, median (IQR)	0.56 (1.06)	0.62 (1.73)	1.43 (1.69)	0.08	0.23	0.03	0.51
PGA, median (IQR)	5 (2)	5 (3)	6 (2)	0.02	0.41	0.004	0.10
Oral aperture, mm, median (IQR)	3.8 (0.7)	3.3 (0.9)	3.4 (1)	0.03	0.01	0.21	0.59
Microstomia, n (%)	46 (59.7)	12 (70.6)	8 (80)	0.45	0.37	0.49	1.00
Gastrointestinal involvement, n (%)	65 (84.4)	16 (94.1)	10 (100)	0.25	0.45	0.34	1.00
Esophageal involvement, n (%)	60 (77.9)	14 (82.4)	9 (90)	0.55	0.72	0.68	1.00
Gastrointestinal symptoms, n (%)	54 (70.1)	15 (88.2)	10 (100)	0.05	0.12	0.04	0.52
Severe gastrointestinal symptoms, n (%)	4 (5.2)	0 (0)	1 (10)	0.48	1.00	0.47	0.37
UCLA GIT, total score, median (min-max)	0.18 (0-2.11)	0.28 (0-1.45)	0.39 (0-1.1)	0.87	0.61	0.82	0.80

p1: low risk vs moderate risk p2: low risk vs high risk p3: moderate risk vs high risk
dcSSc: diffuse cutaneous systemic sclerosis; HAQ: health assessment questionnaire; IQR: interquartile range; lcSSc: limited cutaneous systemic sclerosis; mRSS: modified Rodman skin score; MUST: Malnutritional Universal Screening Tool; PGA: physician global assessment; UCLA GIT: The University of California Los Angles Scleroderma Gastrointestinal tract questionnaire.

DISCUSSION

The majority of SSc patients suffer from GI involvement which can lead to detrimental consequences, such as esophageal stricture, pseudobstruction or malnutrition, and markedly impairment of health related quality of life. The primary aim of treatment modalities and clinical approaches to GI involvement is usually to relieve the symptoms and sustain adequate nutritional status. Although the exact pathogenesis of GI involvement in SSc is obscure, clinical and animal studies are implicated in vascular damage, inflammation, fibrosis, and muscular atrophy which result in hypomobility, the hallmark of GI involvement. Furthermore, recent studies have revealed that autonomic nerve dysfunction contributes to one of the mechanisms underlying dysmotility in GI involvement.[18,19]

In our study, the incidence of GI involvement was found to be 87.5% and upper GI symptoms were found to be more prominent in SSc patients. The cohort study which included 69% of lcSSc patients, similar to our study sample has demonstrated that the predominant GIT complaint is upper GIS symptoms (94%), the most common of ones are reflux and distention, evaluated using SSc-GIT 1.0.5 The EUSTAR database which is the most extensive SSc cohort has shown that upper GI symptoms are more frequently observed than lower GI symptoms, compatible with our results.[20] Besides, our study revealed that 5% of SSc patients had severe GI disease which is related to increased morbidity and mortality.[9]

In SSc, dysmotility is one of the main mechanisms responsible for serious GI manifestations such as reflux esophagitis, gastroparesis, small intestinal bacterial overgrowth, or pseudo-obstructions, all of which might be potential leading causes of malnutrition.[18,21] Beyond severe involvement, various symptoms and associated complications of GI involvement can contribute to the predisposition of malnutrition in SSc patients. In our cohort, SSc patients were found to have a notable frequency of malnutrition risk with 25.6% classified as having moderate (16%) to high (9.6%) risk for malnutrition and %5 patients with underweight. The Canadian SSc cohort with a large number patient size has demonstrated that 30% of patients are at moderate to high risk for malnutrition and the number of GI symptoms is related to higher risk for malnutrition.[22] Similarly, GI symptoms were reported in 76% of SSc patients and the presence of these symptoms was found to be a predictor of higher risk for malnutrition in our study. Nonetheless, our study reported that the UCLA GIT 2.0 total score which reflects the severity of GIS involvement and its related symptoms, did not exhibit a significant increase

in patients at moderate to high risk for malnutrition. The study assessing GI symptoms and nutritional status in SSc has demonstrated a meaningful correlation was not detected between MUST score and UCLA GIT 2.0 total or subscale scores, similar to our results.[23] However, a recent study has indicated that malnourished SSc patients have significantly worse GI symptoms evaluated using UCLA GIT 2.0.[24]

Although dcSSc is considered as a predictor of major organ involvements such as ILD or renal disease, it is noteworthy that GIS involvement is frequently observed in both lcSSc and dcSSc patients.[4] In our study, patients with dcSSc were markedly frequent within the moderate and high-risk group for malnutrition, and a significant association was found between dcSSc and higher risk for malnutrition in SSc. Similar to our result, the Canadian cohort group has reported the relationship between dcSSc and a higher risk for malnutrition.[22] In contrast previous study including ninety-eight SSc patients has shown that mRSS scores are increased in patients with high risk for malnutrition whereas disease subsets are similar between risk groups.[25] In addition disease subset, renal crises/involvement was significantly frequent in patients at high risk for malnutrition in comparison to patients at low risk while there was no detected meaningful association between renal crisis and higher risk for malnutrition in our study. An interesting finding from our study was no obvious effect of major organ involvement on risk for malnutrition.

In the literature, a few clinical studies have detected that disease severity is considered as an independent risk factor for malnutrition.[22,26,27] The results of our study, consistent with prevailing previous reports, emphasized that the disease severity assessed with PGA was a predictor for malnutrition risk. Besides, patients with moderate to higher risk for malnutrition had worse health quality in our study. Microstomia, a common manifestation of SSc, can affect nutritional status in SSc patients through leading to chewing problems, dental health problems, and loss of teeth. Microstomia and a decrease in oral aperture are considered as risk factors for the development of malnutrition in SSc.[22,25] Interestingly, the frequency of microstomia was similar in low and moderate to high risk groups for malnutrition whereas a decrease in oral aperture was significantly related to moderate to high risk for malnutrition in our cohort.

Limitations

The main limitation was a lack of information on treatments related to GI involvement in the study. Therefore, we could not analyze the effect of GI-related treatment on symptoms or nutritional status. Another limitation was the absence of an investigation of laboratory findings related to malnutrition such as hemoglobin, serum folate, vitamin B12, and albumin. Besides, we did not evaluate patients according to localization of GI involvement due to the need of further investigation to detect the definitive localization. Also, we did not perform multivariate analyses to determine the independent risk factors for malnutrition because of the imbalance in the sample size of the groups.

CONCLUSION

In SSc, GI involvement and malnutrition may be overlooked possibly due to a predominant focus on other major organ involvements with their substantial their heavy burdens. However, the evaluation of GI involvement and malnutrition can be facilitated through the straightforward and practical approach of questioning the symptoms and using the MUST score.[10] Furthermore, special attention might be needed to be directed towards patients exhibiting specific features, such as dcSSc, GI symptoms, severe disease, and a decrease in oral aperture for the development of malnutrition.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Gazi University Hospital Ethics Committee (Date: 05.10.2020, Decision No: 664).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1429510

Anatolian Curr Med J. 2024;6(2):150-154

The correlation between serum free light chain levels and plasma cell ratio in bone marrow biopsy in multiple myeloma

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Cite this article as: Yeşilaltay A, Ramadan S. The correlation between serum free light chain levels and plasma cell ratio in bone marrow biopsy in multiple myeloma. *Anatolian Curr Med J.* 2024;6(2):150-154.

Received: 31.01.2024 ◆ **Accepted**: 17.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: MM (Multiple myeloma) is the second most common hematological malignancy. In addition to the recent advances in treatment, new parameters are used in clinical practice in diagnosis and follow-up. sFLC (free chain kappa and lambda) shows the activation of the disease depending on the rate of MM malignant cell secretion in serum. However, the plasma cell (PC) ratio in bone marrow biopsy is still the gold standard in diagnosis. We examined the dynamic correlation between the PC ratio and the number of sFLC-related cells and the secretion rate. We aimed to examine whether a low PC ratio could be in a more aggressive form with a higher sFLC secretion with too much activity, thus examining the correlation between them.

Methods: A total of 62 newly diagnosed MM patients admitted to Başkent University Faculty of Medicine İstanbul Hospital were included in the study. At the time of diagnosis, sFLC values were requested simultaneously with bone marrow biopsy. Radiological images were obtained with PET CT/MRI or CT.

Results: In all MM groups, bone marrow PH percentages were not correlated with sFLC regardless of subtype. IgG kappa type MM had the highest sFLC values despite the lowest number of PHs, while Lambda light chain MM had the lowest sFLC despite the highest PH rates.

Conclusion: These results showed us that sFLC rates are independent of the percentage of PC in MM. We believe that the two are not correlated and should be followed up together in the follow-up of the disease.

Keywords: Multiple myeloma, bone marrow biopsy, serum free light chains, radiological involvement, plasma cell ratio

INTRODUCTION

Multiple myeloma (MM) is the second most common disease with a rate of approximately 1% among all cancers and 10% among all hematological malignancies.[1,2] It is more common in males than females, with a median age at diagnosis of 65 years.[3] In MM, it is considered that the disease emerges after the MGUS (monoclonal gammopathy unknown significance) and SMM (smoldering myeloma) stages.[4] The diagnostic criteria for all plasma cell diseases such as MGUS, SMM, MM, solitary plasmacytoma, POEMS syndrome and systemic amyloidosis have been clearly defined by the consensus report of IMWG (International Myeloma Working Group).[5]

Demonstration of increased monoclonal protein in a patient with suspected MM is the most important parameter in diagnosis and follow-up. Monoclonal (M) proteins produced by MM malignant plasma cells can be analysed by various methods. Serum protein electrophoresis (SPEP) method, which is still frequently used in clinical practice, is a cheap and easy to access test both in screening and follow-up. However, its disadvantage is that although it determines the presence of M protein in the spike, it does not determine its type. Small increases of M protein in IgD or IgE type MM can be easily overlooked, whereas serum protein elements other than immunoglobulins, such as fibrinogen, may falsely suggest the presence of an M protein.[6,7]

Again, secondary hemoglobin-haptoglobin complexes appearing as a broad band in the alpha-2-globulin region in cases of hemolysis, high transferrin concentrations in patients with iron deficiency anaemia forming a localised band in the beta region, increased alpha-2 and beta bands in nephrotic syndrome are among the reasons that cause confusion in the detection of M protein.

The method used to determine the type of M protein is serum immunoelectrophoresis. The difference of this test is its low sensitivity in determining the type of M protein. For example, HDL, bilirubin, LDL cholesterol, CRP, antistreptolysin-O, creatinine, creatinine, glucose, sodium,

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chloride, bicarbonate, urea nitrogen, albumin, iron and inorganic calcium may interfere with measurements and cause erroneous test results.

In recent years, important steps have been taken in the diagnosis and follow-up of MM. Quantitative analyses of serum free light chain (sFLC=free light chain) Kappa and Lambda are accepted as indicators of plasma cell secretion both in diagnosis and follow-up. (FLC) analysis is a sensitive antibody-based system that detects low concentrations of monoclonal FLC (i.e. kappa or lambda) in serum. This method is also important in that it shows monoclonal proteins at concentrations too low to be detected by routine serum immunofixation techniques in approximately 16% of MM patients who produce only Bence Jones protein (FLC not linked to a heavy chain).[8] Normal values of serum free light chains are shown in **Table 1**.[9]

Table 1. Normal values of serum free light chains and ratio

Free serum kappa light chains - 3.3 to 19.4 mg/L
Free serum lambda light chains - 5.7 to 26.3 mg/L
The ratio of kappa to lambda FLCs - 0.26 to 1.65

However, in patients with renal impairment, serum FLC concentrations increase with a decrease in glomerular filtration rate as a result of the normally rapid renal clearance of serum FLCs in the presence of renal impairment (e.g., creatinine clearance <60 mL/min) and may reach values 20 to 30 times normal in end-stage renal failure.[10] Conflicting results have been obtained in studies evaluating the kappa/lambda ratio in patients with renal failure. Some studies show that the ratio is increased in patients with severely reduced renal function.[11] For example, one study showed that the kappa/lambda ratio, which normally ranges from 0.26 to 1.65, can be as high as 3.1 in the presence of renal failure due to dialysis.

Morphological Appearance

The morphological characteristics of plasma cells may differ depending on their maturity and are sometimes morphologically indistinguishable from myeloblasts. Mature plasma cells are oval and have abundant basophilic cytoplasm. The nucleus is round and eccentrically located with a prominent perinuclear hoph or cytoplasmic clearing. The nucleus contains "clock face" or "spoke" chromatin without nucleolus. Plasma cells observed in myeloma vary from mature forms to immature (**Figure 1A**), plasmablastic (**Figure 1B**) and pleomorphic types. Immature plasma cells have scattered nuclear chromatin, prominent nucleoli, and a high nuclear/cytoplasmic ratio. Approximately 10% of cases contain plasmablastic morphology.[12] In some cases, multinuclear, multilobed and pleomorphic plasma cells predominate. The cytoplasm of myeloma cells containing dense endoplasmic reticulum may contain condensed or crystallised cytoplasmic immunoglobulin, resulting in the following unusual findings not limited to MM: Numerous pale bluish-white, grape-like deposits (Mott cells, Morula cells), cherry-red refractive round bodies (Russell bodies), glycogen-rich IgA (Flame cells), Vermillion-stained overfilled fibrils (Gaucher-like cells, tseaurocytes) and crystal rods.

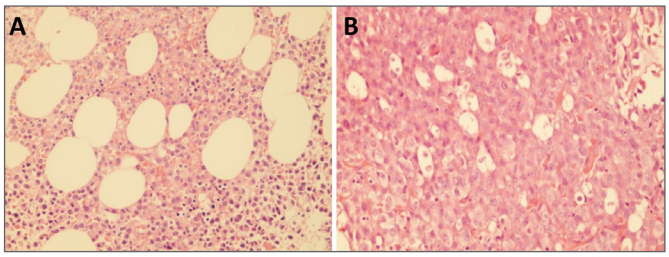

Figure 1. Hematoxylin-Eosin stainx400: Immature plasma cells with eccentric nuclei (A). Hematoxylin-Eosin stainx400: Plasmablastic morphology with distinct nucleoli (B)

Immunophenotype

Immunohistochemical staining, immunofluorescence studies and flow cytometry can be used to determine the immunophenotype of bone marrow plasma cells in patients with MM. The normal kappa/lambda ratio in bone marrow is 2:1. A ratio of more than 4:1 or less than 1:2 is considered to fulfil the definition of kappa or lambda monoclonality, respectively. This finding distinguishes monoclonal gammopathies from reactive plasmacytosis. Neoplastic plasma cells contain Kappa or Lambda monotypic cytoplasmic Ig and usually lose superficial Ig. Myeloma cells express paler CD38 and brighter CD138 than normal plasma cells. In contrast to normal plasma cells, myeloma cells are CD45 negative or expressed at very low levels. CD19, which is usually positive in normal plasma cells, is negative in 95% of myeloma cells. Some antigens, which are absent or detected at very low rates in normal plasma cells, are seen in approximately 90% of neoplastic plasma cells. Among these antigens, CD56 is expressed 75-80% and CD 200 is expressed 60-75% in myeloma cells.[13-15]

METHODS

Our study was approved by Başkent University Medical and Health Sciences Researches Ethics Board (Date: 12.12.2023, Decision No: KA-23408 /12.12.2023). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 62 newly diagnosed MM patients who were admitted to the Hematology Clinic of Başkent University Medical Faculty İstanbul Hospital in the last 5 years were included in the study. Patients were classified as IgG K/L IgA K/L IgD IgM and light chain kappa /Lambda according to the type of MM. The patients included in the study were diagnosed with MM according to the criteria

specified by IMWG. MGUS and Smoldering Myeloma (SMM), AL amyloidosis cases were excluded from the study. In the retrospective analyses of the patients, sFLC values and sFLC k/l ratios at the time of diagnosis were compared with the plasma cell percentages detected in the pathology samples obtained from the bone marrow at the time of diagnosis. Findings in favour of bone involvement in any of the radiological imaging studies (PET, MRI or CT) were accepted as bone involvement.

Statistical Analysis

Statistical analyses were performed using "IBM SPSS Statistics for Windows. Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Spearman correlation test was used to examine the correlation between continuous variables that were not normally distributed. $p<0.05$ was considered statistically significant.

RESULTS

A total of 62 patients (27 (43.55%) males and 35 (56.45%) females) with MM were included in the study. The mean age of female patients was 65.53 years and the mean age of male patients was 68.62 years. The mean age was 67.07 years in the whole group. The youngest patient was female at the age of 30.8 years and the oldest patient was male at the age of 90.3 years. Bone involvement was present in both patients at the time of diagnosis. Bone involvement was 67.74% (42 patients) in the whole group. This rate was 74% (20 patients) in the male patient population and 62.85% (22 patients) in the female patient population (**Table 2**).

Table 2. Demographic data

	Male	Female
Total number of patients (n=62)	27 (43.55%)	35 (56.45%)
Average age	68.62	65.53
bone involvement	27/20 (74%)	35/22 (62.85%)
IgG kappa MM (n=45)	n=21	n=42
IgG lambda MM (n=9)	n=3	n=6
IgA lambda MM (n=1)	n=1	n=0
Kappa light chain MM (n=4)	n=1	n=3
Lambda light chain MM (n=3)	n=1	n=2

IgG-Kappa MM was detected in 45 (72%) of the patients included in the study. Among the patients, 21 were male and 24 were female and bone marrow involvement was present in 60% of this group. The number of patients diagnosed with IgG-Lambda MM was 9 in total, 3 males and 6 females. Bone marrow involvement was found in 88.8% (8 patients). IgA-Kappa MM was observed in 1.6% (1 patient, bone marrow involvement positive), Kappa light chain MM in 6.4% (1 male, 3 female patients; bone marrow involvement positive in 3 patients), Lambda light chain MM in 4.8% (1 male, 2 female patients; bone marrow involvement positive in all). PC ratios of the patients according to MM types are shown in **Table 3**. The correlation between sFLC ratios and plasma cell percentages is shown in the **Table 4**.

