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Instructions to Authors

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Patients' characteristics and procedural outcomes of premature ventricular complex ablation: Data of a single-centre arrhythmia unit experience

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ABSTRACT

Objective: Premature ventricular complexes (PVCs) are common arrhythmias and catheter ablation (CA) is the major treatment in patients with PVCs. In this study, we aimed to share our experience on PVCs patients who had undergone CA.

Patients and Methods: We investigated consecutive patients who had undergone CA because of PVCs between January 2015 and March 2021 in a single centre arrhythmia unit. Patients' characteristics, rhythm Holter recordings, echocardiography results and CA outcomes were noted. Patients were followed up for 22.3±3.7 months. Descriptive statistics were used to demonstrate features of study patients.

Results: Study population consisted of 645 patients; mean age was 51 ± 4.14 and 372 (57.7%) were male. Arrhythmia originated from the right ventricular outflow tract in 279 (46.6 %) patients, coronary cusps in 161 (26.9 %) patients, left ventricle summit in 50 (8.3%) patients and inside of the right or left ventricle in remaining patients. Sustained procedural success was achieved in 526 (88.1 %) patients. Recurrence was observed in 46 (6.7%) patients during follow-up. Major complication occurred in two patients (one procedural mortality due to coronary artery injury and one tamponade).

Conclusion: Premature ventricular contraction ablation can be utilized safely with high success rate. Most PVCs originate from the right or left ventricular outflow tract.

Keywords: Premature ventricular complexes, Catheter ablation, Outflow tract, Mapping

1. INTRODUCTION

Premature ventricular complexes (PVCs) are one of the common arrhythmias observed in routine practice which can be found in many patients undergoing rhythm holter monitoring [1]. While some patients may be asymptomatic, various kind of symptoms may manifest in most PVCs cases. Palpitation, shortness of breath, chest pain and fatigue are common complaints. In addition, some of cases may be asymptomatic even they have large arrhythmia burden demonstrated by holter monitoring. It has been shown that the PVCs patients without structural heart disease have a good prognosis [2]. Premature ventricular complexes may lead to reduced left ventricle ejection fraction (LVEF) which is evaluated by echocardiography. QRS wide, epicardial origin, PVC burden, coupling interval variability and body mass index have been demonstrated as predictors of PVC induced cardiomyopathy [3]. Although, PVCs generally originate from ventricular myocardium, they may sometimes locate in fascicules, aortic cusp or inside of coronary venous system. Arrhythmia mechanism may be re-entry, triggered activity or automaticity [4]. Medical therapy is often used as the first treatment option of PVCs. However, the effectiveness of this treatment is often limited due to its low efficacy or its intolerable side effects [5]. The aim of this study is to demonstrate the patient characteristics, outcomes and complications of catheter ablation (CA) procedures for symptomatic idiopathic PVCs in a single center.

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2. PATIENTS and METHODS

Patient Selection

Data of patients who had undergone radiofrequency CA due to symptomatic PVCs or cardiomyopathy that was assumed to be related with PVCs from January 2015 to December 2020 in our center were included. All of patients were evaluated in terms of structural heart disease prior to the ablation procedure. Patients with significant ischaemic heart disease, significant valvular disease, genetic or infiltrative cardiomyopathy were excluded from the study. Significant coronary artery disease had to be ruled out by coronary angiography or stress testing in all patients. Demographic and clinical data; including age, sex, medication status was collected.

The echocardiographic measurements were performed with an ultrasound system (Epic; Philips Healthcare Medical Systems, Andover, MA, USA) in accordance with the guidelines of the American and European Societies of Echocardiography for cardiac chamber quantification [6]. Standard echocardiographic views were obtained with a 3.5-MHz transducer in all participants.

Premature ventricular complexes burden, the origin and number of PVCs/24hour and complications were collected. Informed consent form was obtained from all patients before the procedure. Patients were older than 18 years, able to read and sign the informed consent form. The study protocol was approved by our institutional review board.

Ablation

All antiarrhythmic drugs were stopped at least 5 half-lives prior to the procedure. Electrophysiological study was performed under local anaesthesia in a fasted state. Sedation was not applied to the patients before the procedure to avoid the risk of suppressing automaticity. If few or no PVCs were observed at baseline, isoproterenol infusion and electrical stimulation techniques were used to induce arrhythmia. Intravenous isoproterenol 1-5 μg/min infusion was given to provide at least 20% heart rate increase. Electrical stimulation was performed using the right ventricular apex or right ventricular outflow tract (RVOT) using burst pacing and triple extra stimuli pacing. Mapping and ablation were guided by Ensite TM Precision (Abbott, Chicago, IL, USA), CARTO electro-anatomic mapping system (Biosense Webster, DiamondBar, CA, USA) or conventional electro-anatomic mapping. For left-sided procedures, systemic heparinization was used to maintain an activated clotting time of 300–350 s. After ablation, patients were monitored for at least 30 minutes to ensure successful ablation. Procedural success was defined as elimination or non-inducibility of the clinical PVCs. After the successful ablation, all antiarrhythmic drugs were withdrawn.

Follow-up

Patients were routinely seen in the outpatient clinic 3 months after the procedure. Holter monitoring was repeated then in many patients. Successful sustained ablation (SSA) was defined as the persistent elimination of at least 80% of the PVC burden or the absence of ventricular tachycardia (VT). Most of the study patients were followed up for 22.3±3.7 months after the procedure.

Statistical analysis

All statistical variables were analyzed with the Statistical Package for the Social Sciences (SPSS 22.0 for Mac; Inc., Chicago, IL, USA) software. The disturbances of variables were examined with analytic Kolmogorov–Smirnov or Shapiro–Wilk's tests. Continuous variables are presented as mean ± standard deviation and categorical variables as numbers and percentages.

RESULTS

A total of 645 consecutive patients were included in our study. Mean age was 51±4.14 and 57.7 % of the patients were male. The patients clinical and demographic characteristics were shown in Table I. There were 489 patients (75.8%) receiving medical therapy prior the ablation procedure. The median PVC burden was 19.4 and left ventricle ejection fraction mean was 57±13 in our study group. Fifty-five (8.7%) of the 645 patients ablation procedure were postponed due to non-inducibility of PVC during the procedure or probable risk of coronary artery or conduction system injury. 85.2 percent of the patients (508) who were ablated during first procedure were successfully ablated. While 50 patients were taken to the ablation procedure twice and 6 patients three times (Table II). After all ablation procedures, successful ablation was achieved in 526 patients (88.1%). Multiple PVC ablations were performed on a total of 24 patients.

Table I. *Demographic and clinical characteristics of patients*

Age	48±14
Male (%)	372 (57.7%)
PVC burden mean/median	20.8±9.1 19.4
PVC number	22700±10000
Hypertension	173 (27.9%)
Hyperlipidemia	89 (14.3%)
Smoking	87 (14.1%)
Diabetes mellitus	47 (7.6%)
Heart failure	118 (19.0%)
Coronary artery disease	70 (11.3%)
Implantable cardioverter defibrillatior	18 (2.9%)
Atrial fibrillation	21 (3.4%)
Palpitation	400 (67.0%)
Beta-blockers	466 (72.2%)
Calcium channel blockers	47 (7.3%)
ACEI/ARBs	142 (27.7%)
Amiodarone	28 (4.3%)
Propafenone	24 (3.7%)
Sotalol	7 (1.1%)
Ejection fraction	56.9±13,1
Body mass index	28±5

Data are presented as mean ± standard deviation while categorical variables were expressed as percentages. ACEI: Angiotensin-converting enzyme inhibitors; ARB: Angiotensin receptor blockers. PVC: premature ventricular complex.

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Table II. *Results of the clinical study*

Total numbers of procedures	
1 procedure	590 (91.5%)
2 procedures	50 (7.8%)
3 procedures	6 (0.9%)
Ablation postponed during first procedure	55 (8.7%)
Causes	51
Noninducibility	3
Parahisian	1
Proximity to LMCA	
Causes of repeat procedure	
Noninducibility	8 (13.3%)
Unsuccessful ablation	26 (43.3%)
Recurrens	26 (43.3%)
Mapping and ablation system	
Conventional mapping system	277 (46.9%)
3D mapping system	313 (53.2%)
Successful sustained ablation	526 (88.1%)
Successful sustained ablation in patients with LV dysfunction	90 (80.5%)
PVC burden after successful ablation mean/median	4.4±8.3????
More than one PVC ablation	24 (4.0%)
Mean ejection fraction post ablation	59±10
Complictions	
Pseudoaneurism	1 (0.2%)
Cardiac tamponade	1 (0.2%)
Death	1 (0.2%)
Recurrence	43 (6.7%)

Data are presented as mean ± standard deviation while categorical variables were expressed as percentages. LMCA: left main coronary artery, LV: left ventricle, PVC: premature ventricular contraction

It was seen that 238 (43.1%) of PVCs originated from the right ventricular outflow tract (RVOT), and 161 (26.9%) of PVCs originated from coronary cusps (Table III). The ratio of success according to location of PVC is shown in Table IV.