Table 3. Plasma cell ratios and sFLC values according to MM types

MM Types	Mean % Plasma Cells	Mean S FLC Kappa/Lambda
IgG kappa MM	37.28%	104.201
IgG lambda MM	44.3%	113.699
IgA kappa MM	70%	30.30
Kappa light chain MM	62.5%	29.2
Lambda light chain MM	60%	139.65

Table 4. Correlation between sFLC ratios and plasma cell percentages

		sFLC/k	sFLC/L	K/L ratio	Diagnosis PC%
sFLC/k	r	1			
	p				
sFLC/L	r	-0.057	1		
	p	0.658			
K/L ratio	r	0.251*	0.119	1	
	p	<0.05	0.356		
Diagnosis PC%	r	0.017	0.058	0.122	1
	p	0.893	0.653	0.343	

*p<0.05; Spearman correlation test: As seen in Table 4, it is possible to say that there is a statistically positive correlation between sFLC/k variable and K/L Ratio (p<0.05). Accordingly, it can be said that as the sFLC/k value increases (or decreases), the K/L Ratio value also increases (decreases). However, no correlation was found between serum free kappa and free Lambda and bone marrow per cent plasma cells.

DISCUSSION

Since the free FLC value to be applied in our study will directly show the pathologically increased PC clone, we aimed to reveal the correlation between the rate of malignant plasma cell clone and free light chains secreted from the clone in our study. Thus, we investigated the correlation of bone marrow PC rates and differences with sFLC.

Due to the monoclonal nature of MM, only one of the kappa or lambda light chain is increased as a monoclonol. The plasma cell prefers light chain production to heavy chain production, which requires less ATP and can be secreted quickly and rapidly. Thus, the initial increase of the light chain before the start of heavy chain production is considered more appropriate for early detection of the rapidly changing character of the disease. With this feature, it is very valuable in the diagnosis and progression monitoring of patients with non-secretory MM and oligosecretory (monoclonal protein in serum <1 g/dL [10 g/L] MM and monoclonal protein in urine <200 mg/day), AL amyloidosis as well as light chain myeloma. Predicting the risk of progression of MGUS. In the follow-up of smoldering MM. Their use is important in the progression of solitary plasmacytoma of bone.[16-18]

MM is a highly heterogeneous disease with different genetic and molecular mechanisms. This different nature of the disease leading to differences during the course

of the disease was demonstrated by B. Barlogie at the Arkansas Myeloma Institute (UAMS) with a classification based on PCR-based gene expression profiling.[19] Even if the patient has the same genetic structure and the same type of MM, it is possible that they may present in different ways. This is due to differences in expression rates and expressed proteins from the underlying pathological gene. Therefore, this heterogeneous group requires a test that is sensitive to the course of the disease and that can rapidly show instantaneous changes, This test is a quantitative measurement of free kappa and lambda in serum and their ratio to each other. The important point that should not be overlooked here is that even if the free chain amounts are normal, the proportional distortions between them may be pathological and therefore may be a very early indicator in disease follow-up.

The gold standard test that we still use in current hematology practice at the time of diagnosis and in case of nuclei is the plasma cell count in bone marrow biopsy and the percentage of these cells in the bone marrow. However, some questions arise here. Is it necessary to increase the PH number quantitatively in order to increase the M protein? Can the secretion rate of PC increase or decrease regardless of the PC rate? Which parameter should be prescribed for treatment in this case? Which should be taken into account in assessing both nucleus and remission status?

Although plasma cell percentage is still a major parameter among the diagnostic criteria for MM, we sometimes encounter unexpectedly high sFLC values due to low plasma cell percentage or vice versa, low serum FLC values despite high PH count. In this case, the question that comes to mind is whether there may be differences in the secretion rate of malignant cells among themselves regardless of the plasma cell ratio or the fact that secretion rates may vary. Thus, a higher proportion of plasma cell numbers may result in less FLC secretion, whereas conversely a lower plasma cell number may have a higher secretory potency. This may affect the complications and survival of the disease. Thus, perhaps a lower proportion of plasma cells may be more destructive. In conclusion, the question of which parameter should be considered is important. Another important point is the MRD (minimal residual disease) tests that we still use in the clinic. The basis of these tests is the quantitative quantification of plasma cell counts in the bone marrow by flow cytometry or PCR-based methods. If plasma cell secretion is independent of the count, the reliability of these tests and MRD may need to be reviewed.

The MM subgroup analysis of the patients in our study was similar to IgG Kappa, which is the most common type in the world. There were more female patients and bone involvement was present in most of the patients.

In our review of the literature, we did not find any study showing a correlation between PH rate and FLC, except for a study conducted in China with a small case group using total FLC.[20] In this study, PH and FLC values were found to be correlated only in Ig G MM and no correlation was found in other MM subtypes. However, total FLC was used in the study and free values were not analysed. This test is not as useful as free FLC, but it is considered to be a very useful test because it is easy to be affected by infection and similar inflammatory processes that are currently increased in the body for other reasons.

In our study, IgG kappa group MM patients were found to secrete the highest sFLC at the lowest cell rate according to the current PC percentages, whereas we found the lowest sFLC values in Lambda light chain patients despite high PC rates.

As a result of our study, we found that there was no correlation between the percentage of plasma cells in the pathologically detected bone marrow biopsy sample and serum free kappa and lambdas. These results suggest that the secretion rate and activity of plasma cell may be independent of the number.

While a low PH number may cause MM to be more aggressive with a high secretion rate, the opposite may be the case. The important point here is that PC is actually in a dynamic process independent of its number, suggesting the possibility that its secretions may increase or decrease in certain periods of the disease.

CONCLUSION

We believe that MM may be better reflected not only by blood and urine M proteins or bone marrow PH count but also by both together. Studies with large patient series including remission and relapse groups with frequent bone marrow biopsies to be performed throughout the course of the disease will be able to define the correlation between PC rate and sFLC more clearly. We believe that the results of these studies may lead to a revision of the diagnostic and remission criteria and MRD concepts.

ETHICAL DECLARATIONS

Ethics Committee Approval

Our study was approved by Başkent University Medical and Health Sciences Researches Ethics Board (Date: 12.12.2023, Decision No: KA-23408 /12.12.2023).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1423588

Anatolian Curr Med J. 2024;6(2):155-160

Clinical characteristics of patients discharged from a palliative care center to home care: a retrospective cross-sectional study

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Cite this article as: Parpucu ÜM, Kahveci K, Döger C, Aydemir S, Küçük O. Clinical characteristics of patients discharged from a palliative care center to home care: a retrospective cross-sectional study. *Anatolian Curr Med J.* 2024;6(2):155-160.

Received: 22.01.2024 ◆ **Accepted**: 18.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: The aim of this study was to establish the clinical characteristics and their impact on the length of stay (LOS) of patients discharged from a palliative care center (PCC) to home care (HC).

Methods: Our cross-sectional study retrospectively analysed 314 patients who were discharged from PCC to HC between 1 January 2015 and 30 September 2018. The patients were divided into two groups based on their LOS in the PCC. Prolonged hospitalisation was defined as hospitalisation for more than 30 days. Clinical characteristics associated with prolonged hospitalization were analyzed.

Results: The study included 314 patients, with 129 (41.08%) female and 185 (58.92%) male. The mean age was 68.41±18.91 years and the LOS in PCC was 36.27±40.34 days. Of the patients, 186 were hospitalized for 30 days or less, while 128 were hospitalized for more than 30 days. The most frequent diagnosis was cerebrovascular event (CVE) (37.57%). The most common accompanying chronic systemic diseases were hypertension (20.70%), followed by diabetes mellitus and heart failure (9.87%; 6.68%, respectively). Out of the total number of patients, 9.87% (n=31) were mobilized. Among them, 55.09% (n=173) were able to receive oral nutrition, 42.03% (n=132) had percutaneous endoscopic gastrostomy (PEG), 23.88% (n=75) had pressure ulcer (PU), and 27.07% (n=85) had tracheostomy. Additionally, 6.68% (n=24) of the patients were receiving respiratory support with a home ventilator. It was observed that the LOS of patients who were mobile, able to feed orally, and diagnosed with cancer was shorter. The presence of CVE (p=0.001), head trauma (p=0.013), hypoxic brain diagnosis (p=0.001), PEG (p<0.001), tracheostomy (p<0.001), PU (p=0.011), and home ventilator (p=0.024) were identified as predictors of long LOS. Hypoxic brain diagnosis was found to be the clinical feature most strongly associated with long-term hospitalization (OR:6.8), followed by PEG feeding (OR:6.6) and the presence of tracheostomy (OR:5.2).

Conclusion: In our study we observed that time to discharge is extended due to training on care and nutrition for patients undergoing tracheostomy, PEG and PU.

Keywords: Palliative care, home care, length of stay, discharge, prolonged hospitalisation

INTRODUCTION

The increase in the number of chronic diseases (CD) requiring care, together with the growing elderly population in the world, is causing a serious increase in the demand for post-hospital care services and healthcare expenditure.[1,2] Patients with CD are known to have the greatest need for palliative care (PC) aimed at improving quality of life, and these patients have complex needs such as symptom relief and end-of-life care.[3,4] The majority of CD are patients with dementia, heart failure (HF) and diseases with high mortality and symptom burden such as cancer.[5,6] Many health systems are developing novel programs by integrating nursing homes and home care (HC) with PC for sustainable health care due to a lack of resources as the need for PC increases.[7]

In our country, the Ministry of Health's Pallia-Turk project recognised PC as a medical discipline in 2010, and community-based PC services were planned to be provided at home and by family practitioners.[8,9] As a result of these plans, the first Palliative Care Center (PCC) was opened for adult patients, and PC services previously provided in oncology clinics are now planned independently of oncology clinics and for all other critically chronic patients in addition to cancer. The number of PCCs are increasing day by day, and HC

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services, such as PC, have gained momentum in the last 10 years and started in 2005 and are offered mainly to bedridden patients, together with respiratory patients, advanced muscle disease patients, terminal cancer patients and newborns in family and home settings.[10,11] HC is provided by family practitioners, hospital-based units and mobile teams formed by community health centers.[10] Despite the rapid expansion of PC and HC in Turkiye and the necessary in-service training, PC services are not provided as part of HC, although they are present in hospitals.

Most people prefer to remain in their familiar home environment for the rest of their lives, even if they are seriously ill.[12] Integrating PC services with HC and providing PC in the home can improve patient satisfaction and reduce the length and cost of hospital stays.[6,13] By better adapting to patients' wishes and goals, hospital stays can be reduced and patient and carer satisfaction increased.[14,15] Effective symptom control can be achieved through a coordinated effort between PC and HC teams. A multidisciplinary approach and a seamless transition from PCC to HC can further improve patient care.[16] In this context, it is important to integrate PC services into HC and to encourage patients to be cared for at home so that they can live comfortably and in line with their values.

This study was designed to provide guidance for the implementation of home-based PC in terms of the clinical characteristics of patients and their transition from PC to HC. Our basic aim was to determine the clinical characteristics and their impact on the length of stay (LOS) of patients discharged from PC to HC.

METHODS

Study Design and Ethics

This retrospective cross-sectional descriptive study was started after obtaining the approval of the Health Sciences University Ankara Numune SUAM Clinical Researches Ethics Committee (Date: 28.03.2019, Decision No: 2625/2019). All procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Declaration of Helsinki as revised in 2013.

Participants

A retrospective analysis was conducted on the data of all patients aged 18 years or older who were discharged from the PCC between 1 January 2015 and 30 September 2018. The study excluded 22 patients, including 4 with missing records, 12 with a LOS of one day or less in the PCC, and 6 with recurrent hospitalisation. **Figure** shows the patient flow chart.

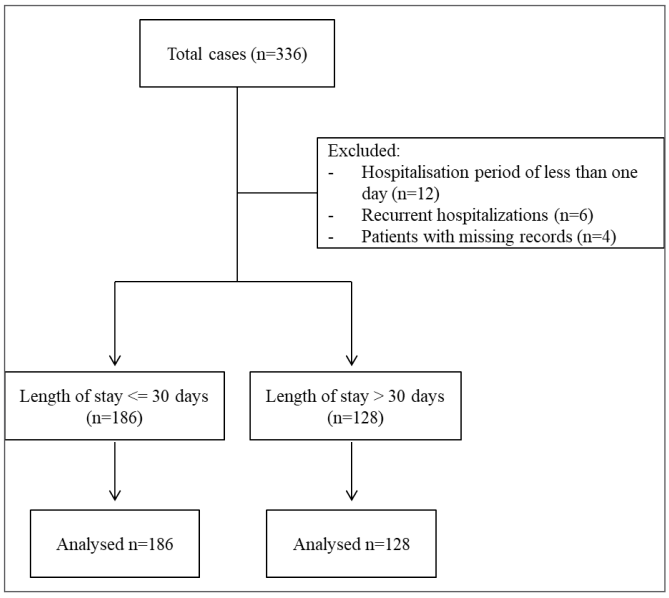

Figure. Flow charts of the patients

Interventions and Clinical Definitions

Age, sex, LOS in PCC, Glasgow Coma Scales (GCS), diagnoses, and chronic systemic diseases such as HF, hypertension (HT) and diabetes mellitus (DM) were recorded. In addition, patients with comorbidities such as mobilization status, oral feeding, percutaneous endoscopic gastrostomy (PEG), tracheostomy, home ventilator, and pressure ulcer (PU) were identified.

A 30-day limit was accepted to determine the clinical characteristics that were effective in long-term hospitalization. The patients were divided into two groups according to the LOS in PCC as LOS of 30 days or less and LOS more than 30 days.

Outcomes

The study's primary outcome measure is the clinical characteristics of PC patients who are discharged home. The secondary outcome measure is the clinical characteristics associated with prolonged LOS (more than 30 days) in patients who are discharged home from PCC.

Statistical Analysis

Statistical analysis and calculations were carried out using MS-Excel 2003 and IBM SPSS Statistics 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) software. Tables were formed for the analysis of demographic data. The data in the tables were shown as number (n), percentage (%), and numerical variables were demonstrated by mean±standard deviation (SD). Cross tables were created for each clinical feature between the two groups for LOS. Chi-square test was used in the evaluation of the cross tables. The odds-ratio (OR) was calculated to evaluate the effect of each factor on hospitalization. $P<0.05$ was considered significant for all calculations.

RESULTS

The study included 314 patients, with 129 (41.08%) female and 185 (58.92%) male. The mean age was 68.41±18.91 years and the LOS in PCC was 36.27±40.34 days. Of the patients, 186 were hospitalized for 30 days or less, while 128 were hospitalized for more than 30 days. The mean GCS was established as 11.6±3.2. Of the 314 patients included in the study, diagnoses were 21.68% (n=68) cancer, 8.28% (n=26) chronic obstructive pulmonary disease (COPD), 37.57% (n=118) cerebrovascular event (CVE), 3.50% (n=11) Parkinson's, 13.37% (n=42) dementia, 5.09% (n=16) hypoxic brain, 2.58% (n=8) motor neuron disease, 7.32% (n=23) trauma, and 6.05% (n=19) infection. CVE (37.57%) was observed as the highest, while the lowest rate was motor neuron disease (2.54%). The most common concomitant chronic systemic diseases were HT (20.70%), followed by DM and HF (9.87%; 6.68%, respectively). While 9.87% (n=31) of patients were mobilized, 55.09% (n=173) of them were able to receive oral nutrition, 42.03% (n=132) patients had PEG, 23.88% (n=75) had PU, and 27.07% (n=85) had tracheostomy and 6.68% (n=24) of the patients were receiving respiratory support with a home ventilator (**Table 1**).

Table 1. Demographic characteristics of patients

Variable	Value
Age (Years)*	68.11±18.91
Gender**	
Female	129 (41.08)
Male	185 (58.92)
Length of stay (days)*	36.27±40.34
GCS*	11.6±3.2
Diagnosis**	
Cancer	68 (21.68)
CVE	118 (37.57)
Parkinson's disease	11(3.50)
Dementia	42 (13.37)
COPD	26 (8.28)
Hypoxic brain	16 (5.09)
MND	8 (2.54)
Trauma	23 (7.32)
Infection	19 (6.05)
Heart failure	21 (6.68)
Hypertension	65 (20.70)
Diabetes mellitus	31 (9.87)
Comorbidity**	
Mobilization	31 (9.87)
Oral nutrition	173 (55.09)
PEG	132 (42.03)
Tracheostomy	85 (27.07)
Home ventilator	24(6.68)
Pressure ulcer	75 (23.88)

*Values are presented as mean±standard deviation; **Values are presented as n (%)
GCS: Glasgow coma scales; CVE: Cerebrovascular event; MND: Motor neuron disease; COPD: Chronic obstructive pulmonary disease; PEG: Percutaneous endoscopic gastrostomy

When the effect of clinical characteristics on the LOS was evaluated, it was found that patients diagnosed with cancer, mobilized patients, and patients with oral nutrition were hospitalized for less than 30 days (p values respectively; 0.003, 0.001, 0.049). However, it was established that patients with CVE (p=0.001), head trauma (P=0.013), hypoxic brain (p=0.001) diagnoses, along with PEG (p<0.001) and tracheostomy (p<0.001), patients that were followed up with home ventilator (p=0.024), patients with PU (p=0.011) spent more than 30 days in the hospital (**Table 2**). Hypoxic brain diagnosis was the clinical feature most associated with long-term hospitalization (OR: 6.8). This was followed by PEG feeding (OR: 6.6) and the presence of a tracheostomy (OR: 5.2). LOS was prolonged 2.9-fold by the diagnosis of head trauma, 2.6-fold by the use of a home ventilator, 2.3-fold by the diagnosis of CVE, and 1.9-fold by the presence of PU.

DISCUSSION

Along with a coordinated work by PC and HC teams, a better quality of life can be ensured for patients through an uninterrupted transition from PC to HC and a multidisciplinary team approach. This study is the first of its kind that investigates the clinical characteristics and LOS of patients that were discharged home from PC and transferred to HC. Similar to international studies,[4,17,18] most patients were male and over 65 years old. The rate of non-cancer patients was higher (78.32%). The patients who were difficult to care for by their relatives, had poor self-care and communication skills, and required training for care had a longer LOS in the PCC. This patient group required more services and hospitalisation time.