Table III. *Distribution of PVC location*

RV out-flow track	279 (46.6%)
RV	8 (1.3%)
Tricuspis annulus	4 (0.7%)
Coronary cusps	161 (26.9%)
Aortomitral continuity	41 (6.8%)
Summit	50 (8.3%)
Left ventricle	42 (7.0%)
Papillary muscle	11 (1.8)
Left ventricle Fascicle	1 (0.2%)
Mitral annulus	14 (2.3%)
Multilocation PVC	27 (4.5%)

Categorical variables were expressed as percentages. RV: right ventricle PVC: premature ventricular contraction

Table IV. *Ratio of success according to location of PVC*

Location of PVC	Successful ablation	Unsuccessful ablation
RVOT	255 (93.1%)	19 (6.9%) *
Right ventricle	5 (62.5%)	3 (37.5%)
Tricuspid annulus	4 (100%)	0 (0%)
Coronary cusps	147 (91.3%)	13 (8.7%)
Aorto-mitral continuity	35 (85.4%)	6 (14.6%)
Summit	29 (58.0%)	21 (42.0%)
Left ventricle	35 (83.3%)	7 (16.7%)
Mitral annulus	13 (92.9%)	1 (7.1%)
Multilocation PVC	(70.8%)	7 (29.2%)

*PVC: premature ventricular contraction, RVOT: right ventricle out-flow track. Categorical variables were expressed as percentages. *5 patients could not be ablated because of parahisian originated PVC.*

DISCUSSION

It has been shown in previous studies that if we monitor apparently healthy individuals by 24-hour ambulatory rhythm Holter, PVCs occurs in 50% to 54% of the subjects [7,8]. Most of the patients are asymptomatic, however as the burden of PVCs increases life qualities of the patients decrease. As palpitation is the most common complaint, drug therapies like beta blockers and calcium channel blockers are aimed to relieve this symptom via decreasing contractility in post PVC beats in addition to recommended lifestyle changes. However, it is not clear which patient groups respond well to this approach as there are some other aspects regarding patient characteristics or course of the disease process.

While medical therapy may mask the symptoms, more emphasis is given recently to catheter-based therapies as it being curative in selected patients and being safe if performed by experienced operators. These developments led to many comparative studies. It has been shown that radiofrequency ablation is more effective than medical therapy for treatment of PVCs [5,9].

Symptoms aside, there are some other entities to take into consideration. Prognosis may change drastically when patients develop PVC-induced cardiomyopathy [10] or malign arrhythmias caused by R-on-T phenomenon [11,12]. Recently, catheter ablation has been preferred as first-line therapy in patients with PVC-induced cardiomyopathy [13].

In this study, we determined the demographic characteristics, PVC localization, success and complication rates of patients who underwent PVC ablation procedure. Our findings showed that PVCs ablation resulted in high success rate in patients with frequent PVCs with a sustained successful ablation rate of 85.2% after first ablation procedure and 88.1% after all ablation attempts. The success rate of sustained successful ablation was 80.1% in patients with left ventricle systolic dysfunction. The complication rate was acceptable with 0.2% of death. When the success was evaluated according to the PVC origin, it was shown that the success rate in RVOT and coronary cusps PVCs ablation was quite higher than summit PVCs ablation.

The results of our study are consistent with the results of previous reports in the literature. As far as recent clinical studies

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were concerned, most of the studies conducted in patients with symptomatic frequent PVCs have reported over 80% success rates and low complication rates of ablation [14-16]. Similarly, successful results are obtained with PVC ablation of patients with left ventricle systolic dysfunction [15]. A metanalysis demonstrated long term success rate of up to 80% and complication rates of no more than 8%.

Conclusion

Premature ventricular contractions are one of the most common arrhythmias encountered in routine cardiology practice. One of the main treatment modality is catheter ablation and premature ventricular contraction ablation can be utilized safely with high success rate. Most of PVCs are originated from right or left ventricle outflow tract. Also, success rates are high when PVCs are originated from outflow tracts.

Compliance with Ethical Standards

Ethical approval: The study protocol was approved by the Kartal Kosuyolu Training and Research Hospital Ethics Committee (approval number: 2020.8/10-224).

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Conflict of interest: The authors have no potential conflicts to declare.

Authors' contributions: MC and AK: Literature search, SD, AK and KG,: Study design, KG and SD: Legislative applicatons, KG, AU, AT, AK and TA: Data collection, AK and CY: Supervision and quality control, AK and BGK: Statistical advice, AK and KG: Statistical data analysis, SA and TA: Data interpretation, KG: Drafting the manuscript. All authors read and approved the final version of the article.

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Original Article

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Classification of hemiplegia through gait analysis and machine learning methods

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ABSTRACT

Objective: Gait analysis is a method that is used for understanding normal walking and determining the stage of the disease as it affects walking. It is important to objectively determine the stage of the disease in order to decide interventions and treatment strategies. This study aims to determine the Brunnstrom Stage of the hemiplegic patients with an analysis of gait data.

Patients and Methods: In the first part of the study, the gait signal data were taken from 28 post-stroke hemiplegic patients and 7 healthy individuals with three-axis accelerometers. In the second part, new gait data were collected from 15 healthy individuals through an accelerometer on the anteroposterior axis.

First the accelerometer signals were decomposed to Daubechies 5 (Db5) level six wavelets using MATLAB software. Subsequently, these attributes were classified through several classifier and machine learning algorithms on WEKA and MATLAB software packages to predict the stages of hemiplegia.

Results: The highest accuracy rate in the prediction of hemiplegia stage was achieved with the LogitBoost algorithm on WEKA with 91% for 35 samples, and 90% for 50 samples. This performance was followed by the RUSBoosted Trees algorithm on the MATLAB software with an accuracy of 86.1% correct prediction.

Conclusion: The Brunnstrom Stage of hemiplegia can be predicted with machine learning algorithms with a good accuracy, helping physicians to classify hemiplegic patients into correct stages, monitor and manage their rehabilitation.

Keywords: Hemiplegia, Stroke, Gait analysis, Brunnstrom, Machine learning

1. INTRODUCTION

Hemiplegia as a result of a stroke affects lots of people every year. Approximately two-thirds of those who have had a stroke do not become ambulatory without assistance and only half of those who were rehabilitated were able to walk independently [1]. The hemiplegic gait is defined as a movement pattern and body posture with a heavy, challenging or weak coordination that the hemiplegic patient experiences during the gait [2]. Although, there are many methods to evaluate hemiplegic patients, the Brunnstrom Staging, is the preferred one amongst all the methods. Therefore, we preferred to evaluate our patients according to the Brunnstrom Staging. Brunnstrom consists of six stages of improvement. However, only the hemiplegic patients at stages III, IV, V and VI were included in the study as the gait is not in question during the first two stages [3].

Gait analysis is widely used in determining human gait disorders. There are two fundamental approaches developed for the gait analysis to analyse the human gait. The first approach uses the marking systems that include video-based systems, active magnetic trackers, and optical marker systems, to acquire the human gait motion. However, as they depend on an artificially-created source, they cannot be used outside a laboratory setting [4]. Muro-De-La-Herran et al., named them as "unwearable sensor systems". The second approach involves wearable sensors. Wearable sensor systems make gait analysis possible outside laboratories and can gather information about the gait during the daily activities of an individual. Wearable systems use the sensors located in various parts of the body such as feet, knees, thighs, or waist [5].

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Through a gait analysis, it is possible to define the gait phases, to determine the kinematic and kinetic parameters of human gait events, and to quantitatively evaluate the musculoskeletal functions. Gait analysis has been used since the 19th century to for sports or safety purposes. For instance, in some types of sports training, the method is applied to recognize the faults in athlete performances so that they can improve. For security purposes, interest may centre on distinguishing and identifying persons based on a general characterization of their silhouette and the movements between the subject's different body segments when walking [5,6].

Today, there is a great variety of signal processing methods, and the Wavelet Transform (WT) technique is one of them. The WT is a mathematical method that gives the time-frequency representation of a signal. The WT is an effective signal processing tool thanks to its features such as the time-frequency localization (obtaining a signal at a specific time and frequency, or extraction of attributes at various locations on different scales) and differential-proportional filtering (distinguishing between the signals with various frequencies) [7,8].

In a study conducted by Lee et al., in 2018, through the use of a wearable system, it was aimed to distinguish the hemiplegic gait by extracting the simple properties of the acceleration signals caused by the asymmetry during the gait. The wearable system designed was equipped with a three-axis accelerometer and a three-axis gyroscope. In the study, which employed a "random forest" algorithm for classification, the accuracy, sensitivity, specificity and positive predictive value were found to be 100% [9].

Another study aimed to determine the symmetry, regularity and stability of the gaits in hemiparetic patients in the post-stroke period by using the stability index that was based on a dynamic time-bending algorithm, a sample entropy method and an empirical mode decomposition. The study was conducted with 15 healthy control subjects and 15 post-stroke hemiparetic patients. A total of four different machine learning methods were used, which included the decision support machine, decision tree, multilayer neural network and k-nearest neighbour (kNN), and the maximum area under curve (AUC) value was given as 0.94 by the kNN classifier [10].

In our study we first used WT to decompose gait data then applied several machine learning algorithms to establish the Brunnstrom Stage of hemiplegic patients accurately.

2. PATIENTS and METHODS

Data collection tools

The signals used in the study were obtained through a three-axis accelerometer from the waist of participants (3031-010, IC-Sensors, USA, size: 4x4x3 mm; weight: 0.3 g; range: ±10 g; frequency reaction: 0-500 HZ). The accelerometer was orthogonally mounted to record the anteroposterior (x), lateral (y) and vertical (z) signals. The accelerometers were calibrated by measuring their outputs under a controlled inclination. Then, they were fixed onto an acrylic plate for the waist belt. An elastic waist belt was put on the lumbosacral area of the vertebral column on the back of the patient in the proximity of the center of gravity while the patient was standing upright. The accelerometer unit was connected to a portable data recorder (Micro 8, Shimadzu, 36 Japan) through an interface circuit. This data recorder consists of one Central Processing Unit (CPU), one 10-bit A/D converter, one Integrated Circuit (IC) card interface and one removable 2-MB IC memory card. The interface circuit contains three amplifiers as an anti-aliasing filter for each direction, as well as three second-degree analog Butterworth low permeable filters. The cut-off frequency is 500 Hz. The accelerometer outputs were digitalized by the data recorder at a sampling rate of 1024 Hz and recorded on an IC memory card. Following the completion of the measurements, the data were transferred to a personal computer through a card reader for analysis purposes [11].

The data set

In the first part of the study, the gait signal data taken from 28 post-stroke hemiplegic patients and 7 healthy individuals were used. Of the patients, 9 were female and 19 were male, while the healthy ones were all female. The gait signal data was taken from post-stroke hemiplegic cases and healthy individuals in 2005-2006 at the University of Chiba, Japan [12]. The approval of the local ethics committee was obtained from the University of Chiba, Japan, and all subjects gave their written informed consent for the data used in the study.

Even though, the Brunnstrom Staging consists of six stages, only the patients at stages III, IV, V, and VI were included in the study as the gait is not in question at the first two stages. Table I gives the distribution of patients and their Brunnstrom Stages.

Table I. *Distribution of patients according to Brunnstrom Staging*

Brunnstrom Stage	Number of Patients	Gender (F/M)
III	12	3/9
IV	9	3/6
V	4	3/1
VI	3	0/3

Table II gives the mean age, weight and height characteristics of the hemiplegic and healthy individuals included in the study.

Table II. *Demographic characteristics of the subjects*

Group	Age	Height	Weight
Patient	67 ± 11	155 ± 8.89	55.27 ± 9.81
Healthy	61 ± 5.1	149±1.41	49.66±1.69

In the second part of the study, the gait signals were collected additionally from 15 healthy individuals through an accelerometer with an anteroposterior axis. The total number of samples was increased to 50 by merging the same attributes obtained through the signals decomposed by the WT method with the attributes of the anteroposterior axis of the data set used in the first part of the study.

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Table III. Classification algorithms and their accuracy rates

		Accuracy rates (%) with 35 samples	Accuracy rates (%) with 50 samples
WEKA Classification algorithms	LogitBoost	91.4	90.0
	Iterative Classifier Optimizer	91.4	90.0
	J48	88.5	86.0
	CVR	85.7	82.0
	OneR	87.7	78.0
	Bagging	85.7	78.0
	REPTree	80.0	76.0
	Random Forest	74.2	72.0
	Random SubSpace	74.2	68.0
	Multi Class Classifier	65.7	76.0
	AdaBoost	57.1	52.0
MATLAB Software Classifiers	RUSBoosted Trees	86.1	86.0
	Complex Tree	83.3	82.0
	Subspace Discriminant	75.0	76.0
	Linear Discriminant	69.4	64.0

Data analysis

Within the scope of the study, the gait signal data taken from 28 post-stroke hemiplegic patients and 22 healthy individuals were used. The MATLAB and WEKA software were used to analyse the signal data.

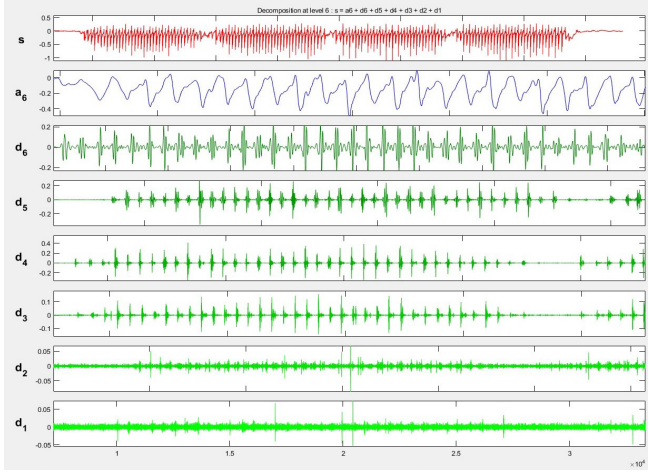

Fig.1. *The Db5 main wavelet was decomposed up to level 6, thus each gait signal was decomposed into d1-d6 detail bands and a6 approximate sub-band (s denotes the original state of the signal, d denotes the detail function, and a denotes the approximation function.)*

Extraction of attributes from the gait signals through discrete wavelet transform

The selection of the proper wavelet and the number of decomposition levels is of utmost importance for the analysis of the signals through discrete wavelet transform. The dominant frequency components are considered while selecting the number of decomposition levels [13].

Initially, every single axis of the signal taken from each subject was saved as MATLAB file format and thus 105 pieces of signal data were obtained. Subsequently, 105 decomposition procedures were carried out to obtain the coefficients to be used in the classification process for each axis. This process was carried out by using the Db5 decomposed main wavelet, which is the most commonly preferred item in the literature for gait signal analysis [14]. The Db5 main wavelet was decomposed up to level 6, thus each gait signal was decomposed into d1-d6 detail bands and a6 approximate sub-band (Figure 1.)

The attributes of the approximation signal at level 6 were selected to create the classification data following the decomposition of the signals into 6 levels through the use of the Db5 main wavelet (Figures 2-5).

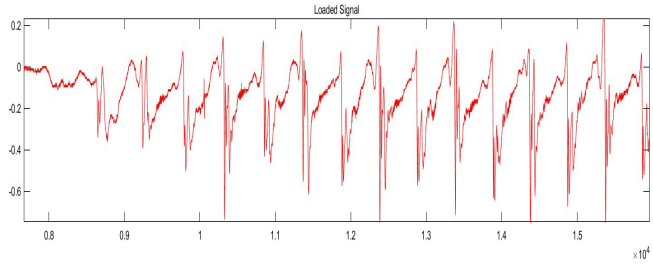

Fig. 2. *The gait signal's amplitude of the anteroposterior axis taken from the healthy individual*

These attributes are minimum, maximum, mean, median, absolute deviation from the mean, absolute deviation from the median, the first norm of the vector (L1 norm), the second norm of the vector (L2 norm), and maximum norm. Table IV shows some examples of the attributes on the anteroposterior axis for each stage.

Figure 2 shows the gait signal of the anteroposterior axis taken from the healthy individual while Figures 4 shows the gait signal of the anteroposterior axis taken from the patient at the 3rd Brunnstrom Stage. Figure 3 shows the approximation signal at level 6 of the Db5 wavelet of the gait signal of the anteroposterior axis taken from the healthy individual while Figure 5 shows the approximation signal at level 6 of the Db5 wavelet of the gait signal of the anteroposterior axis taken from the patient at the 3rd Brunnstrom Stage.

Table IV. The attributes of the approximation signal at level 6 (anteroposterior axis)

Patient no	Stg	Mean	Median	Max	Min	Med Abs Dev	Mean Abs Aev	L1 Norm	L2 Norm	Max Norm
7	healthy	-0.674	0.735	1.202	-2.346	0.532	0.547	1369	39.63	2.346
8	3	-0.554	-0.554	1.038	-1.769	0.330	0.366	1859	39.7	1.769
23	4	-0.746	-0.731	1.712	-3.047	0.576	0.632	2062	52.2	3.047
29	5	-0.938	-0.955	2.154	-3.466	0.926	0.954	2379	65.82	3.466

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Estimation of the Brunnstrom Stage through classification algorithms

The first data set consists of the attributes, which were obtained from the level 6 approximation signal coefficients as a result of the WT of the gait signals taken from the anteroposterior, lateral and vertical axes of 35 individuals (28 patients, 7 healthy), as well as the Brunnstrom stages of those individuals. The second data set, on the other hand, consists of the attributes extracted from the level 6 approximation signal coefficients as a result of the WT of the signals taken from 15 healthy gaits, which were added to the data set that was already present. Nevertheless, this data set only includes the attributes obtained from the signals taken from the anteroposterior axis.

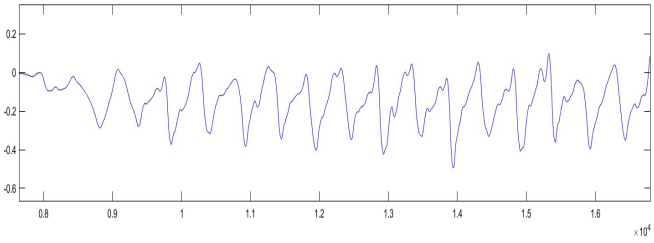

Fig. 3. *The approximation signal's amplitude at level 6 of the Db5 wavelet of the gait signal of the anteroposterior axis taken from the healthy individual*

As specified in the very beginning, the objective of the study was to estimate the Brunnstrom stages based on the gait signal data of individuals. In this context, initially, the classification algorithms on the Weka software were used, which was then followed by those on the MATLAB software, for the classification problem in this study.