In a study by Brian Cassel et al.[19] which investigated the effects of healthcare service use and costs of a home-based PC program, it was found that cancer patients had shorter hospital stays compared to patients with COPD, HF, and dementia. Additionally, the study reported that home-based PC practice during the end-of-life period reduced hospital stays and costs. Additionally, several studies have shown that factors such as diagnosis, tracheostomy, home ventilator, and nutritional status can influence the discharge of PC patients to their homes.[20-22] In our study, it was found that cancer patients had a shorter hospital stay compared to other patient groups. Patients who underwent PEG, tracheostomy, and PU, and required home ventilator support, as well as those who could not be mobilized, had a significantly longer hospital stay compared to patients who could be mobilized and fed orally (P<0.001).

Table 2. Variables related to length of stay in palliative care center

		<=30 days	>30	Total	OR	P
Gender	Male	106 (57%)	79 (61.7%)	185 (58.9%)	0.822 (0.519-1.301)	0.403
	Female	80 (43%)	49 (38.3%)	129 (41.1%)		
Cancer	None	135 (72.6%)	111 (86.7%)	246 (78.3%)	0.405 (0.221-7.741)	0.003
	Yes	51 (27.4%)	17 (13.3%)	68 (21.7%)		
CVE	None	131 (70.4)	65 (50.8%)	196 (62.4%)	2.309 (1.445-3.687)	0.001
	Yes	55 (29.6%)	63 (49.2%)	118 (37.6%)		
Head trauma	None	178 (95.7%)	113 (88.3%)	291 (92.7%)	2.954 (1.213-7.191)	0.013
	Yes	8 (4.3%)	15 (11.7%)	23 (7.3%)		
Hypoxic brain	None	183 (98.4%)	115 (89.8%)	298 (94.9%)	6.896 (1.923-4.723)	0.001
	Yes	3 (1.6%)	13 (10.2%)	16 (5.1%)		
Alzheimer	None	161 (86.6%)	111 (86.7%)	272 (86.6%)	0.986 (0.509-1.912)	0.967
	Yes	25 (13.4%)	17 (13.3%)	42 (13.4%)		
Parkinson's disease	None	181 (97.3%)	122 (95.3%)	303 (96.5%)	1.780 (0.532-5.963)	0.344
	Yes	5 (2.7%)	6 (4.7%)	11 (3.5%)		
MND	None	183 (98.4%)	123 (96.1%)	306 (97.5%)	2.480 (0.582-0.566)	0.205
	Yes	3 (1.6%)	5 (3.9%)	8 (2.5%)		
COPD	None	169 (90.9%)	119 (93%)	288 (91.7%)	0.752 (0.324-1.744)	0.505
	Yes	17 (9.1%)	9 (7%)	26 (8.3%)		
Hypertension	None	145 (78%)	104 (81.3%)	249 (79.3%)	0.816 (0.465-1.433)	0.479
	Yes	41 (22%)	24 (18.8%)	65 (20.7%)		
Heart failure	None	169 (90.9%)	124 (96.9%)	293 (93.3%)	0.321 (0.105-3.977)	0.036
	Yes	17 (9.1%)	4 (3.1%)	21 (6.7%)		
Diabetes mellitus	None	168 (90.3%)	115 (89.8%)	283 (90.1%)	1.055 (0.497-2.238)	0.889
	Yes	18 (9.7%)	13 (10.2%)	31 (9.9%)		
Infection	None	176 (94.6%)	119 (93%)	295 (93.9%)	1.331 (0.525-3.374)	0.546
	Yes	10 (5.4%)	9 (7%)	19 (6.1%)		
Oral nutrition	None	75 (40.3%)	66 (51.6%)	141 (44.9%)	0.635 (0.403-2.999)	0.049
	Yes	111 (59.7%)	62 (48.4%)	173 (55.1%)		
PEG	None	141 (75.8%)	41 (32%)	182 (58%)	6.649 (4.030-8.967)	<0.001
	Yes	45 (24.2%)	87 (68%)	132 (42%)		
Tracheostomy	None	160 (86%)	69 (53.9%)	229 (72.9%)	5.262 (3.063-9.737)	<0.001
	Yes	26 (14%)	59 (46.1%)	85 (27.1%)		
Home ventilator	None	177 (95.2%)	113 (88.3%)	290 (92.4%)	2.611 (1.105-6.166)	0.024
	Yes	9 (4.8%)	15 (11.7%)	24 (7.6%)		
Pressure ulcer	None	151 (81.2%)	88 (68.8%)	239 (76.1%)	1.961 (1.161-3.313)	0.011
	Yes	35 (18.8%)	40 (31.3%)	75 (23.9%)		
Mobilisation	None	156 (85.2%)	120 (96.8%)	276 (89.9%)	0.193 (0.065-6.565)	0.001
	Yes	27 (14.8%)	4 (3.2%)	31 (10.1%)		

Values are presented as n (%); OR: Odds ratio; p<0.05 is significant; CVE: Cerebrovascular Event; MND: Motor Neuron Disease; COPD: Chronic Obstructive Pulmonary Disease; PEG: Percutaneous Endoscopic Gastrostomy

Palliative medicine is a medical speciality that aims to enhance the quality of life of patients with serious or advanced medical conditions.[23] It is appropriate at all stages of illness, including at the time of diagnosis. Neurological conditions often have high symptom burdens, variable disease courses, and poor prognoses, which affect not only patients but also their families and carers. Major contributing factors to the difficulty of care include inadequate communication with patients. Ideally, a comprehensive care approach should manage the complex needs of these patients by addressing their physical, psychological, social, and spiritual aspects of care to reduce suffering.[24] Taylor et al.[25] reported that patients with neurological diseases require more PC than those with cancer. They also found that patients admitted to a PCC with neurological disease had more severe symptoms than those admitted for cancer and had lower Palliative Performance Scale scores. Additionally, the group with neurological disease had longer hospital stays. In our study, the patient group with neurological disease had the highest percentage of diagnosis. The clinical feature most associated with prolonged hospitalisation was the diagnosis of hypoxic brain. We believe that this patient group requires the longest duration of service both in terms of disease treatment and care requirements.

Tracheotomy is recommended for patients with airway obstruction or requiring long-term mechanical ventilation support. The growing number of patients undergoing tracheotomy has resulted in an increase in referrals to hospices and PC.[26] Tracheostomies are often

performed to wean patients off the ventilator. However, in most cases, tracheostomies are placed in patients who are at the end of their life with little hope of meaningful recovery. The use of tracheostomy in PC offers a convenient option for airway control.[27] Tracheostomy care is a complex process that requires knowledge and skills for elderly patients and their caregivers. Caregivers of tracheostomy patients have reported feeling burdened due to the intensive and complex nature of their role. They require guidance and training to carry out this process effectively.[28-30] A study conducted by Nagi et al.[31] in 2014 found that training was necessary for caregivers of elderly patients with tracheostomy, and that the provided training made a significant difference. In previous descriptive studies conducted with caregivers of elderly tracheostomy patients, it was reported that they experienced a heavy burden due to the intensive and complex tracheostomy care required.[29,30] They expressed a need for training to carry out this process and highlighted the time-consuming nature of the care. In our study, we observed that patients with tracheostomy had a longer hospitalisation period. Tracheostomy care and education provided to elderly patients and their caregivers can extend the LOS.

Approximately 40-300 million patients worldwide receive PC, with PU being particularly prevalent due to limited mobility and changes in tissue perfusion caused by antalgic posture, dyspnoea, oedema, anorexia-cachexia syndrome, and impaired sensory perception due to analgesia.[32,33] Studies have shown that the likelihood of PU development is greater in CD such as stroke and in patients over 65 years of age, and that it prolongs hospital stay.[34,35] In our study, we found that patients with PUs had longer hospital stays. Furthermore, a retrospective review that investigates PU prevalence, incidence, and related factors in home PC patients reported that terminal patients were at risk of PU that adversely affected quality of life, and that effective PU prevention and care management was important, and that caregivers should be supported by PC nurses.[18] Hudson[36] emphasized that PC providers should provide caregivers with coping strategies and train them to provide care, and reported that end of life care of patients in the home environment can increase the quality of life and reduce the burden on the health system. In a study evaluating the applicability of a training program on acute symptom management for caregivers of cancer patients receiving home care, they observed that hospital admissions of patients for acute symptoms decreased by 80%.[37] Our study population consisted of patients requiring special care: PEG (42.03%), tracheostomy (27.07%), home ventilator (6.68%), and PU (23.88%). It is challenging to provide HC for this patient group, and therefore, training for carers and HC services provided by healthcare providers are crucial. This approach can reduce the burden on the health system and ensure cost-effective service management.

In our clinic, care planning of the patients is made with a multidisciplinary team approach, and their caregivers are provided with more difficult and specific feeding, PEG, tracheostomy, and wound care trainings as well as routine care of the patient. Thus, it is easier for patients that are discharged home to continue receiving care in the home environment, and for carers to cope with the difficulties while providing care. In our study, the presence of PEG and tracheostomy was found to be the most effective factor on hospital stays for more than 30 days. We believe that the reason for providing caregivers with nutrition, tracheostomy, and wound care trainings is for the possibility of prolonged stays, and in addition, their quality of life may increase by conducting HC in an uninterrupted and more effective fashion.

Limitations

There were several limitations to the present study. Due to PCC patients not being a homogenous patient group, and patients to have a variety of age groups, diagnoses, and clinical characteristics, a standardization could not be fully ensured. Furthermore, it is important to note that our study was conducted retrospectively at a single center and therefore cannot be extrapolated to the wider population. However, our study put forth certain fundamental data on the clinical characteristics of patients discharged from PCC in order to clarify their care needs. Further research is required on this topic, particularly larger multicenter prospective studies.

CONCLUSION

Our study identified the clinical characteristics of patients that were discharged home from PC and some of the main factors affecting hospitalization for more than 30 days.

Clinical features such as PEG feeding, tracheostomy, pressure ulcers, and home ventilator use can significantly prolong hospitalisation and these features can be integrated into HC. In order to provide more effective HC for patients scheduled for discharge, we believe that adequate training in PEG, tracheostomy and wound care is necessary, and that a home-based PC is necessary by integrating PC into HC services.

ETHICAL DECLARATIONS

Ethics Committee Approval

Approval was obtained from the Health Sciences University Ankara Numune SUAM Clinical Researches Ethics Committee (Date: 28.03.2019, Decision No: 2625/2019).

Informed Consent
Ankara Numune SUAM Clinical Researches Ethics Committee did not require informed consent because the study was retrospective.

Referee Evaluation Process
Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1427475

Anatolian Curr Med J. 2024;6(2):161-167

Evaluation of systemic immune-inflammation index, systemic inflammatory response index and hematologic inflammatory parameters in generalized anxiety disorder: a controlled study

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Cite this article as: Canlı D. Evaluation of systemic immune-inflammation index, systemic inflammatory response index and hematologic inflammatory parameters in generalized anxiety disorder: a controlled study. *Anatolian Curr Med J.* 2024;6(2):161-167.

Received: 29.01.2024 ◆ **Accepted**: 18.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: The current study aimed to examine the values of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), and systemic inflammatory response index (SIRI) in generalized anxiety disorder (GAD).

Methods: In this retrospective study, 147 GAD patients who did not receive treatment and 154 healthy controls with similar characteristics to the patient group were included. NLR, PLR, MLR, SII, and SIRI values calculated from the complete blood count values of the GAD group were compared with age- and sex-matched healthy controls.

Results: Neutrophil, lymphocyte, monocyte, platelet counts and NLR, MLR, SII, and SIRI values were significantly higher in the GAD group compared to healthy controls ($p<0.001$, $p=0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.003$, $p<0.001$, $p<0.001$, respectively). The logistic regression analysis revealed that SII and SIRI were identified as significant variables associated with receiving a diagnosis of GAD.

Conclusion: Inflammatory markers such as NLR, MLR, SII, and SIRI are thought to play an important role in the evaluation of inflammatory activity in GAD. However, larger and more comprehensive studies are needed.

Keywords: Generalized anxiety disorder, inflammatory biomarker, systemic immune-inflammation index, systemic inflammatory response index

INTRODUCTION

Generalized anxiety disorder (GAD) is a common mental disorder characterized by persistent worry, restlessness, tension, and somatic symptoms associated with many various events, situations, and activities. These symptoms significantly impact daily life, and individuals with GAD often experience difficulty controlling their apprehensions.[1] Symptoms must persist for at least 6 months for diagnosis. GAD is the most prevalent among anxiety disorders.[2] Although there is no single cause of GAD, a combination of multiple risk factors may influence the development of the disorder. There is increasing evidence that neuroinflammation is also involved in the etiology of psychiatric disorders and that inflammatory processes play a role in psychiatric disorders.[3,4] Evidence for the role of inflammation in GAD is also increasing.[2]

Although there are various blood biomarkers such as cytokines to evaluate inflammation, most of them are difficult and expensive tests. Therefore, there is a growing interest in blood biomarkers that are more accessible, cost-effective, and suitable for routine practice. NLR, PLR, and MLR are biomarkers used to show systemic inflammation in many diseases. These markers have also been investigated in different psychiatric diseases.[5] These ratios, calculated from a complete blood count, are more useful in indicating the status and severity of inflammation compared to a single parameter.

In addition to these markers, newer parameters have come to the forefront in recent years. These are SII and SIRI values. In recent studies, SII and SIRI have been defined as more sensitive markers.[6] Various studies have been conducted in recent years on complete blood count parameters in psychiatric disorders. Studies related to complete blood count data in panic disorder (PD) and GAD, two psychiatric disorders included in the DSM-5 Anxiety Disorders section, have been observed.[7,8] It is thought that the results obtained from this study may contribute to clinical practice in the diagnosis and follow-up of GAD and the evaluation

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of response to treatment by using a combination of systemic inflammation parameters that can be obtained from complete blood count and can be easily calculated. As far as the literature review reveals, there is no study specifically focusing on the role of SII and SIRI in GAD. Therefore, this study aimed to evaluate SII and SIRI along with lymphocyte-related ratios in GAD and compare them with healthy controls.

METHODS

The study was carried out with the permission of the Amasya University Non-interventional Clinical Researches Ethics Committee (Date: 04.01.2024, Decision No: 2023/162). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was planned retrospectively. The files of the patients were examined through the patient record system of our hospital. The patient record system shows patients' past treatments and comorbidities. The files of 324 patients admitted to our hospital between January 2022 and January 2023 and diagnosed with GAD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) by a specialist psychiatrist were examined. 21 individuals with a diagnosis of GAD using medication, 33 individuals with comorbid psychiatric disorders, 96 individuals with comorbid organic diseases, and 27 individuals with both comorbid psychiatric and organic diseases were excluded from the study. Considering the inclusion criteria, 147 patients were included in the study. The patient group was composed of individuals who were seeking help for the first time and were not taking medication. The control group consisted of hospital staff who were sent for routine psychiatric examination by the workplace physician, who were not diagnosed with any psychiatric illness as a result of the psychiatric examination performed by the psychiatry specialist, and who did not have any organic disease.

The complete blood count parameters examined during the initial visit were analyzed. Patients with additional psychiatric or organic diseases that might affect complete blood count data were not included in the study. Thus, those with a diagnosis of GAD who were taking medication, those with comorbid psychiatric diseases, and those with comorbid organic diseases (chronic liver or kidney disease, autoimmune disease, diabetes mellitus, hypertension, heart disease, respiratory system disease, cancer, anemia, endocrine diseases, etc.), and those with active infection were excluded from the study.

The NLR, PLR, and MLR were calculated using the following formula: NLR=neutrophil count/lymphocyte count, PLR=platelet count/lymphocyte count, and MLR=monocyte count/lymphocyte count.[9-11] SII and SIRI values were also calculated using neutrophil, lymphocyte, and monocyte counts. SII="(platelet count x neutrophil count)/lymphocyte count" formula, SIRI="(neutrophil count x monocyte count)/lymphocyte count" formula.[12,13]

Statistical Analysis

IBM SPSS version 22.0 (IBM Corp., Released 2013; IBM SPSS Statistics for Windows, Version 22.0; Armonk, NY: IBM Corp.) was used for statistical analysis. Descriptive statistics and continuous variables were given as mean±standard deviation. Compliance with normal distribution was evaluated by the Kolmogorov-Smirnov/Shapiro-Wilk test. Parametric values were evaluated using the t-test, and non-parametric values were evaluated using the chi-square test. The Student's t-test was employed for comparing groups with data conforming to a normal distribution, and the Mann-Whitney U test was used for data not conforming to a normal distribution. Numerical variables were presented as mean±Standard deviation, and categorical variables were presented as numbers and percentages. Receiver operating characteristic (ROC) analyzes were conducted to evaluate the ability of the NLR, PLR, MLR, SII, and SIRI to predict the diagnosis of GAD. The area under the ROC curve (AUC) values of NLR, PLR, MLR, SII, and SIRI to predict the diagnosis of GAD were given with 95% confidence interval (CI) and with sensitivity and specificity. Variables associated with the diagnosis of GAD were investigated using logistic regression analyses. Since the formulas for SII and SIRI among the variables included the NLR, PLR, and MLR variables, SII and SIRI variables were included in the analysis as variables that could be associated with the diagnosis of GAD. The statistical significance level was determined to be 0.05 and below.

RESULTS

The GAD group consisted of 147 (74 females and 73 males) patients, and the control group consisted of 154 (77 females and 77 males) healthy individuals. The mean age was 40.44±0.84 years in the patient group and 40.19±0.96 years in the control group. There was no significant difference between the groups in terms of mean age, gender, and marital status (p=0.847, p=0.953, p=0.854, respectively) (**Table 1**).

Table 1. Characteristics of patients with generalized anxiety disorder and the control group participants

Characteristics	Patients (n=147) mean±SD	Controls (n=154) mean±SD	p value
Age (years)	40.44±0.84	40.19±0.96	
Gender, n (%)			0.847
Female	74 (50.3)	77 (50.0)	
Male	73 (49.7)	77 (50.0)	
Marital status			0.953
Married	96 (65.3)	99 (64.3)	
Single	51 (34.7)	55 (35.7)	
Occupational status			0.854
Working	70 (47.6)	154 (100.0)	
Not working	77 (52.4)		

mean±SD: mean±standard deviation

Neutrophil, lymphocyte, monocyte, platelet counts, NLR, MLR, SII, and SIRI values were found to be significantly higher in GAD patients than in the control group, while there was no statistically significant difference between the groups in terms of PLR values. The pairwise comparisons of laboratory findings are presented in **Table 2**.