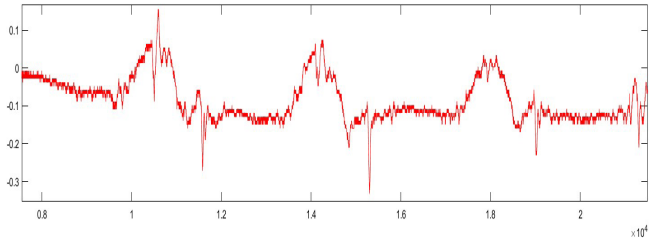

Fig. 4. *The gait signal's amplitude of the anteroposterior axis taken from the patient at the 3rd Brunnstrom Stage*

On the WEKA software the following algorithms were used: The Iterative Classifier Optimizer, AdaBoost, Bagging, Classification via Regression (CVR), LogitBoost, OneR, J48, Random Forest, Random SubSpace, MultiClass Classifier and RepTree classification algorithm.

The WEKA includes various strategies for training and testing. As the dataset is relatively small, the 10-fold cross-validation technique was employed in the study as a test option in all algorithms used for the solution of the problem. As the dataset is relatively small, the 10-fold cross-validation technique was employed in the study as a test option in all algorithms used for the solution of the problem. The cross-validation technique is one of the methods of splitting the data set into parts for training and evaluating the model. In this technique, the dataset is randomly divided into two parts according to a determined k ratio, and the first part is used for both trainings and the second for testing.

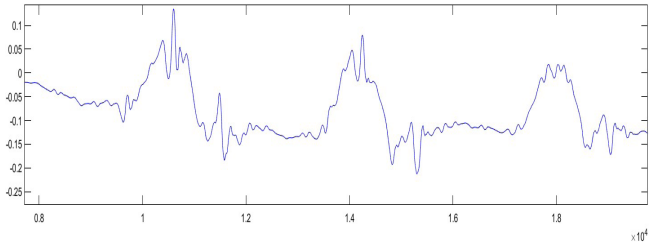

Fig. 5. *The approximation signal's amplitude at level 6 of the Db5 wavelet of the gait signal of the anteroposterior axis taken from the patient at the 3rd Brunnstrom Stage*

3. RESULTS

Results of the classification conducted with a 35-person data set on the WEKA software

The Iterative Classifier Optimizer algorithm yielded the same results as the LogitBoost algorithm, which was caused by the fact that it used the LogitBoost algorithm as an iterative classifier.

Results of the classification conducted with a 50-person data set on the WEKA software

In this section, new data were added to the current data set, and the classification was iterated with the same algorithms. Only the relevant axis of the current data was used as the newly-obtained gait signal covered the gait signal taken from 15 healthy individuals, using an accelerometer with an anteroposterior axis.

In a similar manner to the current signal, the 15 newly-added gait signals were also decomposed at 6 levels through the Db5 main WT. Then again, the attributes of the approximation signals at level 6 were selected and added to the current data set.

As shown in Table III, a decrease was observed in the accuracy rates as a result of the classification process iterated with the new data set.

Results of the classification conducted with a 35-person data set on the MATLAB software

The data set, comprised of the attributes obtained from the gait signals as a result of the WT, was classified by using the classification algorithms available on the MATLAB Software, to estimate the Brunnstrom stages of the individuals. Table III gives the classification algorithms used in the MATLAB software and the accurate classification rates. In a similar manner to the WEKA software, the 10-fold cross-validation technique was also employed in the MATLAB software as a test option in all algorithms used for the solution of the problem.

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The RUSBoosted Trees algorithm yielded the highest accuracy rate for the solution to this problem on the MATLAB software, and the accurate classification rate was 86.1%. The true positive rates of the algorithm were found as 0.92 for the 3rd stage, 0.89 for the 4th stage, 0.75 for the 5th stage, 1 for the 6th stage and 0.86 for the healthy individuals. While, the RUSBoosted Trees algorithm accurately classified all of the stage 6 patients, it made one mistake in other groups.

Results of the classification conducted with a 50-person data set on the MATLAB software

The attributes, which were obtained from the gait signals taken from 15 healthy individuals through an accelerometer with an anteroposterior axis, were added to the current data set, and the classification was iterated by using the same algorithms on the MATLAB software.

As a result of the iterative RusBoosted Trees algorithm, the accuracy rate did not change and was found to be 86.1%. Nevertheless, the True Positive rates varied based on the groups. They were found as 1 for the third stage, 1 for the fourth stage, 0 for the 5th stage, 0 for the 6th stage and 0.77 for the healthy individuals. While the Subspace Discriminant algorithm accurately classified all of the patients at stages 3 and 4, it did not accurately classify any of the patients at stages 5 and 6.

4. DISCUSSION

In our study, the WT technique and classification algorithms were used to estimate the Brunnstrom stages of the hemiplegic patients based on their gait signals. On the MATLAB software, the attributes of the approximation signal at level 6 were selected from the gait signals decomposed into 6 levels through the Db5 main wavelet.

It could be argued that the 28 hemiplegic gait and 7 healthy gait samples are insufficient for the study to generalize the success of the classification results due to the insufficient number of samples. The accuracy rates achieved via cross-validation are good and are improved further by the addition of the gait signal data of the healthy individuals due to the difficulty experienced in finding any data that were similar to the gait signal data of the hemiplegic elderly, whose Brunnstrom stages were known and which also constituted the data set of the study. As a result the number of cases reached 50 following the addition of 15 healthy gait signal data. This caused the drop in the accuracy rates and overall performance of the machine learning algorithms. The probable causes of the decrease in the accuracy rates following the reclassification made through the newly-created data set are as follows:

The current data set used the attributes obtained following the WT of the gait signals taken via a three-axis accelerometer. In the new data set, however, only the gait signal data taken from the anteroposterior axis were used. Despite the increase in the number of cases, it is believed that the accuracy rates decreased as a result of the decrease in the number of attributes.

Each analysis can yield different results in machine learning techniques. It is believed that this is caused by the varying data set and the parameters used in the algorithm.

Besides, despite the decrease observed in the classification accuracy rates in all algorithms, an increase of 11% was observed in the Multi Class Classifier algorithm.

The results of the Boosting algorithm were another striking issue in the classification results. Among the accurate classification rates of the AdaBoost and LogitBoost algorithms, a difference was observed by 34% for the first algorithm, which was followed by a 38% difference for the latter. It is believed that this was caused by the fact that the LogitBoost algorithm is designed to solve the excessive conformity problem stemming from the extremely noisy data, which is a problem for the AdaBoost algorithm. The LogitBoost linearly decreases the training error for the solution to this problem [15].

The LogitBoost algorithm and Iterative Classifier Optimizer algorithm yielded the same results. This was caused by the fact that the Iterative Classifier Optimizer algorithm used the LogitBoost algorithm as an iterative classifier.

The accuracy rate did not change as a result of the iterative RuSBoosted Trees algorithm on the MATLAB software. Nevertheless, it cannot be said that the algorithm yielded the same results because the Accurate Positive rates changed.

Using the time-frequency analysis, Ning Wang et al., carried out an accelerometer-based classification of the gait patterns. To determine five different human gait patterns through the data obtained by the use of a three-axis accelerometer attached to the waist over the iliac spinal cord, 33-dimensional time-frequency field properties were developed and evaluated in the study. In the study, 52 subjects were asked to walk on a flat surface along the hallway, go up the stairs and then come down. The time-frequency properties of the acceleration data were developed in the anterior-posterior (AP), mediolateral (ML) and vertical (VT) directions. The acceleration data in each direction were decomposed into three detailed signals on different wavelet scales by using the wavelet packet transform. The Root Mean Square (RMS) values and standard deviations were calculated for the signals decomposed on scales varying from 5 to 2, which correspond to the frequency band of 0.78 – 18.75 Hz. Although, the MV acceleration did not show any significant difference between the gait patterns, the RMS value of the acceleration signal was shown to be a distinguishing property as it was in previous studies. The RMS values were only calculated in the AP and VT directions for the wavelet coefficients at levels 2 to 5, which correspond to 0.78 – 18.74 Hz [15].

ConclusIons

We believe, WT of gait signals, together with machine learning algorithms presented in this study can be used to classify hemiplegic patients into correct Brunnstrom Stages of hemiplegia with high accuracy, helping physicians to monitor and manage the rehabilitation process of their patients.

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Compliance with the Ethical Standards

Ethics Committee approval: The approval of the local ethics committee was obtained from the University of Chiba, Japan, and all subjects gave their written informed consent for the data used in the study.

Conflicts of interest: Authors declare no conflicts of interest.

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Authors contribution: HT: Undertook the tasks of processing the walking signal and feature extraction, applying machine learning techniques, and writing the article. AY: Collected and normalized the walking signal data used in the article. HU: Provided support with his clinical expert knowledge. UB: Undertook the design of the study, evaluation of the machine learning results used in the study, and consultancy of the study. All authors approved the final manuscript.

The authors do not report any conflict of interest regarding this manuscript.

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Evaluation of pulmonary artery stiffness in patients with systemic sclerosis

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ABSTRACT

Objective: The study aims to investigate the use of pulmonary artery stiffness (PAS) parameter in early diagnosis of systemic sclerosis (SSc) and pulmonary hypertension in SSc patients.

Patients and Methods: The study involved 102 SSc patients and 45 control group patients, who underwent transthoracic echocardiographic evaluations.

Results: Pulmonary artery stiffness was measured as 25.7±7.6 (Hz/msn) in the SSc cases and 13.7 ±1.6 (Hz/msn) in the healthy subjects (P< 0.001). TAPSE/sPAP ratio, which we used as an indicator of RV-PA coupling, was calculated as 0.65+0.28 in SSc cases and 1.12+0.33 in the control group (P<0.001).

When we evaluated PAS values of subgroups PAS was significantly higher in SScPH(-) patients without pulmonary hypertension than control subjects (respectively; 21.67 ± 3.9; 13.7 ± 1.6, P<0.001). The relationship of PAS with the parameters in which pulmonary hypertension and right ventricular functions were evaluated, there was a positive correlation with sPAP(r: – 0.396, P <0.001), while a negative correlation was observed with TAPSE/sPAP (r: 0.456, P<0.001).

Conclusion: We observed higher PAS values in SScPH(-) patients compared to the control group and found a positive correlation between the increase in PAS and sPAP and a negative correlation between them and TAPSE/sPAP.

Keywords: Pulmonary hypertension, Systemic sclerosis, Transthoracic Echocardiography, Pulmonary arterial stiffness, TAPSE/sPAP ratio

1. INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by multi-organ involvement. It leads to increased morbidity and mortality depending on the degree of organ involvement. Pulmonary involvement most commonly leads to pulmonary hypertension (PH) and interstitial lung disease. The incidence of pulmonary hypertension in SSc patients is between 8-12% [1]. Several mechanisms exist for the development of pulmonary hypertension. According to the latest pulmonary hypertension guideline, it may be in the group of connective tissue-related pulmonary arterial hypertension (PAH) (Group 1.4.1) or in the group of lung-associated pulmonary hypertension (Group 3) due to interstitial lung disease [2].

Patients who develop PH may present with symptoms such as asymptomatic or shortness of breath. Many scoring systems have been developed for the early detection of PH development and the most commonly used one is the DETECT algorithm. This algorithm is a simple and reliable tool for PAH detection in SSc [3]. According to this algorithm, transthoracic echocardiography stands out as the first test to be screened.

Myocardial involvement in SSc causes fibrosis and impaired microcirculatory function [4]. The worsening morbidity and mortality of SSc-PAH compared with other PH cases may be partially related to inadequate compensation of the right ventricle due to increased afterload. The inability of the right ventricle to cope with SSc-PAH leads to an inadequate link between right ventricular contractile function and increased pulmonary afterload due to PAH. This results in deterioration of the right ventricle to pulmonary artery (RV-PA) coupling.

Transthoracic echocardiography (TTE) has a crucial role in the diagnosis and screening strategy of PH [5]. Ultrasonographic evaluation of the RV and pulmonary vascular bed is very useful

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to identify detectable pathological changes in the pulmonary circulation in the early stage of the disease. As a determinant of pulmonary vascular bed functions, pulmonary arterial stiffness (PAS) can be measured echocardiographically [6].

In our study, we aimed to investigate whether we can use the PAS parameter to predict the development of PH and deterioration in RV-PA coupling early in SSc patients.

2. PATIENTS and METHODS

The study was undertaken at Marmara University, Pendik Training and Research Hospital, a tertiary center for echocardiography laboratory. A local ethical committee approval was obtained, and the study was undertaken in accordance with the declaration of Helsinki. Consecutive SSc patients who applied to our outpatient clinic between January 2017 and January 2023 were included in the study retrospectively. The SSc diagnoses of the patients were set to meet the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classifications during their follow-up in the Rheumatology Department of our hospital [7]. Exclusion criteria were hemodynamically significant valvular disease (any stenosis and/or regurgitation greater than mild in severity), coronary artery disease (documented coronary atherosclerosis, segmental wall motion abnormality, history of myocardial infarction), ischemic, dilated or hypertrophic cardiomyopathy, pulmonary embolism, evidence of intracardiac shunting or congenital heart disease and poor echocardiographic images. The control group included 45 consecutive age and gender-matched individuals who were referred for echocardiography from the Cardiology Outpatient Clinic at the discretion of their physician over the study period. Baseline clinical properties, such as age, gender, and body mass index (BMI) and laboratory findings were recorded. Skin, lung and other organ involvements of the patients were evaluated in terms of SSc involvement. Diffusing capacity of the lungs for carbon monoxide (DLCO) test was performed for respiratory functions.

TTE evaluation

In this research, TTE was performed by two experienced echocardiographers who were unaware of the patients' medical data. For all measurements an Epiq 7 system (Philips Medical Systems, Andover, MA, USA) equipped with a 3.5 MHz transducer (S5-1 probe) were applied. The American Society of Echocardiography's recommendations for conventional echocardiographic measurements were followed [8]. Modified biplane Simpson's method was used to determine left ventricular ejection fraction (LVEF) [8]. In order to record either mitral or tricuspid flow velocities, the sample volume was oriented at the tip of both valve leaflets in an apical four-chamber window by applying the pulsed-Doppler method. The sample volume was positioned in the apical four chamber window either on the mitral lateral annulus or the tricuspid lateral annulus to acquire the LV and right ventricle (RV) tissue Doppler variables using the pulsed-wave Doppler method. The RV myocardial performance index (MPI) was calculated using the following equation: tricuspid valve closure to opening time RV – ejection time (ET) /RV-ET [8]. RV mid and annular diameters were obtained at the end-diastole from the apical four-chamber window. The RV annular segment's systolic motion across the lateral free wall of the tricuspid annulus in the apical four-chamber view was seen using M-mode imaging, and this motion was used to measure the tricuspid annular plane systolic excursion (TAPSE). The diameter and collapsibility of the inferior vena cava and the tricuspid regurgitant velocity were used to calculate the systolic pulmonary artery pressure (sPAP). RV-fractional area change (FAC) is calculated that distinction between RV end-diastolic and end-systolic areas measured through ideally RV-focused apical view [8]. RV – PA coupling was calculated according to the following formula: TAPSE /sPAP and when a ratio <1.6 was obtained, it was characterized as impaired coupling [9].

Pulmonary Hypertension Definition in Echocardiographic Parameters

Regardless of the underlying etiology, PH causes right ventricular pressure overload and dysfunction, which can be detected by echocardiography. Echocardiography is also a valuable tool in identifying suspected or confirmed causes of PH. However, echocardiography alone is not sufficient to confirm the diagnosis of PH, and right heart catheterisation (RHC) is required. Given the heterogeneous nature of PH and the unique geometry of the RV, there is no single echocardiographic parameter that reliably provides insight into the condition and underlying etiology of PH. Therefore, comprehensive echocardiography when PH is suspected includes estimation of sPAP and identification of additional signs suggestive of PH, with the aim of assigning an echocardiographic grade to the probability of PH.

Estimates of sPAP are based on peak tricuspid regurgitation velocity (TRV) and TRV-derived tricuspid regurgitation pressure gradient (TRPG) after excluding pulmonary artery stenosis, and are based on the non-invasive measurement of RA pressure (RAP). Given the inaccuracy of RAP estimation and the amplification of measurement error through the use of derived variables, the key variable for assigning echocardiographic PH probability is peak TRV (and not estimated sPAP). Peak TRV at 0.2.8 m/s may indicate PH. However, TRV alone cannot reliably determine the presence or absence of PH. Therefore, additional variables related to RV morphology and function can be used to define the echocardiographic probability of PH, which can be determined as low, intermediate, or high.

Right ventricular outflow tract acceleration time (RVOT) is measured from flow onset to peak flow rate. Since the objective is to assess the time to reach peak velocity, not diffusion, it is important to first place the marker at the peak and then work back to the beginning of the flow. Results greater than 130 ms are considered normal, whereas results less than 100 ms are highly suggestive of pulmonary hypertension. The formula for calculating mean pulmonary pressure is mPAP = 90 ($0.62*AT_{RVOT}$).

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Measurement of Pulmonary Arterial Stiffness

We first found the pulmonary artery in the parasternal short-axis window. The semilunar pulmonic valve was then exposed for the Doppler recordings of pulmonary blood flow. For at least 5 subsequent beats, the pulmonary flow's maximum frequency shift (MFS) was recorded. Pulmonary flow accleretion time (PfAT) was recorded for at least five subsequent beats and is defined as the interval between the beginning of SPA flow and peak flow rate. Next, the average MFS and average PfAT were divided to yield the PAS value [10] (Figure 1).