Table 2. Comparison of complete blood count values and some laboratory findings of generalized anxiety disorder and control group

Variables	GAD (N=147) mean±SD	Control (N=154) mean±SD	p value
Neu (10^3/μl)	5.19±0.08	3.57±0.06	<0.001*
Lym (10^3/μl)	2.44±0.05	2.23±0.04	0.001*
Mono (10^3/μl)	0.63±0.02	0.50±0.01	<0.001*
Plt (10^3/uL)	279.67±5.10	245.51±4.23	<0.001*
NLR	2.27±0.06	1.68±0.04	<0.001*
PLR	192.45±54.35	114.52±2.44	0.444
MLR	0.27±0.01	0.23±0.01	0.003*
SII	637.17±22.93	408.73±11.10	<0.001*
SIRI	1.42±0.05	0.84±0.03	<0.001*
Alanine amino transferase (U/L)	18.56±4.28	19.36±4.97	0.137
Aspartate amino transferase (U/L)	21.42±4.64	22.34±4.87	0.094
Blood urea nitrogen (mg/dL)	23.01±10.10	25.17±10.08	0.065
Creatinine (mg/dL)	0.83±0.16	0.80±0.17	0.097

*p<0.05 statistically significant; The data were compared using Student's t-test and Mann-Whitney U-test .
Abbreviations: mean±SD: mean±standard deviation, GAD: Generalized anxiety disorder, Neu: Neutrophil count, Lym: Lymphocyte count, Mono: Monocyte count, NLR: Neutrophil-to-lymphocyte ratio, PLR; Platelet-to- lymphocyte ratio, MLR; Monocyte-to- lymphocyte ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index

ROC curve analyses for NLR, PLR, MLR, SII, and SIRI to predict the diagnosis of GAD are demonstrated in **Figure 1**. The AUC values for NLR, PLR, MLR, SII, and SIRI to predict diagnosis of GAD were 0.75 (95% CI: 0.69-0.80, p<0.001), 0.53 (95% CI: 0.46-0.59, p=0.444), 0.60 (95% CI: 0.53-0.66, p=0.003), 0.80 (95% CI: 0.75-0.85, p<0.001) and 0.82 (95% CI: 0.77-0.87, p<0.001), respectively. The cut-off value of SIRI (1.01) was associated with 74.0% sensitivity and 75% specificity. The cut-off value of SII (472.38) was associated with 75.0% sensitivity and 75% specificity.

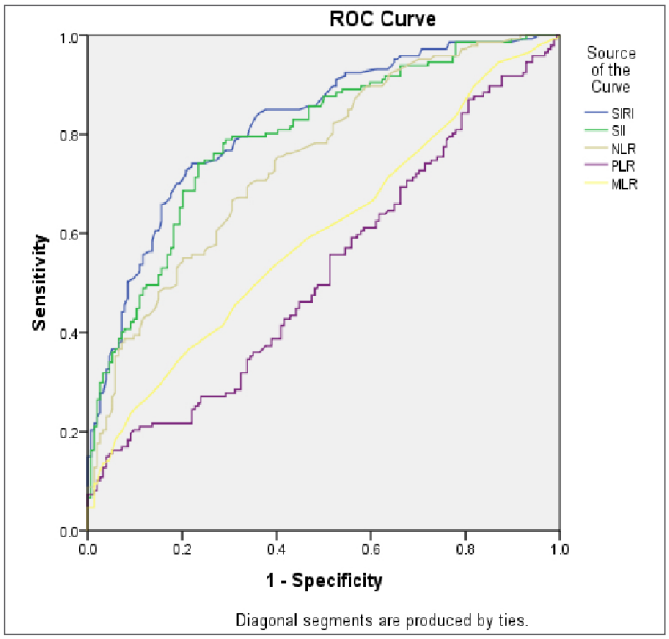

Figure 1. ROC curve analyses for NLR, PLR, MLR, SII and SIRI to predict diagnosis of GAD
ROC: Receiver operating characteristic, GAD: Generalized anxiety disorder, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MLR; Monocyte-to- lymphocyte ratio, SIRI: Systemic inflammatory response index, SII: Systemic immune-inflammation index,

Logistic regression analysis was performed separately for the SII and SIRI variables, which were the most comprehensive among the significant parameters obtained. According to the results of the analysis, it was determined that when the SIRI variable value increased by one unit, the probability of being diagnosed with GAD increased 26.687 times, and when the SII variable value increased by one unit, the probability of being diagnosed with GAD increased 1.007 times. According to the data obtained, SIRI was found to be an important variable in the diagnosis of GAD. The logistic regression analysis conducted to assess the predictors of GAD diagnosis is presented in **Table 3**.

Table 3. Logistic resgresion analysis model for the effect of SIRI and SII values on generalized anxiety disorder

Variables	B (S.E.)	df	Sig.	95% C.I.for Exp(B)		
				Lower	Exp(B)	Upper
Constant	-3.540 (0.455)	1	0.000			
SIRI*	3.284 (0.426)	1	0.000	11.584	26.687	61.483
Constant	-3.511 (0.480)	1	0.000			
SII**	0.007 (0.001)	1	0.000	1.005	1.007	1.009

A p-value less than 0.05 was considered significant for statistical analysis.
* Nagelkerke R square: 0.394; Hosmer-Lemeshow test's chi-square value: 5.590
** Nagelkerke R square: 0.344; Hosmer-Lemeshow test's chi-square value: 6.101
SIRI: Systemic inflammatory response index, SII: Systemic immune-inflammation index, C.I.: Confidence interval

DISCUSSION

In this study, ratios related to neutrophils, lymphocytes, monocytes, and platelets were compared between the GAD and control groups. While there are studies evaluating NLR and PLR levels in GAD patients, this is the first study in which the newer parameters SII and SIRI values were examined together. In our study, it was determined that untreated patients with GAD had higher neutrophil, lymphocyte, monocyte, platelet counts, NLR, MLR, SII, and SIRI values compared to healthy controls.

Recently, the usability of complete blood count parameters to examine inflammatory processes in psychiatric diseases has been the subject of research. Especially schizophrenia, schizoaffective disorder, bipolar disorder, depression, and substance use disorders are the most studied psychiatric disorders.[14-17] These parameters have also been investigated in obsessive-compulsive disorder (OCD) and PD, which are anxiety-related psychiatric disorders.[8,18,19]

White blood cells and their subtypes along with platelets are significant biomarkers that play an important role in inflammation, and the activation of these cells results in the release of inflammatory cytokines. Neutrophils are important components of innate immunity and are the first line of defense against tissue inflammation. They lead to the release of various cytokines that induce oxidative stress and inflammation. Lymphocytes play a key role in the adaptive immune response, primarily contributing to functions like antibody production.[16,20] On the other hand, monocytes are vital for the innate immune response, releasing pro-inflammatory and pro-oxidant cytokines during inflammation.[21] Platelets, akin to neutrophils, produce and release cytokines influencing inflammation.[22] These cell parameters are easily accessible through a complete blood count. The combined evaluation of these cells and ratios such as NLR, PLR, MLR, SII, and SIRI calculated based on these cells is thought to be more valuable in evaluating inflammation.[23]

The pathophysiology of GAD is complex, and the role of systemic inflammation has not yet been fully elucidated. However, although it has not yet been proven, it has been shown that inflammatory activation increases in GAD patients independently of the accompanying depression.[24] In their study, Hou et al.[25] reported a relatively increased pro-inflammatory response, a decrease in anti-inflammatory response, and a change in cytokine balance in GAD patients.

It has been shown that stress induction in humans leads to an increase in the neutrophil count.[26] Additionally, NLR is an important indicator reflecting the activation of inflammatory cells. Therefore, NLR may be an indicator of inflammatory response reflecting stress intensity and systemic inflammation.[27] The relationship between NLR and inflammatory markers such as inflammatory cytokines and CRP has also been reported.[28] Therefore, studies especially on NLR, have been intensified. Higher NLR levels have been linked to higher mortality in psychiatric patients, suggesting a potential role of neuroinflammation in the development of psychiatric diseases.[23,29,30] Platelets play a modulatory role in activating neutrophils and monocytes. PLR is a value calculated based on the ratio of platelet to lymphocyte counts. This value is being researched for its utility in monitoring individual immune processes through changes in the number of platelets, which have a modulatory role in the immune system, and the ratio of changes in lymphocytes, which are elements of acquired and innate immune responses. Activated monocytes play a role in paracrine signaling and are involved in the release of various proinflammatory cytokines and chemokines. MLR provides an understanding of the relationship between acquired and innate immune responses. There are a limited number of studies related to MLR.[5]

In this study, all parameters related to neutrophil count were found to be higher in the GAD group compared to the control group. Our study aligns with Orum[8], who found increased neutrophil count and NLR values in patients with GAD compared to healthy controls while investigating inflammatory parameters in GAD patients. The higher NLR levels may have resulted from the higher neutrophil count. In another study, PLR was found to be lower in patients with GAD compared to controls.[31] In a study conducted with children and adolescents with anxiety disorders, it was reported that NLR, MLR, and PLR were higher compared to healthy controls.[32] In children and adolescents with anxiety disorders and depression, high NLR and PLR have also been associated with suicidal behavior.[33] Brinn and Stone[29] reported elevated NLR in nonphobic anxiety disorder. Studies on cells involved in the immune response and lymphocyte-related ratios in PD and OCD, which are anxiety-related disorders, are noteworthy. In a study comparing PD patients with healthy controls, no significant differences were found between the groups in terms of NLR and PLR values, as well as neutrophil and platelet counts, while lymphocyte counts were significantly higher in the PD group.[19] In another study, it was reported that lymphocyte counts were higher in PD patients compared to controls, while there was no difference in terms of neutrophil, monocyte, and platelet counts.[34] When we look at the studies conducted in OCD, which is another anxiety-related disease, it was found that NLR levels were higher in patients diagnosed with OCD compared to healthy controls.[18] Additionally, it was found that NLR levels were higher in adolescent OCD patients with anxiety disorder

compared to controls, and neutrophil count increased when accompanied by anxiety disorder.[35]

In our study, SII and SIRI values were found to be higher in the GAD group compared to healthy controls. SII, a novel biomarker related to inflammation and immunity, is calculated based on neutrophil, platelet, and lymphocyte counts. Studies have reported that SII is a marker reflecting inflammation and immune response. This index includes important components of the immune response, including neutrophils, lymphocytes, and platelets.[36] Previously, SII has been investigated to predict the prognosis and severity of disease in physical conditions such as pancreatitis and ischemic stroke.[37,38] It has been demonstrated that SII can show systemic inflammation and immune response better than NLR, PLR, and MLR.[39] However, SII has been less investigated in psychiatric diseases. There is increasing evidence that SII, NLR, and PLR can be used as markers of disease severity in psychiatric diseases.[40,41] SII has been reported to be significantly associated with depression and anxiety symptoms in patients with tuberculosis.[41]

Dionisie et al.[42] found that SII was higher in patients with bipolar depression than in patients with unipolar depression. Inaltekin and Yağci[43] also reported that NLR, PLR, and SII values were significantly higher in patients with bipolar manic episodes and schizophrenia. Wei et al.[23] reported higher values of NLR, PLR, MLR, SII, and SIRI in patients with schizophrenia and bipolar disorder compared to healthy controls. There is also evidence showing a positive significant relationship between SII and depression and anxiety scores in individuals who have recovered from COVID-19.[44] In addition to the findings of studies that did not find significant differences in SII values between patients with first-episode schizophrenia and healthy controls, there are also study findings reporting that patients with schizophrenia have higher SII values than healthy controls.[23,45] In a study evaluating lymphocyte-related ratios and SII index in sleep-related disorders, sleep-related disorders were shown to have a stronger association with SII than PLR and NLR.[46]

SIRI is a new index of inflammation calculated based on neutrophil, lymphocyte, and monocyte counts. Conceptually, it indicates the ratio of innate immune response cells to adaptive immune response cells. SIRI was initially investigated as a predictor of prognosis in malignancies and later continued to be investigated in different diseases.[47,48] However, it is observed that SIRI is less studied in psychiatric diseases compared to other biomarkers. In the literature, there are reports of abnormal numbers of circulating immune cells in patients with major depressive disorder and bipolar disorder.[49,50] In a study, it has been shown that SII and SIRI significantly influence the risk of depression.[51]

Another finding of our study is the results of logistic regression analysis. With the obtained results, it is considered that SII and SIRI may be associated with the diagnosis of GAD. Therefore, it is believed that calculating SII and SIRI, along with the existing parameters, will be crucial in assessing inflammation. Our findings demonstrate the association of GAD with NLR, MLR, SII, and SIRI.

Limitations

An important limitation of our study is its retrospective nature. In addition, the fact the smoking status, alcohol use, nutrition, and exercise status of the participants could not be evaluated, and the data were not supported by scales are other important limitations. Another limitation of our study is that C-reactive protein (CRP) levels were not available for all participants and this parameter could not be evaluated. Therefore, we need longitudinal studies that evaluate all these parameters together and are supported by scales.

CONCLUSION

In light of the data obtained from the study, it can be suggested that low-grade inflammation is present in GAD. To better understand the role of inflammation GAD's development, we require larger-scale studies with larger sample groups, excluding confounding factors. A better understanding of the role of inflammation in anxiety disorders will shed light on the development of new treatment strategies.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Amasya University Non-interventional Clinical Researches Ethics Committee (Date: 04.01.2024, Decision No: 2023/162).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1395276

Anatolian Curr Med J. 2024;6(2):168-174

The relationship between melatonin level, oxidative stress, fatigue and sleep disorders in multiple sclerosis patients

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Cite this article as: Dağcı Y, Bilge N, Ceylan M, Özkan Hİ. The relationship between melatonin level, oxidative stress, fatigue and sleep disorders in multiple sclerosis patients. *Anatolian Curr Med J.* 2024;6(2):168-174.

Received: 24.11.2023 ◆ **Accepted**: 19.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Our study aimed to investigate the relationship between oxidative stress and melatonin levels, sleep disturbances and fatigue in persons with MS (pwMS).

Methods: The study included 50 pwMS and 30 healthy controls. Levels of serum melatonin, glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured in both groups. Persons with MS (pwMS) were evaluated using the extended disability status scale (EDDS) while Pittsburgh sleep quality index (PSQI), Epworth sleepiness scale, insomnia severity index, fatigue severity scale and Beck depression scale were used for both groups.

Results: Persons with MS (pwMS) exhibited significantly higher sleep disturbances (p<0,001), PSQI score (p<0,001), sleep latency (p=0,014), insomnia severity (p=0,001), fatigue (0,001) and fatigue severity (p<0,001), and Beck depression scale scores (p<0,001) and SOD levels (p<0,001) compared to the control group, while exhibiting significantly lower levels of melatonin(p=0,004). In pwMS, patients who experienced difficulty sleeping had significantly lower melatonin levels compared to those who did not (p=0,049). In pwMS, the melatonin level showed a negative correlation with age (r=-0,341; p=0,015) and EDSS (r=-0,386; p=0,006). Persons with MS (pwMS) with fatigue had significantly higher EDSS (p=0,003), PUQI (p=0,001), Epworth sleepiness score (p=0,028) and insomnia scores (p=0,002), compared to those who didn't.

Conclusion: Our results showed that the melatonin levels were lower, presence of fatigue, and fatigue severity were higher in pwMS with sleep disorders than in those without sleep disorders. The frequent occurrence of fatigue(indirectly) and sleep disturbances in pwMS can be attributed to low melatonin levels.

Keywords: Multiple sclerosis, melatonin, sleep disturbances, oxidative stress, fatigue

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disorder with neuroinflammatory and neurodegenerative features, primarily characterized by multifocal inflammatory-demyelinating lesions within the central nervous system. Etiology-wise, though exact causality remains elusive, the pathogenesis is thought to involve a complex interplay of genetic predisposition and environmental factors.[1]

Regions with lower sunlight exposure tend to have a higher prevalence of multiple sclerosis (MS). Additionally, MS often exhibits a seasonal pattern in terms of the occurrence of flare-ups.[2] The pineal gland serves as a neuroendocrine transducer that receives photoperiodic information from both the retina and the circadian suprachiasmatic nucleus oscillator. Melatonin is thought to potentially contribute to the physiopathology of multiple sclerosis (MS) and is directly influenced by the effects of sunlight in individuals without MS.[3] Recent studies have revealed that oxidative stress may have a significant role in the pathophysiology of MS.[4] Numerous studies have demonstrated that melatonin serves as an antioxidant and regulates lipid metabolism in MS. Moreover, it is involved in immunomodulation, neuroprotection, and neurogenesis in the context of MS.[5] Melatonin exerts its effects in MS by regulating gene expression, influencing antioxidant defense systems, and stimulating the activities of various antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx).[5]

Sleep disorders are more commonly observed in individuals with MS compared to the general population.[6] The role of melatonin as a sleep regulator is well-established, and it is documented that sleep disturbances

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in MS may share common underlying mechanisms. These mechanisms include circadian rhythm disruptions caused by impaired visual pathways, leading to compromised melatonin secretion and reduced input to the suprachiasmatic nucleus. Additionally, increased levels of proinflammatory cytokines may contribute to the connection between MS and sleep disturbances.[7] In our study, we aimed to investigate the relationship between melatonin level and oxidative stress, and disease and sleep disorders in persons with MS (pwMS).

METHODS

The study was carried with the permission of the Atatürk University Medical Faculty Clinical Researches Ethics Committee (Date: 29.03.2018, Decision No: 02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. No financial or other conflict of interest was declared by the authors.

Patients eligible for this study were those who were referred to the MS outpatient clinic within the timeframe from April 2018 to September 2018. Inclusion criteria dictated that individuals had to be diagnosed with MS based on the 2010 McDonald criteria. Patients experiencing MS exacerbations, those with an accompanying disease that may be confused with MS (SLE, antiphospholipid antibody syndrome, Sjogren's syndrome, Behcet's disease, SSS vasculitis, Sarcoidosis, etc.), those under the age of 18, those who had an MS attack in the last 3 months, those using vitamin supplements or steroids in the last 3 months, patients with chronic heart, liver, kidney or lung diseases, patients with endocrine disorders (diabetes mellitus, thyroid and parathyroid disorders, adrenal, pituitary insufficiency), those with malignancy, those previously diagnosed with sleep apnea or primary insomnia, those with a previous psychiatric condition and associated medication use and those who did not agree to participate were excluded from the study. A total of 50 pwMS were recruited in the study. A non-patient second group consisting of 30 healthy volunteers was created. All participants filled in a voluntary consent form. Demographic characteristics (gender, age, marital status, educational status, duration of MS diagnosis, number of MS attacks, MS subtype, presence of another disease, use of medication, body mass index) were questioned in face-to-face interviews while extended disability status scale was used to evaluate the severity of the disease[8] and fatigue severity scale[9], Pittsburgh sleep quality index[10], Epworth sleepiness scale[11], insomnia severity index[12] and Beck depression scale[13] were used to assess sleep disorders among control and/or patient groups by an experienced neurologist.

After an overnight fasting period, venous blood samples were collected from both the participating patients and control subjects by skilled personnel between 08:00 and 09:00 in the morning. Following a 30-minute resting period, the centrifugation process was carried out at 4000 rpm for a duration of 10 minutes. Subsequent to centrifugation, the samples were preserved at a temperature of -80°C until the point of analysis.[14]

Melatonin (Biont, Cat No: YLA0321HU) was analyzed with the ELISA (enzyme-linked immunosorbent assay) method according to the standard protocol recommended by the manufacturer.

Superoxide dismutase was analyzed with the Cayman brand superoxide dismutase assay kit (catalog number: 706002).

Glutathione peroxidase was analyzed with the Cayman brand glutathione peroxidase assay kit (catalog no: 703102).

Malondialdehyde is a product of lipid peroxidation and measured based on the absorbance of the pink-colored adduct of MDA with thiobarbituric acid (TBA) at 532 nm 15.

Statistical Analysis

The statistical analysis was conducted utilizing the Statistical Package for Social Sciences (SPSS) (software version 20 for Windows (IBM SPSS Inc., Chicago, IL). The normal distribution of data was examined using the Kolmogorov-Smirnov test. For normally distributed numerical variables, the results were presented as mean±standard deviation, whereas variables not adhering to normal distribution were presented as median (min-max). Categorical variables were reported as numbers and percentages. To compare normally distributed numerical variables across groups, the Student's t-test and ANOVA test were applied. Conversely, the Mann Whitney U and Kruskal Wallis H tests were utilized to compare non-normally distributed numerical variables between groups. Categorical data were compared using the Chi-square test and Fisher's exact test where applicable. Pearson and Spearman's rank correlation analyses were employed to explore associations between data variables. Statistical significance was established at a p-value of<0.05.

RESULTS

Our study cohort comprised 80 participants, including 50 patients diagnosed with MS and 30 healthy controls. Notably, no statistically significant differences were discerned in terms of gender, age, body mass index, and other socio-demographic characteristics between

the patient and control groups (**Table 1**). The mean expanded disability status (EDSS) Scale score for pwMS was 2. Among individuals with MS, the prevalence of sleep disturbances was marked at 88% and fatigue at 44%, whereas the control group exhibited a prevalence rate of 46.7%, resulting in a substantial divergence between the two groups (p<0.001). In regard to the Pittsburgh sleep quality index (PSQI) score, the MS group yielded an average score of 11.5, whereas the control group's average score was 4, indicating a noteworthy distinction between the two groups (p<0.001). Sleep latency exhibited a significant increase within the patient group (p=0.014). A considerable variance in the severity of insomnia between the groups was evident (p=0.001); nevertheless, no significant distinction was observed in terms of the clinical categorization of insomnia (p>0.05) (**Table 2**). Daytime sleepiness was found in 32% (n:16) of pwMS. Fatigue severity was higher in patients with increased daytime sleepiness than in those without (p=0.007).

Table 1. Groups' socio-demographic characteristics

	MS n=50	Control n=30	All population n=80	p
Age (mean±SD)	35.2±10.6	35.7±9.5	35.4±10.1	0.819
Gender (n)				0.605
Female	38	21	59	
Male	12	9	21	
BMI kg/m² (Mean±SD)	26.0±4.6	25.7±5.4	25.9±4.9	0.840
BMI distribution (n)				0.724
Normal	23	17	40	
Overweight	14	7	21	
Obese	13	6	19	
Smoking (n)	11	5	16	0.963
Alcohol (n)	1	0	1	
Marital status (n)				0.980
Married	30	24	54	
Single	16	4	20	
Divorced	3	1	4	
Widow	1	1	2	
Educational background (n)				0.085
No	2	1	3	
≤8 years	24	3	27	
9-12 years	17	9	26	
>12 years	7	17	24	
Disease type				
RRMS	44	-	44	
SPMS	5	-	5	
PPMS	1	-	1	
Treatment agents				
Interferon β1-a	12	-	12	
Fingolimod	11	-	11	
Teriflunomide	7	-	7	
Interferon β1-b	6	-	6	
Glatiramer acetate	6	-	6	
Natalizumab	5	-	5	
Dimethyl fumarate	3	-	3	

MS: Multiple Sclerosis, RRMS: Relapsing-Remitting MS, SPMS: Secondary Progressive MS, PPMS: Primary Progressive MS

Table 2. Distribution of sleep scales in MS patients and the control group

	All population n=80	MS n=50	Control n=30	p
PSQI: median (min-max)	7.5 (0-26)	11.5 (2-26)	4 (0-13)	<0.001*
Sleep disturbance (n%)				<0.001*
No	22 (27.5)	6 (12)	16 (53.3)	
Yes	58 (72.5)	44 (88)	14 (46.7)	
Sleep latency median (min-max)	15 (2-60)	20 (5-60)	10 (2-60)	0.014*
Insomnia severity median (min-max)	4 (0-20)	5.5 (0-20)	2 (0-10)	0.001*
Clinically insignificant insomnia n (%)	58 (72.5)	33 (66.0)	25 (83.3)	0.261
Insomnia lower threshold n (%)	20 (25.0)	15 (30.0)	5 (16.7)	
Clinical insomnia n (%)	2 (2.5)	2 (4.0)	-	
Epworth sleepiness scale median (min-max)	4 (0-15)	3 (0-12)	4.5 (1-15)	0.074
Daytime sleepiness n (%)				0.750
Normal	54 (67.5)	34 (68.0)	20 (66.7)	
Normal but increased daytime sleepiness	19 (23.8)	12 (24.0)	7 (23.3)	
Increased but moderate daytime sleepiness	6 (7.5)	4 (8.0)	2 (6.7)	
Increased but moderate daytime sleepiness	1 (1.3)	-	1 (3.3)	
Increased but severe daytime sleepiness	-	-	-	

MS: Multiple sclerosis, PSQI: Pittsburgh sleep quality index

Fatigue was found in 44% (n:22) of the pwMS. Mean age (40.7±10.3 vs. 30.8±8.7; p=0.001), female gender ratio (95.5% vs. 60.7%; p=0.012), median MS duration (6.5 vs. 3; p=0.005) in patients with fatigue compared to those without fatigue was found to be high. In patients with fatigue, median EDSS (3.3 versus 1; p=0.003), median PSQI (13.5 versus 7.5; p=0.001), median insomnia severity (8 versus 3.5; p=0.002), The median Epworth sleepiness score (4.5 versus 2.5; p=0.028) was found to be high. The rate of clinical insomnia was found to be higher in patients with fatigue compared to those without it (9.1% versus 0%; p=0.017) (**Table 3**).

Clinical depression was found in 28% (n:14) of the pwMS. It was found that all those with depression were women (p=0.035). Age, BMI, MS duration, number of attacks and medication distribution did not differ significantly according to the presence of depression. Melatonin and oxidative stress parameters did not show an association in pwMS with depression compared to those without depression. Median PSQI score (15 versus 9.5; p=0.009), median insomnia severity (8.5 versus 5; p=0.045), median Epworth sleepiness score (4.5 versus 3; p=0.050) in pwMS compared to those without depression were found to be high.

Table 3. The relationship between fatigue and related scales in MS patients

	Fatigue		p
	No n=28	Yes n=22	
EDSS	1 (0-6.5)	3.3 (0-6)	0.003*
PSQI	7.5 (2-22)	13.5 (4-26)	0.001*
Sleep disturbance			0.318
No	5 (17.9)	1 (4.5)	
Yes	23 (82.1)	21 (95.5)	
Sleep latency	15 (5-60)	20 (5-60)	0.075
Sleep duration	7.7±1.3	7.3±1.7	0.497
Severity of insomnia	3.5 (0-13)	8 (1-20)	0.002*
Clinically insignificant insomnia	23 (82.1)	10 (45.5)	0.017*
Insomnia lower threshold	5 (17.9)	10 (45.5)	
Clinical insomnia	-	2 (9.1)	
Epworth sleepiness scale	2.5 (0-12)	4.5 (1-12)	0.028*
Beck depression invantory	10 (0-24)	13 (7-27)	0.06
Clinical depression			0.395
No	22 (78.6)	14 (63.6)	
Yes	6 (21.4)	8 (36.4)	

EDSS: Extended disability status scale, PSQI: Pittsburgh sleep quality index

The patient group exhibited a notably diminished melatonin level (p=0.004). The SOD level demonstrated a marked increase within the patient group (p<0.001) (**Table 4**). Furthermore, in both the patient and control groups, no significant correlations were identified between melatonin levels and GPx, MDA, or SOD levels.

Within the control group, no significant correlations were identified between melatonin and oxidative stress parameters, as well as age, BMI, EDSS, PSQI score, sleep latency, sleep duration, insomnia severity index, and Epworth sleepiness scale (**Table 5**). However, among pwMS, a negative correlation was observed between melatonin levels and both age (p=0.015) and EDSS score (p=0.006). Conversely, a positive correlation emerged between GPx levels and age (p=0.026), duration of disease (p=0.046), and EDSS score (p=0.007) (**Table 4**). Notably, a negative correlation was established between MDA levels and parameters such as PSQI (p=0.003), sleep latency (p=0.049), and insomnia severity index (p=0.001) (**Table 5**).

In the subgroup analysis involving overweight individuals, a distinct pattern emerged, underscoring a noteworthy diminution in melatonin levels among subjects diagnosed with MS as opposed to the control cohort (415.8 vs. 586.4; p=0.010). Moreover, the median SOD16 level exhibited a discernible elevation (0.58 to 0.48; p=0.012), concomitant with a heightened mean malondialdehyde (MDA) level in pwMS (31.6±3.1 vs. 27.4±1.9; p=0.004). Of particular note was the nuanced correlation unveiled between the presence of sleep disorders and melatonin concentrations within the patient cohort. Specifically, melatonin levels exhibited a significant decrease in pwMS grappling with sleep difficulties in contrast to those without such issues (674.1 vs. 429.1; p=0.049). Additionally, individuals manifesting heightened daytime sleepiness displayed a tendency towards lower melatonin levels, though this relationship failed to reach statistical significance (p=0.479).

Table 4. Relationship between melatonin, GPx, SOD and MDA values in MS patients and controls

	All Population (n=80)	MS (n=50)	Control (n=30)	p
Melatonin (ng/L) median (min-max)	483.1 (266.8-1272.1)	438.9 (266.8-1180.7)	599.3 (307.9-1272.1)	0.004*
MDA (nmol/ml) median (min-max)	29.53 (24.8-56.2)	30.2 (24.8-56.2)	28.2 (26.2-36.5)	0.136
GPx (nmol/ml) median (min-max)	15282 (1018.8-35658)	15027.3 (1018.8-25979.4)	15282 (2037.6-35658)	0.176
SOD (U/ml) median (min-max)	0.57 (0.25-0.82)	0.6 (0.38-0.82)	0.5 (0.25-0.68)	<0.001*

MS: Multiple sclerosis GPx: Glutathione peroxidase, SOD: Superoxide dismutase, MDA: Malondialdehyde

Table 5. Findings of melatonin and oxidative stress parameters

Variables	Control								MS							
	Melatonin		GPX		SOD		MDA		Melatonin		GPX		SOD		MDA	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Melatonin (ng/L)	-	-	0.029	0.878	-0.316	0.089	-0.124	0.513	-	-	-0.141	0.329	0.221	0.124	-0.145	0.315
GPX (nmol/ml)	0.029	0.878	-	-	0.284	0.129	0.292	0.118	-0.141	0.329	-	-	-0.006	0.965	0.050	0.731
SOD (U/ml)	-0.316	0.089	0.284	0.129	-	-	0.313	0.092	0.221	0.124	-0.006	0.965	-	-	0.058	0.689
MDA (nmol/ml)	-0.124	0.513	0.292	0.118	0.313	0.092	-	-	-0.145	0.315	0.050	0.731	0.058	0.689	-	-
Age (n)	-0.304	0.103	-0.118	0.533	0.102	0.592	0.093	0.625	-0.341	0.015*	0.315	0.026*	0.001	0.994	0.171	0.234
BMI (kg/m²)	0.104	0.585	-0.100	0.599	0.044	0.818	0.134	0.479	-0.259	0.070	0.277	0.072	-0.071	0.623	0.272	0.108
Duration of disease (year)	-	-	-	-	-	-	-	-	-0.241	0.092	0.280	0.046*	-0.158	0.273	-0.038	0.793
Number of attacks (n/per year)	-	-	-	-	-	-	-	-	0.192	0.181	-0.263	0.065	-0.007	0.961	0.124	0.390
EDSS (n)	-	-	-	-	-	-	-	-	0.386	0.006*	0.374	0.007*	-0.072	0.621	0.018	0.900
PSQI a (n)	-0.259	0.167	-0.288	0.123	-0.097	0.611	-0.156	0.410	-0.219	0.126	-0.030	0.837	-0.189	0.189	-0.406	0.003*
Sleep latency (n)	-0.120	0.527	-0.130	0.495	-0.333	0.072	-0.332	0.073	-0.054	0.707	0.014	0.921	-0.161	0.264	-0.280	0.049*
Duration of sleep (n)	0.065	0.733	0.249	0.184	-0.165	0.385	0.085	0.656	0.004	0.977	0.012	0.932	-0.118	0.413	0.111	0.445
Insomnia severity index (n)	0.066	0.727	0.184	0.329	-0.267	0.153	-0.394	0.031	-0.086	0.554	-0.032	0.827	-0.154	0.286	-0.442	0.001*
Epworth score (n)	-0.051	0.790	0.081	0.669	0.322	0.082	-0.019	0.922	-0.093	0.521	-0.044	0.763	0.159	0.269	-0.226	0.114

DISCUSSION

Multiple sclerosis (MS) is a chronic neurological disease that has a significant impact on daily life activities. Studies have consistently shown that pwMS often encounter difficulties with sleep, and the prevalence of sleep disturbances in this population is higher compared to the general healthy population.[17] Various studies have reported the prevalence of sleep disturbances in individuals with MS to be within the range of 42-65%. Similarly, the prevalence of daytime sleepiness among pwMS has been estimated to be approximately 10-40%.[18] In our study, the patient group exhibited a higher rate of sleep disorders according to the Pittsburgh sleep quality Index compared to the control group, with a rate of 88%. Daytime sleepiness was found similar to the literature, with a rate of 32%.

The pathophysiology of MS is not fully understood, but oxidative stress in MS is characterized by the excessive production of reactive oxygen species and decreased antioxidant defense mechanisms, are both known to be involved in the pathogenesis of MS.[19] The disruption of antioxidant systems or increased production of reactive oxygen species (ROS) can contribute to lipoprotein peroxidation in MS. Lipoprotein lipid peroxidation products are neurotoxic and possess proinflammatory properties, which may play a role in demyelination and axonal injury in MS.[20] Studies involving melatonin have yielded remarkable findings.[3,4] Melatonin has been shown to enhance the activities of SOD 16 and glutathione peroxidase (GPx), while inhibiting the activity of the pro-oxidative enzyme nitric oxide synthase (NOS). It has been reported that melatonin therapy can lead to a decrease in malondialdehyde levels.[21] MDA is the primary and extensively studied product of peroxidation of polyunsaturated fatty acids. Studies have reported a significant increase in lipid peroxidation products, including MDA, in the brain, plasma, and cerebrospinal fluid of individuals with MS.[20] Indeed, different studies have reported varying results concerning the levels of GPx in pwMS.[22] It has been reported that individuals with MS exhibit higher levels of SOD 15, catalase (CAT), and glutathione reductase (GR) compared to healthy controls.[22] In our study, it was observed that the level of melatonin was significantly lower in pwMS compared to the control group, which aligns with existing literature. Furthermore, the production of melatonin was found to be negatively correlated with age and the expanded disability status scale[23], indicating a potential relationship between melatonin levels, age, and disease severity in MS. These findings contribute to our understanding of the role of melatonin in MS and its potential implications for disease progression. Our findings are consistent with the existing literature in terms of melatonin levels, GPx, and SOD in pwMS. The study results indicate that there was no significant difference in GPx levels between the patient and control groups, although GPx levels were lower in the patient group. On the other hand, SOD levels were higher in the patient group compared to the control group, which aligns with previous research.[24] Free radicals directly or indirectly activate endogenous detoxification mechanisms. Increased cytokines increase the expression of SOD.[25] These findings contribute to the understanding of the antioxidant status and oxidative stress markers in pwMS and support the notion of altered antioxidant defense mechanisms in the disease. In our study, no statistically significant difference was observed in the mean serum MDA levels between pwMS and the controls.

Melatonin is indeed a key regulator of the sleep-wake cycle. In healthy individuals, exogenous melatonin can aid in initiating sleep and improve sleep quality in various clinical conditions. Recent studies have provided evidence suggesting that melatonin therapy may have a role in regulating sleep patterns.[26] A different study has proposed that melatonin may exert chronobiological effects on sleep. It suggests that melatonin release is associated with the onset, quality, and latent stage of sleep rather than total sleep time. This effect is thought to be achieved through the hypothermic effect and thermoregulation mechanism of melatonin.[27] In our study, melatonin levels were lower in pwMS who experienced difficulty sleeping compared to those who did not. In pwMS, no significant correlation was found between melatonin levels and PSQI and Epworth sleepiness scale scores, sleep latency, duration of sleep and insomnia severity. Our findings were consistent with the literature. There is a limited number of studies examining the relationship between oxidative stress markers and sleep disorders. MDA level was found to be negatively correlated with PSQI, sleep latency and insomnia severity index. Again, SOD and GPx levels were not found to be significantly correlated with PSQI and Epworth sleepiness scale scores, sleep latency, duration of sleep and insomnia severity. These findings can be explained by the fact that oxidative stress markers can be affected by different physiological and pathophysiological processes. It is widely believed that melatonin can enhance the gene expressions and/or activities of various antioxidant enzymes, including SOD[16], peroxidase, glutathione (GSH), and lipid peroxidase. By doing so, melatonin is thought to suppress oxidative stress.[21] In our study, melatonin levels did not exhibit a significant correlation with oxidative stress parameters in patient and control groups. In our opinion, this supports the fact that the antioxidant mechanism is affected not only by melatonin, but also by different endogenous processes.