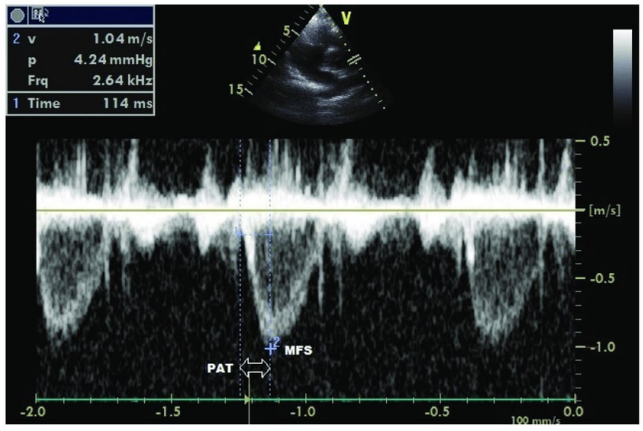

Figure 1. *An illustration of a patient showing PAS measurement. PAS indicates pulmonary artery stiffness, PfAT indicates pulmonary flow acceleration time, and MFS indicates maximum frequency shift*

Statistical Analysis

For all analyses, SPSS (version 26.0; SPSS Inc., Illinois, USA) statistical software was used. Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were expressed as numbers (percentage). The Kolmogrow-Smirnow test was used to determine if continuous variables are normally distributed. SSc and the control group were compared using independent variables by the chi-square, Fischer's exact test, Student's *t* test, Man-Whitney *U*, and Kruskal-Wallis tests, if necessary. SSc PH(+), SSc PH(-) and the control groups were compared using independent variables using ANOVA test. To assess the correlation between PAS and the right ventricular echocardiographic parameter, we applied a Pearson correlation test. For all statistics, a P value below 0.05 was considered significant.

3. RESULTS

Initially, 114 SSc patients were included in the study. Twelve patients were excluded from the study due to inadequate echocardiographic image (n:6), history of coronary artery disease (n: 2), and moderate-to-advanced valve insufficiency (n:4). According to the rheumatological examinations of SSc patients, skin involvement was found in 72 patients and lung involvement (interstitial lung disease) was found in 46 patients. Patients were classified as limited cutaneous SSc (lcSSc, n = 45) or diffuse form (dcSSc, n = 27) according to the degree of skin involvement. 32 SSc patients were classified as SSc PH (+) if they had tricuspid regurgitant velocity > 2.8 m/s along with further echocardiographic PAH signs. PH(-) was assigned to the remaining patients (70 SSc with tricuspid regurgitation velocity < 2.8 m/s and no other echocardiographic PH signs). The functional capacity of all patients was 1-2. Electrocardiographic evaluations of the patients were performed. Atrial fibrillation was detected in 8 of the patients, frequent supraventricular premature beats in 4 patients, and frequent ventricular extra beats were detected in 5 patients. In the other patients, including the control group, all were in sinus rhythm, and no specific findings of right ventricular hypertrophy or dilatation were observed. In the rheumatological evaluation of the patients, antiScl antibody was detected positive in 38 patients, while ANA was negative in 41 patients.This information was also added to the results section. All 32 PH patients were using endothelin receptor antagonists, and 11 were also taking phosphodiesterase inhibitors. Inhalers (27 individuals), methotrexate (10 patients), azathioprine (11 patients), danasumab (4 patients), hydroxychloroquine (55 patients), and mycophenolate mofetile (25 patients) were among the drugs taken by the patients. In terms of basic characteristics , no statistical difference was found between the groups which were shown in Table I. Only N-terminal pro-brain natriuretic peptide (NT-proBNP) was found to be significantly higher in the SSc group than in the control group.

Table I. *Baseline characteristics**

	Systemic Sclerosis (n=102)	Control group (n=45)	P value
Age, years	54.7±13.2	56.4±10.2	0.567
Female,n (%)	81 (79)	34 (76)	0.782
BMI, $kg.m^{-2}$	27.4±4.4	28.1±3.7	0.686
Heart rate, $beats.min^{-1}$	82.1±13.9	77.7±12.1	0.435
SBP, mmHg	120.1±17.7	124.9±12.4	0.259
Comorbidities			
Hypertension, n (%)	61 (60)	28 (62)	0.543
Diabetes, n (%)	24 (23)	10 (22)	0.897
Dyslipidemia, n (%)	19 (18)	9 (20)	0.321
Functional status			
WHO class I, n (%)	76 (75)	45 (100)	
WHO class II, n (%)	26 (25)	-	
WHO class III, n (%)	-	-	
WHO class IV, n (%)	-	-	
Laboratory parameters			
Creatinin, $mg.dL^{-1}$	0.73±0.2	0.66±.0.14	0.076
Hemoglobin, $g.dL^{-1}$	12.6±1.7	13.5±1.68	0.856
CRP, $mg.L^{-1}$	7.1±22.1	6.1±14.3	0.879
hs-cTnT, $ng.L^{-1}$	9.8±8.2	5.6±3.9	0.032
NT-proBNP, $ng.L^{-1}$	448.1±823.9	51.7±47.7	**0.008**
DLCO	74.8±23.6	-	-

**Values are mean ± standard deviation, number (percentage) .*
BMI: body mass index, CRP: C-reactive protein, DLCO: diffusing capacity of the lungs for carbon monoxide, hs-cTnT: high-sensitivity cardiac troponin T, NT-proBNP, N-terminal pro-brain natriuretic peptide, SBP: systolic blood pressure, WHO: World Health Organization.

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Echocardiographic parameter results are summarized in Table II. No significant differences were detected in LV diameters and LVEF between the groups. RA and LA areas were higher in SSc patient. Mitral E, A was higher in SSc patients, E/e' were not different between groups. RV/LV ratio was significantly shorter in control patients. RV TDI parameters were impared in SSc patients. Although, RVFAC, TAPSE, and RVs' were shorter in SSc patients, RV-MPI was similar between groups. SSc patients had significantly higher sPAP and shorter pulmonary acceleration time.

Pulmonary arterial stiffness was measured as 25.7±7.6 (Hz/msn) in the SSc cases and 13.7 ±1.6 (Hz/msn) in the healthy subjects (p < 0.001). TAPSE/sPAP ratio, which we used as an indicator of RV-PA coupling, was calculated as 0.65+0.28 in SSc cases and 1.12+0.33 in the control group (P<0.001).

Table II. *Echocardiographic parameters**

	Systemic Sclerosis (n=102)	Control group (n=45)	P value
Echocardiographic parameters			
LVEF, %	61.4 ± 4.8	60.9±2.7	0.565
LVEDd, mm	44.7±4.7	43.9±4.6	0.343
IVSd, mm	9.5±1.7	9.6±2.1	0.545
RA area, cm^2	16.1±4.3	13.3±2.2	**<0.001**
LA area ,cm^2	16.5±4.1	14.5±2.1	0.002
E_M, cm/s	0.88±0.24	0.78±0.16	0.03
A_M, cm/s	0.83±0.19	0.71±0.14	**0.001**
E/e'_M	9.2 ± 3.2	9.1 ± 3.1	0.894
RV mid, mm	38±5.6	28.9±2.29	0.007
RV annuler, mm	44.1±5.4	34.9±1.7	**<0.001**
E_T, cm/s	0.79±0.7	0.59±0.15	**0.023**
A_T, cm/s	0.74±0.89	0.47±0.09	**0.005**
RVs', cm/s	12.4±2.8	13.3±1.7	**0.027**
RV/LV	0.75±0.19	0.68±0.09	**0.024**
TR V_{max}, $m.sec^{-1}$	2.79±0.65	2.05±0.4	**<0.001**
sPAP, mmHg	38.7±17.1	21.9±6.1	**<0.001**
mPAP, mmHg	22.4±10.2	14.3±3.5	**0.002**
RV MPI	0.33±0.04	0.31±0.06	0.124
RV FAC, (%)	46.9±17.9	67.8±16.4	**<0.001**
PAd, mm	19.6±5.1	19.2±2.2	0.734
PfAT, msn	110.6±18.5	155.1±14.9	**<0.001**
MFS, Hz	2730±424	2122±172	**<0.001**
PAS, Hz. msn^{-1}	25.7±7.6	13.7±1.6	**<0.001**
TAPSE, mm	20.1±3.6	24.5±2.7	**<0.001**
TAPSE/sPAP, $mm.mmHg^{-1}$	0.65±0.28	1.12±0.33	**<0.001**
IVC, mm	16.8±5.3	12.7±3.2	**0.002**

** Values are mean ± standard deviation or number (percentage).*
AM: mitral flow A-wave, AT: tricuspid flow A-wave, EM: mitral flow E-wave, ET: tricuspid flow E-wave, e': tissue Doppler e' wave, IVC: inferior vena cava diameter, FAC: fractional area change, IVS: interventricular septum thickness, LA: left atrium, LVEDD: left ventricle end-diastolic diameter, LV: left ventricle, LVEF: left ventricle ejection fraction, MFS: maximum frequency shift, Pad: pulmonary artery diameter, PAS: pulmonary arterial stiffness, PfAT: pulmonary flow acceleration time, RV annular: right ventricle annular diameter, RV mid: right ventricle midregion diameter, RV MPI: right ventricle myocardial performance index, RV s': tricuspid anulus systolic velocity, mPAP: mean pulmonary arterial pressure, sPAP: systolic pulmonary arterial pressure, TR Vmax: tricuspid regurjitation maximal velocity, TAPSE: tricuspid annular plane systolic excursion.

When we evaluated PAS values of subgroups (SSc patients with and without pulmonary hypertension and control subjects), PAS was significantly higher in SSc patients with pulmonary hypertension than SSc patients without pulmonary hypertension (respectively; n = 32, mean = 34.6 ± 5.9; n = 37, mean = 21.67 ± 3.9; P =<0.001). Moreover PAS was significantly higher in SScPH (-) patients without pulmonary hypertension than control subjects (respectively; n = 70, mean = 21.67 ± 3.9; n = 45, mean 13.7 ± 1.6, P<0.001).