Although the etiology of fatigue in multiple sclerosis is not clearly known, a multifactorial etiology is mentioned, including individual, environmental and developmental factors. It is reported that 50-90% of pwMS experience fatigue.[28] In our study, which showed that 44% of people with MS have fatigue, there was a positive correlation between fatigue severity scores and EDSS, PSQI, Epworth sleepiness score and Beck depression score. There was no significant relationship between depression and fatigue severity or presence.

Depression is the most common psychiatric disorder in MS. Studies have shown that depression scores are higher in female patients than in male patients.[29] In our study, in agreement with the literature, 28% of pwMS were found to have clinical depression and all those with depression were female. In our study, there was no association between melatonin in pwMS with depression compared to those without depression. PSQI score, insomnia severity and Epworth sleepiness score were found to be significantly higher in pwMS with depression compared to controls.

Sleep disorders are highly prevalent among individuals with MS, and the findings of our study indicate that melatonin levels are lower in pwMS compared to the control group. Furthermore, those with pwMS with sleep difficulties exhibited lower melatonin levels and higher fatigue severity compared to those without sleep difficulties. However, no significant relationship was observed between melatonin levels and sleep latency, sleep duration, insomnia severity and fatigue in pwMS. Additionally, melatonin levels did not appear to have a direct impact on the oxidant/antioxidant system in this context. Considering the relationship between MS and sleep disorders, it can be thought that the decreased melatonin levels observed in pwMS may contribute to the frequent occurrence of sleep disorders and thus increase fatigue.

CONCLUSION

Sleep disturbance and fatigue are common in pwMS and should be a priority among clinical evaluations. However, further research is needed to fully understand the complex interplay between melatonin, sleep disorders, fatigue, and the oxidant/antioxidant system in MS.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was initiated with the approval of the Atatürk University Medical Faculty Clinical Researches Ethics Committee (Date: 29.03.2018, Decision No: 02).

Informed Consent

Written free informed consent was obtained from all participants in this study.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1430895

Anatolian Curr Med J. 2024;6(2):175-180

Etoposide hypersensitivity reactions and outcomes of desensitizations in immediate-type hypersensitivity reactions

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Cite this article as: Köycü Buhari G, Nazik Bahçecioğlu S, Gültuna S, et al. Etoposide hypersensitivity reactions and outcomes of desensitizations in immediate-type hypersensitivity reactions. *Anatolian Curr Med J.* 2024;6(2):175-180.

Received: 02.02.2024 ◆ **Accepted**: 19.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: This study aims to define characteristics of hypersensitivity reactions with etoposide, and outcomes of desensitizations in immediate-type hypersensitivity rections

Methods: This is a retrospective observational study of patients who had hypersensitivity reactions with etoposide from January 2019 to December 2023.

Results: A total of 39 patients with lung cancer were included in the study. Ten (25.6%) patients had known other drug allergies and three (7.7%) patients had previous chemotherapeutic hypersensitivity two with paclitaxel and one with docetaxel. Most of the initial hypersensitivities were in the first or second cycle (n=29, 74.4%). Ten (25.4%) patients had hypersensitivity reactions at the first application of etoposide. Thirty (76.9%) patients had immediate-type hypersensitivity reactions. There was no significant difference in terms of patient and initial hypersensitivity characteristics between patients who had immediate or non-immediate type hypersensitivity reactions. Of the 30 patients with immediate-type hypersensitivity reactions, initial reaction was mild in 16 (53.3%) and moderate in 14 (46.7%) patients. Most common symptoms were erythema in 29 (96.7%), dyspnea in 13 (43.3%), chest tightness in 8 (26.7%), discomfortness in 7 (23.3%), and hypertension in 6 (20%). Skin tests were negative in five patients who underwent skin testing. A total of 98 desensitization courses were performed in 27 patients and 3 (11.1%) patients had breakthrough reactions.

Conclusion: Most of the hypersensitivity reactions to etoposide are immediate-type and not severe. Desensitization is an effective and safe procedure to manage these reactions. Further research is needed to elucidate the mechanisms of hypersensitivity reactions.

Keywords: Etoposide, hypersensitivity, desensitization, reaction, immediate, chemotherapy

INTRODUCTION

Hypersensitivity reactions (HSRs) to chemotherapeutic agents and their management are important in clinical practice because they can not be easily replaced or exchanged to an alternative agent, and also alternative regimens may be less effective, more toxic, or more expensive than first-line chemotherapeutics.[1]

Etoposide is a semisynthetic derivative of epipodophylotoxin, which is effective against several types of malignancies, including lung cancer.[2] HSRs to etoposide are uncommon; the incidence is estimated to be between 1% and 3%.[3,4] The clinical presentations can vary from mild cutaneous to severe life-threatening reactions.[4-6]

Mild reactions may be prevented by premedication with corticosteroids and antihistamines or by prolonging the infusion time in some patients.[7] There are also reported cases of etoposide hypersensitivity managed by switching etoposide to etoposide phosphate.[8,9] However, patients who could not tolerate these methods were also reported.[10,11]

HSRs limit the use of chemotherapeutic agents because of their potential to cause more severe reactions or even death in the next administration.[12,13] In immediate-type HSRs, rapid drug desensitization can provide tolerance and reuse of the offending agent, thus giving patients a chance to be treated with first-line chemotherapeutics.[14] Although chemotherapeutic desensitization has been shown to be safe and effective, sometimes breakthrough reactions (BTRs) can be encountered during the procedure.[15]

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Data about etoposide HSRs is limited. This study aims to define characteristics of HSRs with etoposide, and outcomes of desensitizations in immediate-type HSRs.

METHODS

This is a retrospective observational study of patients who had HSRs with etoposide from January 2019 to December 2023 and were referred to our allergy and clinical immunology clinics. The study was approved by the Ankara Atatürk Sanatorium Training and Research Hospital Clinical Research Ethics Committee (Date: 27.12.2023, Decision No: 2012-KAEK-15/2863). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Inclusion criterias were patients who had symptoms compatible with HSR to etoposide and older than 18 years old. Exclusion criterias were insufficient medical records.

Baseline data including patients' characteristics (age, gender, diagnosis, comorbid diseases, previous drug allergy), treatment characteristics (therapy line, cycle number, day number of cycle), initial HSR characteristics (chronology, symptoms, severity), skin test results if performed, number of desensitization courses, occurrence of BTR, BTR characteristics (chronology, symptoms, severity) collected from medical records.

Chronologically, HSRs were classified as immediate and non-immediate reactions. Reactions that occurred during etoposide infusion or within 6 hours after the end of the infusion are classified as immediate-type hypersensitivity reactions. Reactions occurring more than 6 hours after the end of infusion are classified as non-immediate type HSRs.[16]

The severity of initial HSRs and BTRs were classified according to Brown's classification. The reaction was considered as mild if there was only cutaneous involvement, as moderate if there were symptoms suggesting respiratory, cardiovascular or gastrointestinal involvement and as severe if hypoxia, hypotension or neurologic compromise were considered.[17]

Skin tests with etoposide were conducted as follows: for the positive control, a prick test with a solution of histamine hydrochloride (10 mg/ml), whereas for the negative control, a physiological saline (0.9% saline) solution was used. A skin prick test was performed with a concentration of 20 mg/ml etoposide. After a negative skin prick result, an intradermal test was performed with a concentration of 0.2 mg/ml and 2 mg/ml etoposide. The prick test result was considered positive when the cutaneous response was a wheal of at least 3 mm with a surrounding flare, whereas the intradermal test result was considered positive with a wheal of at least 5 mm with a surrounding flare.

A 3-bag 12-step desensitization protocol described by Brigham and Women's Hospital was implemented 18. Written informed consent was obtained before each desensitization procedure. Thirty minutes before starting the desensitization, premedication with methylprednisolone 40 mg, H1- antihistamine (pheniramine 45,5 mg) and H2-antihistamine (famotidine 20 mg or ranitidine 50 mg) was administered as a routine practice of the oncology team before chemotherapy course. All desensitizations were carried under close observation with one-on-one nurse-to-patient care in the allergy unit. If any BTR occured during the protocol, infusion was suspended and the reaction was treated.

Statistical Analysis

All statistical analyses were performed using the SPSS (Statistical Package of Social Sciences) for Windows 18.0 software package. In evaluating the data, mean and standard deviation for normally distributed data, the median and interquartile range for data that did not show normal distribution, values, and percentages for ratios were determined by descriptive statistical method. In univariate analyses, Chi-square, Fisher, Student's t-test, and Mann-Whitney U tests were used, as appropriate. All p-values lower than 0.05 were considered to be statistically significant.

RESULTS

Patient Characteristics

A total of 39 patients, 35 (89.7%) male and 4 (10.3%) female with mean age 59.08±7.8 (range 47-76) were included in the study. The pathological diagnosis was small cell lung cancer (SCLC) in 30 (76.9%), non-small cell lung cancer (NSCLC) in 6 (15.4%) and combined small and non-small cell lung cancer in 3 (7.7%) patients. Metastatic disease was present in 20 (51.3%) patients.

Systemic comorbidities were present in 19 (48.7%) patients; 12 (30.8%) had hypertension, 5 (12.8%) had coronary arterial disease, 4 (10.3%) had diabetes mellitus, and each one patient (2.6%) had hyperlipidemia, hypothyroidism and chronic hepatitis B virus infection. Fifteen (64.1%) of the patients were receiving chronic obstructive pulmonary disease treatment. Median smoking duration was 40 (25-110) pack years in 19 patients for whom smoking information was available.

Ten (25.6%) patients had known other drug allergies; four had beta-lactam, two had paclitaxel, one had docetaxel, one had radiocontrast media, one had lansoprazole allergy, and another patient had a history of multi-drug allergy to nonsteroidal anti-inflammatory drugs, beta-lactam antibiotics, and fentanyl.

Treatment and Initial HSR Characteristics

The initial HSR was observed in 36 (92.3%) patients during the first-line therapy and 3 (7.7%) patients during the second-line therapy. Of the three patients who received second-line treatment, two developed a reaction in the first cycle, and one in the second cycle.

In evaluating all patients the initial HSR was observed in most patients in the first or second cycle with a median value of 2 (range 1-8). It was in the first cycle in 18 (46.15%) patients, second cycle in 11 (28.20%), third cycle in 3 (7.69%), fourth cycle in one (2.56%), fifth cycle in 3 (7.69%) and sixth, seventh and eight cycle in each one (2.56%) patient. Ten (25.4%) patients had HSR at the first application of etoposide.

In evaluating the day of the cycle that the reaction developed, it was on the first day in 23 (59.0 %) patients, on the second day in 14 (35.9%) patients, and on the third day in 2 (5.1%) patients.

According to the reaction chronology, 30 (76.9%) patients had immediate-type HSRs. Twenty-five (83.3%) of these reactions occurred during etoposide infusion, and 20 (66.6%) of them were during the first half of the infusion. Five (20%) of the immediate-type HSRs occurred within the first hour after infusion. Nine (23.1%) patients had non-immediate type HSRs, which were developed at least 6 hours after the etoposide infusion.

Of the 30 patients with immediate-type HSR, initial HSR was mild in 16 (53.3%) and moderate in 14 (46.7%) patients. Most common symptoms were erythema in 29 (96.7%), dyspnea in 13 (43.3%), chest tightness in 8 (26.7%), discomfortness in 7 (23.3%), and hypertension in 6 (20%). All patients with non-immediate type HSR had mild reactions with erythema in all 9 (100%) patients and also angioedema in 3 (33.3%) cases. Clinical symptoms of the HSRs are shown in **Table 1**.

Table 1. Clinical symptoms of hypersensitivity reactions

Clinical symptoms	Immediate-type HSR n=30	Non-immediate type HSR n=9
Erythema n (%)	29 (96.7)	9 (100)
Dyspnea n (%)	13 (43.3)	-
Chest tightness n (%)	8 (26.7)	-
Discomfortness n (%)	7 (23.3)	-
Hypertension n (%)	6 (20)	-
Angioedema n (%)	4 (13.3)	3 (33.3)
Warmth n (%)	4 (13.3)	-
Sweating n (%)	3 (10)	-
Back pain n (%)	2 (6.7)	-
Abdominal pain n (%)	1 (3.3)	-

HSR: Hypersensitivity reaction

There was no significant difference in terms of patient and initial hypersensitivity characteristics between patients who had immediate or non-immediate type HSRs (**Table 2**).

Table 2. Patient and initial hypersensitivity reaction characteristics

	All patients n=39	Patients with Immediate-type HSR n=30	Patients with non-immediate type HSR n=9	p
Age (mean±SD)	59.08±7.8	58±7.65	56±8.61	0.973
Sex n (%)				
Female	4 (10.3)	3 (10)	1 (11.1)	1.000
Male	35 (89.7)	27 (90)	8 (88.9)	
Diagnosis n (%)				
SCLC	30 (76.9)	23 (76.7)	7 (77.8)	0.886
NSCLC	6 (15.4)	5 (16.7)	1 (11.1)	
Combined	3 (7.7)	2 (6.7)	1 (11.1)	
Metastatic disease n (%)	20 (51.3)	18 (60)	2 (22.2)	0.065
Systemic comorbidity n (%)	19 (48.7)	17 (56.7)	2 (22.2)	0.127
Drug allergy n (%)	10 (25.6)	7 (23.3)	3 (33.3)	0.669
Therapy lines n (%)				
First line	36 (92.3)	27 (90)	9 (100)	
Second line	3 (7.7)	3 (10)	-	
Cycle number n (%)				
1st	19 (48.7)	12 (40.0)	7 (77.8)	
2nd	10 (25.6)	8 (26.7)	2 (22.2)	
≥3rd	10 (25.6)	10 (33.3)	-	
Total cycle number, median (min-max)	2 (1-8)	2 (1-8)	1 (1-2)	0.070
Day of reaction on the cycle n (%)				
1st day	23 (59.0)	16 (53.3)	7 (77.8)	
2nd day	14 (35.9)	12 (40.0)	2 (22.2)	
3rd day	2 (5.1)	2 (6.7)	-	
Day of reaction on the cycle, median (min-max)	1 (1-3)	1 (1-3)	1 (1-2)	0.178

HSR: Hypersensitivity reaction, NSCLC: Non-small cell lung cancer, SCLC: Small cell lung cancer

Management of Immediate-type HSRs and Outcomes of Desensitizations

Skin prick and intradermal tests with etoposide were performed on five patients with immediate-type HSRs; all were negative.

Re-administration of etoposide with a slow infusion rate was tried in 2 patients with mild reactions but was not successful.

Etoposide was discontinued in 3 patients after hypersensitivity reactions. In the remaining 27 patients, etoposide was given with desensitizations. A total of 98 desensitization courses were performed during the study period. The median number of desensitization courses was 2 (range 1-12).

A total of 3 BTRs developed in three (11.1%) patients. Two of these patients had mild initial reactions; after desensitizations, they had erythematous cutaneous reactions in the late period (≥6 hours,; one after the first desensitization and the other after the second desensitization course. The third patient with a moderate initial reaction had a mild breakthrough reaction in the last step of the first desensitization course. The procedure was interrupted, and the reaction was treated, but the patient subsequently refused to continue the procedure.

DISCUSSION

In this study, we retrospectively reported the characteristics of initial HSRs with etoposide and the outcomes of desensitizations in patients with immediate-type hypersensitivity. We found that most of the reactions were immediate-type HSRs. There was no significant difference in terms of patient and initial hypersensitivity characteristics between patients with immediate or non-immediate type HSRs.

The most common symptoms of immediate-type HSRs were erythema, dyspnea, and chest tightness. Similarly, previous studies have reported dyspnea, erythema, flushing, angioedema, throat tightness, chest pain or tightness, wheezing, cough, and cyanosis as the most common symptoms.[4,19,20]

The exact mechanism of etoposide HSRs are not fully known. The fact that most of the reactions in our study were observed during the first and second cycle, and even in 25.4% of the patients during the first application, and no skin test positivity was detected in any of the patients tested, suggests that these reactions may not be IgE-mediated.

HSRs to etoposide were assumed to be secondary to its diluent polysorbate 80.[9] Polysorbate 80 consists of a mixture of fatty acid esters of sorbitol-derived cyclic ethers and polyethylene glycol. It induces immediate-type non-IgE-mediated hypersensitivity reactions via complement activation and basophile degranulation.[10]

Polysorbate 80 is also used as a solubilizing agent in the docetaxel formulation.[21] The fact that one of the patients in our study had previous docetaxel hypersensitivity suggests that polysorbate 80 may be the responsible component in this patient. In our study, two patients also had a history of paclitaxel hypersensitivity. Although a different solubilizer, cremophor EL, is used in the paclitaxel formulation Friedland et al. reported possible cross-reaction between paclitaxel and etoposide.[21,22] Caution should be exercised against etoposide hypersensitivity in patients with a history of hypersensitivity to taxanes.

Etoposide phosphate is a water-soluble prodrug of etoposide that does not contain polysorbate 80 but contains dextran 40.[23] There are previous reports of patients who had HSR with etoposide but tolerated etoposide phosphate.[9,24] There are also reports of patients who tolerated etoposide after a hypersensitivity reaction to etoposide phosphate.[5,23]

However, there are reported cases of hypersensitivity to both etoposide and etoposide phosphate, suggesting that HSRs may not be related to diluents but to etoposide itself.[10,25] Although they are not standardized, there are also reports of skin test positivity in patients with etoposide hypersensitivity, suggesting an IgE-mediated mechanism.[26,27] Skin testing protocols with etoposide should be standardized with further studies.