In the analysis performed by separating SSc patients according to whether they have pulmonary hypertension or not, the sPAP, PAS, RV-MPI values were significantly higher in the group with pulmonary hypertension, while RV-FAC, PfAT and TAPSE/sPAP ratios were significantly lower (Table III).

Table III. *Comparison of conventional echocardiography variables of the patients with systemic sclerosis according to the presence or absence of pulmonary arterial hypertension*

	Systemic Sclerosis PH (-) (n=70)	Systemic Sclerosis PH(+) (n=32)	Control group (n=45)	p value
Echocardiographic parameters				
PfAT, msn	118.2±14.3	92.7±13.2	155.6±14.9	**<0.001**
MFS, Hz	2541±329	3143±296	2122±172	**<0.001**
PAS, Hz/msn	21.6±3.9	34.6±5.9	13.7±1.6	**<0.001**
TR V_{max}, $m.sec^{-1}$	2.42±0.36	3.48±0.6	2.05±0.4	**<0.001**
mPAB, mmHg	19.9±4.8	31.2±10.6	15.4±3.7	**0.009**
RV MPI	0.26±0.99	0.42±0.13	2.6±9.9	**<0.001**
RVFAC, %	50.5±17.4	38.6±15.8	67.8±16.4	**<0.001**
PAd, mm	3.85 ± 0.8		4.23 ± 0.69	**0.001**
TAPSE, mm	21.9±2.6	16.7±2.9	24.5±7.7	**<0.001**
TAPSE/sPAP, $mm.mmHg^{-1}$	0.82±0.26	0.38±0.14	1.12±0.33	**<0.001**
E_T, cm/s	0.86±1.2	0.97±1.3	0.59±0.15	0.536
A_T, cm/s	0.72±1.03	1.04±1.5	0.47±0.09	0.186
RVe', cm/s	11.9±2.6	10.2±2.6	11.8±3.3	**0.014**
RVa', cm/s	12.5±3.8	11.1±2.8	16.1±3.8	**<0.001**
RVs', cm/s	12.8±2.7	11.4±2.9	13.7±1.7	**0.003**
IVC ,mm	15.6±4.6	19.8±5.5	12.7±3.2	**0.032**

** Values are mean ± standard deviation.*
AT: tricuspid flow A-wave, a': tissue Doppler a', ET: tricuspid flow E-wave, e': tissue Doppler e' wave, IVC: inferior vena cava diameter, FAC: fractional area change, MFS: maximum frequency shift, PAd: pulmonary artery diameter, PAS: pulmonary arterial stiffness, PfAT: pulmonary flow acceleration time, RV MPI: right ventricle myocardial performance index, RV s': tricuspid anulus systolic velocity, Mpap: mean pulmonary arterial pressure, TR Vmax: tricuspid regurjitation maximal velocity, TAPSE: tricuspid annular plane systolic excursion.

When the patients were separated according to whether the RV-PA coupling was impaired or not, the sPAP, PAS and RV-MPI values were significantly higher in the impaired group, while RV-FAC, PfAT and TAPSE/sPAP ratios were significantly lower (Table IV).

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Table IV. *Comparison of conventional echocardiography variables of the patients with systemic sclerosis according to the presence or absence of RV-PA coupling*

	RV-PA coupling(-) (n=18)	RV-PA coupling(+) (n =129)	p value
Echocardiographic parameters			
PfAT, msn	121.6±27.1	143.4±16.6	**<0.001**
MFS, Hz	2215±275	2590±463	**<0.001**
PAS, Hz/msn	22.9±8.5	15.7±3.4	**<0.001**
TR V_{max}, $m.sec^{-1}$	2.64±0.66	1.82±0.12	**<0.001**
mPAB, mmHg	23.5±9.9	12.2±1.1	**<0.001**
RV MPI	0.37±0.12	0.32±0.11	**0.041**
RVFAC,%	48.4±19.1	62.5±14.8	**0.011**
PAd, mm	19.5±4.5	18.9±1.8	0.64
TAPSE, mm	21.1±3.8	25.2±2.6	**<0.001**
E_T, cm/s	0.76±0.74	1.5±2.9	**0.021**
A_T, cm/s	0.71±0.84	1.24±2.4	0.114
RVe', cm/s	11.5±2.9	11.1±1.4	0.654
RVa', cm/s	12.5±3.8	14.4±4.5	0.113
RVs', cm/s	12.7±2.7	12.5±2.1	0.826
İVC ,mm	14.2±3.8	9.5±5.6	**0.049**

** Values are mean ± standard deviation*
AT: tricuspid flow A-wave, a': tissue Doppler a', ET: tricuspid flow E-wave, e': tissue Doppler e' wave, IVC: inferior vena cava diameter, FAC: fractional area change, MFS: maximum frequency shift, Pad: pulmonary artery diameter, PAS: pulmonary arterial stiffness, PfAT: pulmonary flow acceleration time, RV MPI: right ventricle myocardial performance index, RV s': tricuspid anulus systolic velocity, mPAP: mean pulmonary arterial pressure, TR Vmax: tricuspid regurjitation maximal velocity, TAPSE: tricuspid annular plane systolic excursion.

When the relationship of PAS with the parameters in which pulmonary hypertension and right ventricular functions were evaluated, there was a positive correlation with sPAP(r:-0.396, P<0.001), while a negative correlation was observed with TAPSE/sPAP (r: 0.456, P<0.001) (Figure 2).

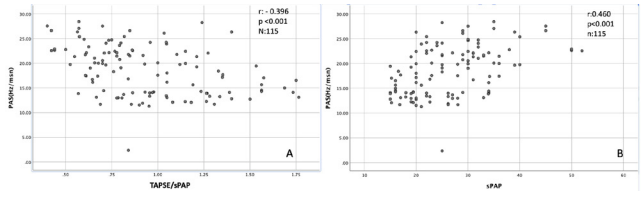

Figure 2. A: *Correlation analysis between PAS and TAPSE/sPAP.* ***B****: Correlation analysis between PAS and sPAP. PAS indicates pulmonary artery stiffness, TAPSE indicates tricuspid annular plane systolic excursion, and sPAP indicates systolic pulmonary pressure*

4. DISCUSSION

To our knowledge, this is the first study indicating higher PAS values in SSc patients. In the present investigation, we observed that SSc patients without PH had higher PAS values than healthy people, which may indicate an early shift in the pulmonary vascular bed among these instances.

The development of pulmonary hypertension has been shown to be the highest risk factor associated with the survival of SSc patients. In a study evaluated for the first time by Koh et al., the 1-year survival rate of PH associated with untreated SSc was approximately 50%; this rate is >90% in patients with lung involvement without PH or without any significant organ involvement [11]. In addition to direct pulmonary artery involvement, right ventricular failure owing to myocardial fibrosis plays a role in the development of pulmonary hypertension. TTEs from PH patients indicate reduced right ventricular systolic and diastolic functioning [12]. In our study, 32 SSc patients had pulmonary hypertension, and we discovered a substantial increase in sPAP values and a significant drop in RV-MPI, RV-FAC, TAPSE, and RVs values. Although, this finding is generally accepted, it is known that deterioration in right ventricular functions begins in the early period in patients who are not found to have PH with conventional echocardiographic parameters. In a study by Demirci et al., SSc patients had lower RV longitudinal strain (RV-LS) and higher right ventriculer dyssyncrony (RV-Dys) than controls, although, there was no significant difference in conventional echocardiographic variables related to RV function [13]. In our study, no significant difference was found in conventional echocardiography parameters in SSc patients who did not develop PH compared to the control group, except for PAS.

The pulmonary circulation is a low pressure, low resistance, high distensibility system. The majority of the total vascular bed compliance is located in the proximal arterial branches. Therefore, the predictive value of pulmonary vascular bed compliance in chronic PAH is also related to increased stiffness of the proximal pulmonary artery. PAH is a disease of the small distal pulmonary arteries characterized by vasoconstriction resulting in increased pulmonary vascular resistance (PVR) and, as a result, increased pulmonary artery pressure. Increased pressure causes dilation and stiffness of the proximal pulmonary artery and can also cause remodeling of the vessel wall, which can subsequently influence stiffness. Therefore, both pressure and changes in the vessel wall contribute to proximal pulmonary artery stiffness. Therefore, PAS evaluation may be important in the early diagnosis of PH. PAS is a Doppler echocardiographic parameter used to evaluate pulmonary artery stiffness [10]. Although, right heart catheterization is the gold standard method for evaluating vascular stiffness and elasticity invasively, it is also possible to evaluate it with non-invasive methods such as TTE, CT and MRI due to the difficulty of clinical application [14,15]. TTE is preferred among non-invasive methods because of its easy accessibility, no need for radiation exposure and low cost. Several investigations have verified the clinical significance of PAS. Early detection of increased PAS evaluated in Behçet's disease patients by Yasar et al. [16] , obstructive sleep apnea syndrome (OSAS) patients by Ozkececi et al. [17], asthma patients by Baysal et al [18], polycystic ovary syndrome patients by Abacioglu et al. [19], cirrhosis patients by Oz et al. [20], heart failure patients by Yenercag et al. [21], systemic lupus eritematosus (SLE) patients by Duman et al [22], and in human immunodeficiency virus-infected patients by Cerik et al. [23]. In

our study, PAS was found to be associated with right ventricular dysfunction. While a positive correlation was observed between increased sPAP and PAS, a negative correlation was detected between decreased PAS in patients with impaired RV-PA coupling. This finding was demonstrated for the first time in SSc patients without PH.