In our study, patients with immediate-type HSRs to etoposide had mild to moderate reactions; however, severe or life-threatening reactions have been reported previously.[4,19]

If an HSR occurs with chemotherapeutics, the physician must decide whether to continue treatment or not. Re-administration of the culprit drug carries the risk of a potentially fatal anaphylactic reaction; however, changing to an alternative drug can have a negative effect on patients' outcomes.[1]

Etoposide hypersensitivity was found to be associated with higher infusion rates and may be prevented by slow infusion.[19] In our study, re-administration of etoposide with slow infusion was tried in 2 patients with mild reactions; however, it was unsuccessfull. Another management option is challanging etoposide with a prophylactic regimen containing corticosteroids and antihistamines.[7,28] Hudson et al.[7] reported 78% of patients were rechallenged successfully to intravenous etoposide.

Desensitization, which allows temporary tolerance to a drug, is another option to continue the therapy.

Several etoposide desensitization protocols have been reported in the literature.[4,29,30] We used a 3-bag 12-step desensitization protocol described by Brigham and Women's Hospital 18. During the study period, a total of 98 desensitizations were performed in 27 patients with immediate-type HSRs and only three BTRs were observed in three (11.1%) patients. All of the BTRs were mild graded; two of them were developed in the late period (≥6 hours) after desensitization. These results suggest that desensitization is an effective and safe method in managing patients with etoposide HSRs.

Limitations

However this study has some limitations. First limitation was the retrospective design of the study. Second limitation was slow infusion or premedication escalation was not attempted in all patients with mild reactions. Drug tolerance could not be achieved in two cases in which slow infusion was attempted. In our hospital, 12 mg dexamethasone is given in routine practice before an etoposide cure, and no increase in premedication has been tried in patients.

CONCLUSION

Most of the hypersensitivity reactions to etoposide are immediate-type and not severe; however, the mechanism is not clear. Further research is needed to elucidate the mechanisms. Desensitization is an effective and safe procedure to manage these reactions.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Clinical Research Ethics Committee (Date: 27.12.2023, Decision No: 2012-KAEK-15/2863).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1404137

Anatolian Curr Med J. 2024;6(2):181-184

Factors affecting need for hormone replacement after thyroid lobectomy

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Cite this article as: Şentürk A, Harmantepe AT, Cantürk AÖ. Factors affecting need for hormone replacement after thyroid lobectomy. *Anatolian Curr Med J.* 2024;6(2):181-184.

Received: 12.12.2023 ◆ **Accepted**: 24.02.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: The aim of this study is to determine the incidence and risk factors of postoperative hormone replacement in patients who underwent thyroid lobectomy.

Methods: Patients who underwent thyroid lobectomy in our clinic between January 2015 and January 2021 were retrospectively scanned. Age, gender, preoperative hemogram and thyroid function tests (TFT) were screened. During postoperative follow-up, current TFT, height, weight, thyroid hormone replacement status and hypothyroidism symptoms were questioned. Pathology reports were examined.

Results: The pathology (patological examination results of specimen of the patients) were nodular hyperplasia in 81.1% (n=30) and Papillary thyroid carcinoma (PTC) in 18.9% (n=7). While the need for hormone replacement developed in 37.8% (n=14) of the patients in the postoperative follow-up, it did not develop in 62.2% (n=23). It was determined that high preoperative thyroid stimulating hormone (TSH) significantly increased the need for postoperative hormone replacement ($p<0.05$). In addition, it was found that the need for hormone replacement increased significantly in patients whose pathology results were compatible with malignancy ($p<0.05$).

Conclusion: Malignancy and preoperative high TSH are important predictors of postoperative levothyroxine need.

Keywords: Thyroid lobectomy, hormone replacement, levothyroxine, risk factors

INTRODUCTION

Though 20% of individuals have palpable thyroid nodules on physical examination, approximately half of the adult population has thyroid nodules incidentally on imaging.[1] Thyroid carcinoma occurs less frequently, with a malignancy rate of 5-15% of all thyroid nodules.[2]

Thyroid lobectomy is generally indicated for the treatment of benign symptomatic nodules, intermediate nodules, or low-risk well-differentiated carcinoma less than 4 cm.[2,3] The need for lifelong thyroid hormone supplementation is an important consideration when deciding on the extent of surgery. Thyroid hormone supplementation is required after total thyroidectomy. Preservation of approximately half of the thyroid gland and eliminating the need for permanent thyroid hormone supplementation is thought to be an important advantage of thyroid lobectomy. In the literature, approximately 10-50% of patients require thyroid hormone supplementation after thyroid lobectomy.[4-7] There are some studies in the literature that can be used to calculate the risk of hiopthyroidism after lobectomy.[8-16] Numerous studies have focused on the prediction of thyroid hormone supplementation after thyroid lobectomy. Historically, rates of thyroid hormone supplementation after thyroid lobectomy have been studied from patients with benign pathology, as those with malignant outcomes were recommended complementary thyroidectomy according to previous guidelines.[17]

This study was designed to analyze the need for thyroid hormone replacement (levothyroxine) in patients who underwent thyroid lobectomy for benign and malignant reasons, and to analyze the relationship of post-lobectomy hypothyroidism with preoperative parameters and histopathological findings.

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METHODS

Our study is a retrospective study, and the study was started after receiving approval from the ethics committee (Date: 26.06.2020, Decision No: E-71522473-050.01.04-368). The Helsinki Declaration of Principles was complied with during the study. The files of patients who underwent thyroid lobectomy in our clinic between January 2015 and January 2021 were retrospectively scanned and their age, gender, preoperative hemogram, and thyroid function tests (TFT) were scanned. Current TFT, height, weight, thyroid hormone replacement status and hypothyroidism symptoms during postoperative follow-up were questioned. Pathology reports were examined.

Patients who underwent completion thyroidectomy, patients who received hormone replacement therapy (levothyroxine) before surgery, patients whose pathology report reported chronic lymphocytic thyroiditis, and patients with missing data were not included in the study.

The patients were divided into two groups: benign-malignant, with and without postoperative levothyroxine. Preoperative and postoperative values and hormone replacement needs were compared between the groups. The postoperative follow-up period of the patients was 2 years. The values at the 2nd year after surgery were compared. Additionally, patients were contacted and their current height, weight, and hypothyroidism symptoms were questioned. BMI (Basal metabolic index) of the patients was calculated. Hypothyroidism symptoms were considered to be present if at least two of the symptoms of fatigue, weight gain, constipation, decreased menstruation and sensitivity to cold were present.

Thyroid lobectomy protocol was performed in accordance with the 2015 ATA (American Thyroid Association) guidelines. Lobectomy was performed in unilateral benign pathologies. However, thyroid lobectomy was performed in patients with malignancy or suspected malignancy in FNAB, if the tumor diameter was smaller than 4 cm, if it was a unifocal lesion, if the patient did not have a history of radiotherapy, and if there was no malignant lymph node or distant metastasis. Levothyroxine replacement was performed in all cases with a serum TSH level of 10 mU/L in the postoperative follow-up. Levothyroxine replacement was performed in patients with serum TSH levels of 4-10 mU/L and hypothyroidism symptoms.

Statistical Analysis

Descriptive statistical analysis was performed to determine the general characteristics of the patients. The data were analyzed using the SPSS 23.0 program. The Kolmogorov-Smirnov test was used to determine whether the quantitative variables were normally distributed. Accordingly, Student-t test was used for parametric data and Mann-Whitney u test was used for non-parametric variables. Categorical variables were analyzed with the Chi-square test. Significance level was accepted as <0.05. SPSS statistical software (IBM SPSS Statistics, Version 26.0. Armonk, NY: IBM Corp.) was used for analysis.

RESULTS

Thirty-seven patients whose long-term information could be accessed and whose symptoms could be questioned were included in the study. 43.2% (n=16) of the patients were male and 56.8% (n=21) were female. The mean age was 46.6 (21-73). Preoperative FNAB of 7 (20.5%) patients had suspected malignancy, 10 (29.5%) patients had atypia of uncertain significance (AUS), and 17 (50%) patients had benign diagnosis. While 59.5% (n=22) of the patients had right lobectomy, 40.5% (n=15) had left lobectomy. The pathology result was nodular hyperplasia in 81.1% (n=30) and PTC in 18.9% (n=7). While the need for hormone replacement developed in 37.8% (n=14) of the patients in their postoperative 2-year follow-up, it did not develop in 62.2% (n=23). In the postoperative follow-ups, 37.8% (n=14) had hypothyroidism complaints independent of hormone replacement, while 62.2% (n=23) did not.

When evaluated according to the need for postoperative hormone replacement, it was determined that the higher TSH than the preoperative values significantly increased the need for postoperative hormone replacement (p<0.05) (**Table 1**). In addition, it was determined that the need for hormone replacement increased significantly in patients with a malignant pathology report (p<0.05) (**Table 1**).

When the patients were divided into groups according to the lobectomy side, it was observed that the lobectomy side did not affect postoperative calcium (Ca), hypothyroidism complaints, and hormone replacement dose (**Table 2**).

Table 2. Comparison of postoperative features according to the lobectomy side

Clinicopathological parameters	Right lobectomy (n=22)	Left lobectomy (n=15)	p value
Postop Ca	8.35 (7.8-10.0)	8.30 (7.6-9.4)	0.641*
Hypothyroidism complaints			0.823**
Yes	8 (57.1%)	6 (42.9%)	
No	14 (60.9%)	9 (39.1%)	
Hormone replacement dose (mcg)	75 (25-125)	66,6 (25-175)	0.770*

* Mann-Whitney-U test, ** Chi-square test

There was no correlation between levothyroxine dose and height, weight, and BMI (p=0.981, p=0.24, p=0.445, respectively).

Table 1. Comparison of demographic, laboratory and pathology results of all patients, those who received and did not receive postoperative hormone replacement

Clinicopathological parameters	All Patients (n=37)	Hormone Replacement (-) (n=23)	Hormone Replacement (+) (n=14)	p value
Age	46.6 ± 12.3	47.2 ±11.2	45.7 ±14.4	0.738*
Gender				0.733**
Female	21 (56.8%)	14 (66.7%)	7 (33.3%)	
Male	16 (43.2%)	9 (56.3%)	7 (43.8%)	
Preoperative Hgb	13.5 ±1.7	13.4 ±1.8	13.7 ±1.4	0.582*
Height (cm)	166.7 ±8.8	165.8 ±7.9	168.1 ±10.2	0.456*
Weight (kg)	78.3 ±13.4	76.6 ±12.0	81.2 ±15.5	0.311*
BMI (kg/m²)	28.2 ±4.8	27.9 ±4.8	28.7 ±4.9	0.632*
Preoperative Ca (mg/dl)	8.3 (7.6-10)	8.4 (7.6-10)	8.3 (7.7-9.4)	0.912***
Preoperative TSH (mU/L)	0.99 (0.02-5.19)	0.72 (0.03-3.8)	1.7 (0.02-5.1)	<0.05***
Preoperative T3 (pmol/L)	4.5 (3.6-10)	4.5 (3.9-5.9)	4.5 (3.6-10)	0.603***
Preoperative T4 (pmol/L)	13.1 (8.4-25.5)	13.3 (8.4-14.5)	12.9 (11.3-25.5)	0.963***
Lobectomy				0.546**
Right	22 (59.5%)	14 (63.6%)	8 (36.4%)	
Left	15 (40.5%)	9 (60%)	6 (40%)	
Pathology				<0.05***
Benign	30 (81.1%)	22 (73.3%)	8 (26.7%)	
Malign	7 (%18.9%)	1 (14.3%)	6 (85.7%)	

* Independent samples t-test, ** Chi-square test, ***Mann Whitney-U test

DISCUSSION

In the postoperative period, 37.8% of the patients required hormone replacement. This rate is consistent with the studies reported in the literature.[18] In our study, it was determined that the need for hormone replacement increased significantly in patients with higher preoperative TSH values and in patients whose pathology report was compatible with malignancy.

Patients can be ascribed that the advantage of lobectomy over total thyroidectomy is that it does not require hormone replacement throughout their life. However, we found that although the treatment was in accordance with the ATA guideline, some of the patients may still need hormone replacement.

In the study of Lee et al.[19] with 276 patients with lobectomy, it was observed that the preoperative TSH level and also the presence of microsomal antibodies were effective on the postoperative hormone replacement requirement, similar to our result. In the study of Wilson et al.[17] with 100 patients with lobectomy, it was determined that the preoperative increased TSH level also increased the need for postoperative hormone replacement. Likewise, Stoll et al.[6] It was determined in the study conducted by TSH that increased TSH level increased the need for postoperative hormone replacement. However, Hashimoto patients were not excluded in this study. In addition, it was observed that thyroiditis and remnant small-volume thyroid independently increased the postoperative hormone requirement.[17] Another study found that the volume of the remaining thyroid lobe increased the need for postoperative hormone replacement.[20] In this study, remnant thyroid volume was estimated from preoperative ultrasonography images. Similar to our study, in the study conducted by Wilson et al.[17] it was determined that the need for hormone replacement may develop after lobectomy if preoperative TSH is >2 mIU/L and the pathology result is related to malignancy.

Most studies are based on lobectomies performed due to benign diseases. We guessed that one of the reasons for this was the limited indication of lobectomy in patients diagnosed with malignancy. The biochemical mechanisms that will explain malignancy's effect on the remnant tissue in the resected tissue are still not fully elucidated.

As seen in the studies mentioned above, the results of our study were consistent with the literature. In patients planned for lobectomy, the current TSH level and the possibility of malignancy should be taken into consideration. TSH value and malignancy should be taken into consideration once again when determining lobectomy indications in the guidelines.

Limitations

The limitation of our study was that it was retrospective and single-center. Due to the lack of data, the number of our cases was small.

CONCLUSION

We found that preoperative TSH level is an important predictive factor. In addition, we determined that the presence of malignancy as a result of pathology is an important predictive factor. More comprehensive prospective randomized studies are needed to evaluate the effect of preoperative TSH level and malignancy on postoperative levothyroxine requirement.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by Sakarya University Ethics Committee (Date: 26.06.2020, Decision No: E-71522473-050.01.04-368).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1416658

Anatolian Curr Med J. 2024;6(2):185-190

Prolonged hospitalization in intensive care unit; contributing factors and impact on mortality

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Cite this article as: Tekir Yılmaz E, Şahin Y, Olgun Keleş B, Altınbaş A. Prolonged hospitalization in intensive care unit; contributing factors and impact on mortality. *Anatolian Curr Med J.* 2024;6(2):185-190.

Received: 08.01.2024 ◆ **Accepted**: 01.03.2024 ◆ **Published**: 08.03.2024

ABSTRACT

Aims: Prolonged stay in the intensive care unit (ICU) is a significant problem. It contributes to increased costs, scarcity of resources, morbidity, and mortality. This study aims to investigate the factors contributing to prolonged ICU stay and its association with mortality.

Methods: We retrospectively analysed 312 patients who stayed in the ICU between January 2020 and September 2023. Patients were divided into 2 groups according to the days of ICU stay: 14 days or more (Group 1) and 30 days or more (Group 2). The effects of APACHE II, SOFA, GCS, age, gender, duration and reason for hospitalization, mechanical ventilation type and duration, renal replacement therapy, tracheotomy, blood transfusion, procalcitonin and acute phase reactants on the length of stay in the ICU were analyzed.

Results: A total of 299 patients were enrolled in the study. There were 112 patients who stayed in ICU for longer than 14 days (Group 1) and 187 patients who stayed for longer than 30 days (Group 2). The mean age of Group 1 was 68.6 years and the mean age of Group 2 was 70.9 years. In Group 1, male gender predominated with 62.5%, and in Group 2, it was 56.7%. Among the patients, 29.4% were hospitalized in the ICU for surgical reasons and 70.6% for non-surgical reasons. There were statistically significant differences between the groups regarding GCS, SOFA scores and PaO_2, duration of mechanical ventilation, mechanical ventilation method, ICU mortality, renal-replacement therapy, tracheostomy status, and transfusion status ($p<0.05$). APACHE, expected mortality, lactate, procalcitonin, albumin, pH, $PaCO_2$, HCO_3^-, and GFR, the reasons for ICU admission, comorbidities, and the existence of any infection were not significantly different between the groups.

Conclusion: Age, MV duration, and SOFA score were found to be associated with both prolonged ICU stay and mortality. Regardless of mortality, there was a significant difference between the two groups in terms of GCS, tracheostomy, and the need for RRT.

Keywords: Intensive care unit, length of stay, mortality

INTRODUCTION

Intensive care units (ICU) are specialized and advanced medical care units for critically ill patients. Providing that patients receive care in these units as indicated and as long as necessary plays an essential role in the efficient utilization of the available inpatient bed potential. The length of hospitalization in the ICU has been extending in recent years with the increase in the elderly population and the use of advanced treatment modalities. Length of stay (LOS) in intensive care unit is used as a measurement benchmark to ensure efficient use of health resources and to reduce costs.[1]

The type of intensive care unit and the patient profile results in different definitions of prolonged stays. Therefore, there is no complete consensus on the LOS. Despite the differences in definitions, an intensive care stay longer than 14 days has been associated with an increased risk of infection and costs, the duration of hospitalization before intensive care, age, and severity of the disease in different studies.[2-6]

Various studies have indicated that as little as 4-11% of patients in the ICU are hospitalized for prolonged periods. Yet, this constitutes the majority of intensive care unit bed occupancy.[7,8]

Several studies have investigated the relationship between various factors and LOS in the ICU, most of which focused on specific populations or uniform ICUs.[9,10] In our study, 3rd level 4 ICU hospitalizations were analyzed.

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These were the Internal Medicine ICU, Neurology ICU, and General ICU where postoperative patients are followed up.

Identifying the associated factors of prolonged ICU stays has become a priority for clinicians, hospital administrations, and healthcare policy, as it allows early identification of these patients.

We believe that defining common criteria for different patient populations is important for standardisation. We hypothesised that ICU scoring systems, patients' comorbidities, renal function, inflammatory markers and blood gas parameters may predict prolonged hospital stay. Our primary aim was to investigate the role of these factors in predicting prolonged hospital stay. Our secondary aim was to evaluate the predictive factors on mortality during prolonged hospitalisation.

METHODS

Following the approval of the Giresun Training and Research Hospital Ethics Committee (Date: 27.12.2023, Decision No: 18.12.2023/19), patients who were hospitalized for more than 14 days for the last 3 years in a total of 48 beds of 4 3rd-Level ICU Units of a Giresun Training and Research Hospital were retrospectively screened. Data were mainly obtained from 2 ICUs with 24 beds where an attending anesthesiologist was available 24 hours a day and postoperative patients were admitted. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We identified 312 patients who had been in intensive for more than 14 days from the hospital records. Patients were divided into 2 groups according to the days of ICU stay: 14 days or more (Group 1) and 30 days or more (Group 2). Age, gender, reasons for hospitalization (surgical, non-surgical), length of ICU stay, comorbidities, mechanical ventilator support (invasive, noninvasive), mechanical ventilation duration (21 days and more). Sequential Organ Failure Assessment (SOFA) score, Glasgow coma scale (GCS), Acute Physiology and Chronic Health Evaluation (APACHE) II score, expected mortality, glomerular filtration rate (GFR), CRP, procalcitonin, albumin, Renal Replacement Therapy (RRT) and blood gas parameters such as pH, PaO_2, $PaCO_2$, HCO_3^-, lactate, and culture positive current infections were retrospectively analyzed using electronic patient files and survival status of the patients were recorded.

The comorbidities of the patients at the time of admission were classified and recorded as cardiovascular system diseases (CVD), respiratory system diseases, neurological diseases, diabetes, and chronic renal failure (CRF).

Statistical Analysis

Statistical analyses were performed using IBM SPSS v23. Normality analyses of quantitative data were performed using the Kolmogorov-Smirnov test. Comparison of normally distributed data was performed using the independent samples t-test, and comparison of non-normally distributed data was performed using the Mann-Whitney U test. Comparison of qualitative data was performed using the Pearson chi-square test. Univariate and multivariate logistic regression analyses were performed to identify predictors of mortality. Data are presented as n (%) and mean (95% CI). Statistical significance was accepted as $p<0.05$.

RESULTS

The study was completed with 299 patients, 13 patients were excluded from the study due to inaccessibility of all data. Long stays accounted for 7.5% of total ICU admissions. Here were 112 patients hospitalized longer than 14 days (Group 1) and 187 patients hospitalized longer than 30 days (Group 2) (**Figure**). The mean age of Group 1 was 68.6 years and the mean age of Group 2 was 70.9 years. The male gender predominated in Group 1 with 62.5% and in Group 2 with 56.7% (**Table 1**). Mortality was 23.2% in Group 1 and 52.4% in Group 2, and there was a significant difference between the two groups ($p<0.001$) (**Table 1**) Statistically significant difference in SOFA scores between groups. SOFA score was 5 in Group 1 and 7 in Group 2 ($p<0.001$) (**Table 2**). In the regression analysis to evaluate the predictors of mortality, age ($p=0.001$), SOFA score ($p=0.002$), and MV duration ($p<0.001$) were found to be the most important predictors (**Table 3**).

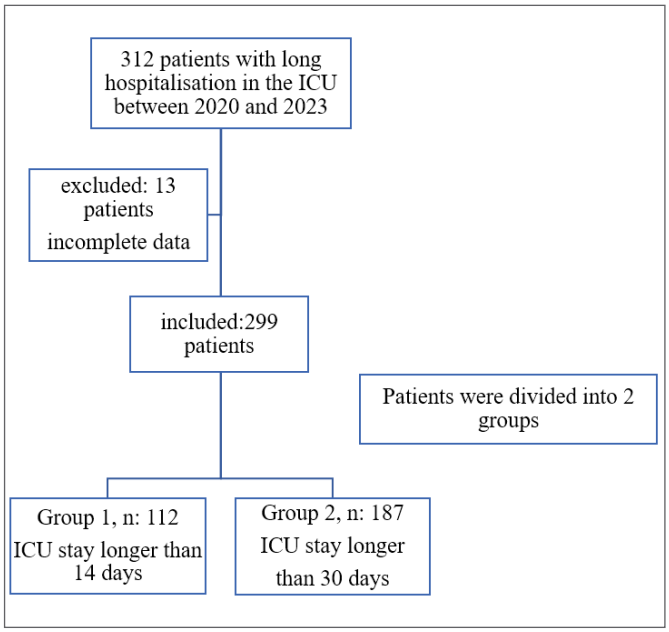

Figure. Flow chart
ICU: Intensive care unit

Table 1. Demographic data and clinical characteristics of the patients and groups

	Grup 1(n%)*	Grup 2(n%)*	p
Sex			0.323
Male	70 (62.5)	106 (56.7)	
Female	42 (37.5)	81 (43.3)	
Case of ICU admission			0.065
Surgical	40 (35.7)	48 (25.7)	
Non-surgical	72 (64.3)	139 (74.3)	
Comorbidity			0.433
-	11 (9.8)	24 (12.8)	
+	101 (90.2)	163 (87.2)	
Cardiovascular diseases			0.865
-	45 (40.2)	77 (41.2)	
+	67 (59.8)	110 (58.8)	
Neurological diseases			0.887
-	90 (80.4)	149 (79.7)	
+	22 (19.6)	38 (20.3)	
Respiratory system diseases			0.635
-	94 (83.9)	149 (79.7)	
+	18 (16.1)	38 (20.3)	
Diabetes mellitus			0.925
-	101 (90.2)	168 (89.8)	
+	11 (9.8)	19 (10.2)	
Chronic renal failure			0.060
-	109 (97.3)	172 (92)	
+	3 (2.7)	15 (8)	
MV duration			<0.001
>21	25 (22.3)	71 (38)	
<21	43(38.4)	95(50.8)	
MV requirement			<0.001
none	44 (39.3)	21 (11.2)	
NIMV	20 (17.9)	20 (10.7)	
IMV	48 (42.9)	146 (78.1)	
Mortality			<0.001
+	26 (23.2)	98 (52.4)	
-	86 (76.8)	89 (47.6)	
Tracheotomy			<0.001
+	11 (9.8)	51 (27.3)	
-	101 (90.2)	136 (72.7)	
RRT			0.031
+	7 (6.3)	27 (14.4)	
-	105 (93.8)	160 (85.6)	
Transfusion			<0.001
+	40 (35.7)	117 (62.6)	
-	72 (64.3)	70 (37.4)	
Infection			0.577
+	29 (25.9)	54 (28.9)	
-	83 (74.1)	133 (71.1)	

*n (%), ICU: Intensive care unit, MV: Mechanical ventilation, NIMV; Noninvasive mechanical ventilation, IMV; Invasive mechanical ventilation, RRT: Renal replacement therapy

The proportion of patients with more than 21 days of mechanical ventilation (MV) was 22.3% in Group 1 and 38% in Group 2. Patients with MV duration below 21 days were 38.4% in Group 1 and 50.8% in Group 2. There was a significant difference between the two groups. (p<0.001) (**Table 1**). Patients not receiving mechanical ventilator care was 39.3% in Group 1, which was significantly higher than in Group 2. (p<0.001) (**Table 1**). The need for tracheotomy, RRT, and blood transfusion was significantly different between the groups. The presence of tracheotomy in Group 2 was significantly higher at 27.3%. (p<0.001) (**Table 1**). The requirement for RRT was 14.4% significantly higher in Group 2 (p=0.03). The need for blood transfusion was significantly higher in Group 2 by 62.6%. (p<0.001) (**Table 1**).

Among the patients, 29.4% were hospitalized in the ICU for surgical reasons and 70.6% for non-surgical reasons. The number of non-surgical ICU admissions was higher in both groups (**Table 1**). 64.3% in Group 1 and 74.3% in Group 2. Statistically significant difference in GCS and PO_2 between groups (**Table 2**). GCS was 11 in Group 1 and 10 in Group 2 (p=0.007). PaO_2 was 81.9 in Group 1 and 117.2 in Group 2 (p=0.007).

APACHE, expected mortality, lactate, procalcitonin, albumin, pH, $PaCO_2$, HCO_3-, and GFR were not significantly different (**Table 2**).

Comorbidities were present in 88.3% of the patients. The most common associated comorbidity causing the longest hospitalization in the ICU was found to be cardiovascular disease. The rate was 59.8% in Group 1 and 58.8% in Group 2 and there was no statistically significant difference between the two groups (**Table 1**). There was no significant difference between the groups for reasons for ICU admission, comorbidities and presence of infection.

Table 2. Patients' findings on admission

	Grup 1 (n=112)*	Grup 2 (n=187)*	p
Age	68.5 (65.9-71)	70.9 (68.3-73.4)	0.026
CRP	107.71 (86.46- 128.96)	109.56 (94.51-124.61)	0.538
GKS	11 (10-12)	10 (9-11)	0.007
SOFA	5 (4-6)	7 (6-7)	<0.001
APACHE	15 (13-16)	16 (15-18)	0.280
PDR	25.24 (21.52-28.96)	29.4 (25.92-32.89)	0.309
Laktat	1.9 (1.7-2.2)	2.8 (1.4-4.1)	0.490
Procalcitonin	4.05 (1.63-6.47)	3.52 (1.93-5.11)	0.727
Albumin	29.76 (28.62-30.9)	29.98 (28.79-31.17)	0.927
pH	7.37 (7.35-7.39)	7.38 (7.37-7.4)	0.509
PO_2	81.9 (69.6-94.1)	117.2 (101.7-132.6)	0.007
PCO_2	43.8 (41.1-46.5)	44.5 (42.3-46.7)	0.875
HCO_3	25.2 (24.1-26.3)	26.1 (25.1-27.1)	0.166
GFR	63 (57-69)	65 (60-70)	0.601

*Mean (95% CI) CRP: C -reactive protein, GCS: Glasgow Coma Scale, SOFA: Sequential Organ Failure Assessment, APACHE: Acute Physiology and Chronic Health Evaluation, PDR: Predicted Death Rate, GFR: Glomerular Filtration Ratio p<0,05:

v 3. Univariant-multivariant regression analysis between independent variables with significant results

	Univariant regression		Multivariant regression*		Multivariant regression**	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age	1.028 (1.011-1.044)	0.001	1.041 (1.015-1.068)	0.002	1.033 (1.013-1.053)	0,001
GCS	0.935 (0.886-0.986)	0.012	1.160 (1.037-1.298)	0.009	1.168 (1.058-1.289)	0,002
SOFA	1.290 (1.185-1.405)	<0.001	1.420 (1.202-1.678)	<0.001	1.402 (1.211-1.623)	<0,001
APACHE	1.033 (1.006-1.060)	0.016	0.992 (0.952-1.034)	0.712		
Albumin	0.955 (0.921-0.991)	0.014	0.972 (0.934-1.011)	0.157		
GFR	0.992 (0.985-0.999)	0.025	1.007 (0.995-1.019)	0.236		
Case of ICU admission	0.416 (0.242-0.715)	0.002	0.838 (0.413-1.701)	0.625		
MV duration	5.340 (3.457-8.248)	<0.001	4.615 (2.601-8.189)	<0.001	5.341 (3.248-8.782)	<0,001
Tracheotomy	2.780 (1.563-4.943)	<0.001	0.924 (0.408-2.093)	0.850		
RTT	3.960 (1.818-8.625)	0.001	1.664 (0.602-4.597)	0.326		
Transfusion	2.934 (1.813-4.750)	<0.001	1.467 (0.775-2.777)	0.239		

*Enter method, ** Backward: Wald method GCS: Glasgow coma scale, SOFA: Sequential organ failure assessment, APACHE: Acute pysiology and chronic health evaluation, GFR: Glomerular filtration ratio, MV: Mecanic ventilation, RRT: Renal replacement therapy

DISCUSSION

This study evaluated a group of patients with long stays in the ICU. Hospitalizations over 14 days in the ICU were compared with very prolonged hospitalizations over 30 days. Studies do not contain sufficient information on the outcomes of very long hospitalizations in the ICU. As these studies focus on a specific group of patients, they enroll a smaller number of patients.[10-12] In this study, we wanted to determine the predictive factors and their relationship with mortality in patients with prolonged ICU stays.

Most patients were being followed up in the ICU for non-surgical reasons in our study. There was a statistically significant difference between the mean ages of the two groups. The prevalence of critical illnesses in elderly patients was found to be high and the LOS in the ICU before recovery was long. Moreover, it has also been reported that prolonged ICU stay is associated with increased mortality and morbidity.[2,4,5,13,14] Our results are similar to studies indicating that advanced age is associated with prolonged hospitalization in the ICU.

The APACHE II score is an ICU scoring system used to indicate the degree of physiologic impairment. It is used to estimate mortality. However, there are different opinions that it can be used in the prediction of conditions such as ICU LOS and separation from MV. Martini et al.[4] have associated the APACHE II score with prolonged hospitalization in their studies. Conversely Suistomaa et al.[15] demonstrated that the predictive value of scoring systems such as APACHE and SOFA diminished in hospitalizations over 7 days. Schönhofer et al.[16] indicated that APACHE II did not predict mortality in prolonged hospitalizations. Several studies have argued that the APACHE II score is an indicator of success or failure in the separation of the patient from the ventilator.[17,18] There was no difference in APACHE II scores between Group 1 and Group 2 in our study. It is toughted that the predictive value of the APACHE II score decreases as the LOS ICU increases.

SOFA is a system for scoring organ failure and dysfunction. There is evidence that the SOFA score in the ICU is a good predictor of patient's separation from mechanical ventilation, LOS, and mortality. Antonelli et al.[19] reported that it may also be useful in identifying patients with a poor prognosis as well as patients who are likely to stay in the ICU for a long time. In our study, we found SOFA scores significantly different between the two long hospitalization groups. In ICU over 30 days, the SOFA score was higher than the other group. We suggest that the SOFA score is predictive for prolonged hospitalizations and remains predictive as the length of hospitalization increases.

The GCS used to assess the level of consciousness, has been reported to predict the LOS in the ICU and mortality in studies conducted on specific groups.[20-24] Despite the heterogeneous group in our study, GCS was significantly lower in very longstay group.

Previous studies have shown that prolonged MV duration leads to prolonged ICU hospitalisation.[2,5,13] Higgins et al.[2] associated prolonged MV duration with infection and poor prognosis. In our study, there was a significant difference between the two groups in terms of the number of patients not on MV. In group 2, the number of patients not on MV was significantly lower with 11.2%. In addition, the number of patients followed up in MV for more than 21 days was 38% and the number of patients followed up in MV for less than 21 days was 50.8% in Group 2, which was significantly higher than in Group 1.

When we examined the method of MV, the number of patients followed up with invasive mechanical ventilation was higher in both groups. The number of patients who did not receive invasive or noninvasive MV treatment was 39.3% in the group of patients hospitalized longer than 14 days. In the group hospitalized longer than 30 days, it was 11.2%. Regarding the MV method, there was

a significant difference between the two groups. From a clinical point of view, it is not surprising that the LOS of patients undergoing invasive MV is prolonged as this period is prolonged.

In our study, the number of tracheotomies was significantly higher in patients who stayed longer than 30 days, similar to other studies.[6,25]

Several studies have associated the prevalence of infection with prolonged hospitalization in the ICU.[3,26] In this study, we found no difference in the prevalence of infection, procalcitonin, and CRP values between the groups. We believe that this is because of the comparison of the two long hospitalization groups. The predictive value of the prevalence of infection may be decreasing beyond 14 days. We suggest that this finding should be supported by more studies.

The need for RRT has been associated with LOS in the ICU in several studies.[4,5,13] We recorded both the need for RRT and the GFR of the patients during our study. We did not find any difference between the GFR of the patients on the day of admission to the ICU. However, we determined that the need for RRT increased with the number of days. In a multinational study of the development of AKI in critically ill patients, sepsis, medication use and MV were found to be predictors of the development of AKI.27 In our study, the need for RRT during prolonged hospitalisation may be related to prolonged mechanical ventilation, sepsis and antibiotherapy.

Nozawa et al.[28] reported that cardiac dysfunction, low ejection fraction, left ventricular dysfunction, cardiogenic shock and cardiac surgery were among the factors that had a negative impact on removing patients from MV. Several studies have shown that CVS diseases increase the risk of long hospitalization in the ICU.[29,30] Similarly, in our study, we also observed a high number of patients with additional CVS diseases other than the reason for ICU admission. Those with CVS disease constituted 59.8% of Group 1 and 58.8% of Group 2 second place followed those with neurological diseases and third place was taken by those with respiratory system diseases.

The mortality rate was 52.4% in Group 2 and was significantly higher than in Group 1. Friedric et al.[13] reported a mortality rate of 42% in patients with very long hospitalization in the ICU. This rate varies between 40-53% in studies investigating hospitalizations in the ICU over 28-30 days.[4,5] The mortality rate is similar in our study.

When multivariate logistic regression analysis was used, age, SOFA score, and MV duration were identified as predictors of mortality. There are studies in which age and MV duration were identified as predictors of mortality. Friedrich et al.[13] found age, ventilation for more than 90 days, dialysis, and inotrope support for more than a minimum of 3 days as predictors of mortality. In contrast, Laupland et al.[7] found advanced age and previous comorbidities as predictors of mortality. In our study, SOFA score was found to be a predictor for both prolonged ICU hospitalisation and mortality. We believe that this should be supported by multicentre studies with larger patient samples.

Multivariate regression analysis revealed an inverse association between GCS and mortality. Mortality increased as the GCS increased. We think that this result is because trauma patients were also monitored in a mixed ICU and early mortality of patients with very low GCS.

Limitations

This study is single-centre. Although it includes ICUs with different patient populations, it only reflects our results. The reasons for long ICU stays may vary in different regions due to many factors, such as the number of beds, lack of palliative care centres, management of non-resuscitated patients. Multicentre studies are needed to understand the reasons for long ICU stays.

CONCLUSION

Age, MV duration and SOFA score were associated with both prolonged ICU stay and mortality. Independent of mortality, GCS, need for tracheotomy and RRT were significantly different between the two groups. Patients who stayed in the ICU for more than 30 days had higher mortality rates. Despite the differences in opinion regarding the SOFA score, we concluded that the SOFA score may be predictive of prolonged ICU stays. We think that more studies are needed to better define the clinical outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study protocol was approved by the Giresun Training and Research Hospital Ethics Committee (Date: 27.12.2023, Decision No: 18.12.2023/19).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Acknowledgments

Special thanks to Assistant Professor İskender Aksoy.

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