The RV-PA coupling describes the RV's adaptation to afterload. Progressive pulmonary vascular remodelling in PH causes an increase in pulmonary vascular resistance and pulmonary artery pressure, an extra strain on the contracting RV, and a change in RV-PA coupling [24]. TAPSE/sPAP ratio is a confirmed non-invasive assessment of RV-PA coupling that can be acquired simply during routine Doppler echocardiography. The TAPSE/sPAP ratio has been included to the list of additional echocardiographic indications suggestive of PH in the updated 2022 pulmonary hypertension (PH) recommendations. In a study by Colalilla et al., TAPSE/sPAP ratio < 0.55 mm/mmHg was a predictive risk factor for PH, and TAPSE/sPAP ratio ≤ 0.32 mm/mmHg was found to be a high predictor for all-cause mortality [25]. In our study, significant deterioration was observed in echocardiographic parameters evaluating right ventricular functions, including PAS, in patients with impaired RV-PA coupling. A significant relationship was also detected between the increase in PAS and the impairment in RV-PA coupling.

Limitation

Our study has several limitations. First of all, our study was planned retrospectively, we do not have information about the clinical course of the patients, we do not have information about whether there is a response to PH treatments. Any quantitative method was used when evaluating the functional capacity of the patients.Our data on effort capacity was made by NHYA classification according to the answers given by the patients to questions about their daily activities. The 6-minute walk test could not be performed on the patients. If the gold standard method, RHC, was used instead of TTE, the diagnosis and follow-up of patients with PH would be more reliable. In addition, PH could be induced by exercise. It has been reported in the literature that the presence of PH can be investigated by exercise echocardiography in SSc patients and may contribute to the diagnosis [26]. In addition to this, it is a weakness that no studies using the more reliable speckle tracking echocardiography method, which can assess right ventricular systolic functions, have been done.

Conclusion

In our study, we observed higher PAS values in SScPH (-) patients compared to the control group and found a positive correlation between the increase in PAS and sPAP and a negative correlation between them and TAPSE/SPAP. In light of these findings, we recommend that PAS measurement, in addition to conventional methods, can be used in the early diagnosis of PH and evaluation of right ventricular dysfunction in the echocardiographic evaluation of SSc patients. Since, TTE can be used easily clinically, we think that a prospective controlled study in larger populations is needed to evaluate its long-term clinical benefits.

Compliance with the Ethical Standards

Ethics Committee approval: The present study complies with the principles outlined in the Declaration of Helsinki. The study was approved by the Marmara University School of Medicine Research Ethics Committee. (approval number: 09.2017.613), and written informed consent was obtained from all participants.

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Conflict of interest: The authors have no potential conflicts of interest to disclose.

Authors contributions: Both authors contributed to the study conception and design.DA was the primary investigator of the study, DA: Design of the study, data collection, analysis of the data and drafting of the article, MD: Analysis and collecting of the data and drafting of the article. Both authors read and approved the final version of the article.

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Diagnostic performance between RT-PCR and chest CT in outpatients with clinically suspected COVID-19

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ABSTRACT

Objective: To investigate the diagnostic performance between chest computed tomography (CT) and reverse transcription-polymerase chain reaction (RT-PCR) in outpatients with suspected coronavirus disease 2019 (COVID-19).

Patients and Methods: Between March and June 2020, a total of 812 patients with clinically suspected COVID-19 who underwent both chest CT and initial-single RT-PCR on admission to outpatient units were retrospectively enrolled. CT severity-score (CT-SS) was calculated and data were matched with PCR results.

Results: Of 812 patients, 54% (439/812) had positive RT-PCR results, and 47% (425/812) had a positive chest CT scan. With RT-PCR results as reference, the sensitivity, specificity, accuracy of chest CT in defining COVID-19 infection were 60%, (95% CI 56-65%, 265/439 patients), 57% (95% CI 52-62%, 213/373), 59% (95% CI 55-62%, 478/812), respectively. Three hundred eighty-seven (47%) patients had no CT findings, 380/812 (46.8%) had mild, 45/812 (5.5%) had moderate, and no patients in the severe group

Conclusion: Chest CT did not show high sensitivity for the diagnosis of COVID-19 for outpatients. We suggest RT-PCR should be the primary diagnostic tool. Chest CT might be considered if there is a strong clinical suspicion with repeatedly negative RT-PCR test results, ensuring infection prevention and control measures can be preserved.

Keywords: Chest CT, RT-PCR, COVID-19, Outpatient, Diagnosis

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first reported in China [1] and declared a pandemic on February 28, 2020. The best strategy for management of the pandemic had been a timely diagnosis of COVID-19 until a vaccine would be available for the population. The World Health Organization (WHO) has emphasized sampling for polymerase chain reaction (PCR) as a part of the assessment of suspected COVID-19 cases in primary care [2]. According to the guideline published by the Republic of Turkey Ministry of Health, all symptomatic patients and asymptomatic contacts are required to admit to hospitals for COVID-19 diagnostic testing. They do not have to apply to primary health care as the diagnostic tests are not available in the Family Health Centers in Turkey. However, outpatient COVID-19 patients are followed by their Family Physicians after the diagnosis.

Real-time-polymerase chain reaction (RT-PCR) is the reference standard test in COVID-19 diagnosis. However, RT-PCR positivity has changed between 0-60% at admission to the hospital [3]. False-negative RT-PCR results may be confusing. The major reason for a false-negative RT-PCR result is improper sampling. The low sensitivity of RT-PCR may result in a failure to diagnose on time, which may cause the transmission of the virus to a larger population. Therefore, alternative diagnostic tools have been researched. Chest CT examination seems to be an alternative diagnostic tool in patients, especially with false-negative RT-PCR results according to many studies [4-6]. Reported CT imaging findings include a bilateral distribution of ground-glass opacities (GGO), crazy paving patterns, reversed halo signs, and airway changes [7-9]. Yet, some small scaled studies have also documented limited RT-PCR sensitivity

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despite having pulmonary abnormalities correlated with COVID-19 [4,5]. The discorrelations between chest CT and RT-PCR to diagnose COVID-19 infection have been reported in other studies as well [4-7]. Guan et al., did not document CT abnormalities in 2.9% of patients with severe and 17.9% of patients with non-severe disease at the time of admission [10]. Waller et al., reviewed that chest CT had limited clinical utility, especially in patients without symptoms at the beginning of the disease [11]. In this study, we investigated the diagnostic performance of RT-PCR tests and chest CT images of patients with suspected COVID-19 outpatients at the time of admission.

2. PATIENTS and METHOD

The study protocol was approved by the Institutional Review Board and the Clinical Research Ethics Committee of Marmara University School of Medicine (approval number: 09.2020.578).

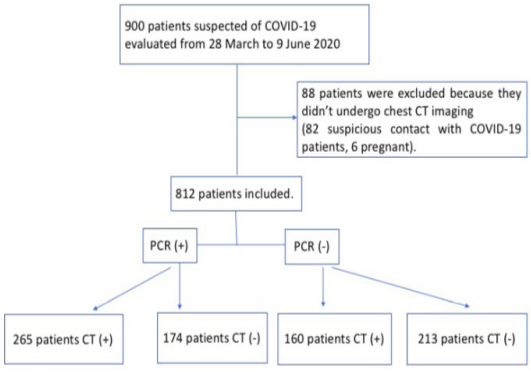

Figure 1. *The flowchart of the study*

Study population

At the beginning of the novel coronavirus pandemic, the Turkish Ministry of Health made an algorithm to take care of patients to offer quality healthcare services to the public in our country. From March 28 to June 9, 2020, a total of 900 outpatients suspected of COVID-19 disease were retrospectively recorded (Figure 1). Patients with mild symptoms of COVID-19, according to the WHO scale <4 who did not need oxygen support [11] were organized as outpatients. After the exclusion of 88 patients (82 patients had suspicious contact with COVID-19-infected patients, and 6 women were pregnant) due to lack of chest CT imaging, the remaining 812 patients who underwent both chest CT imaging and RT-PCR assay were included in the study. Chest CT and RT-PCR tests were done on the same day.

Viral RNA was taken out by using Bio-speedy1 (Bioexen LTD, Turkey)

Chest CT protocol and image analysis

Chest CT imaging was examined on a picture archiving and communication system (PACS) independently by two radiologists who were blinded to RT-PCR results of the patients. Their data were then cross-matched and if there were any conflicts, the images were reevaluated, and an agreement was provided to resolve the inconsistencies. CT-severity score (CT-SS) was used for evaluating infection burden at admission to the hospital [12]. CT-SS was counted up by enumerating the infected lobe. Lung lobes were scored ranging between 0-4 and, categorized as none, minimal, moderate, or severe, (0%), (1-25%), (26-50%), (51-75%), (76-100%) respectively. CT-SS was calculated by summing these affected lung lobes and ranged from 0-20. According to this count, CT-SS score analysis was grouped into four levels none (0), mild (1-5), moderate (6-10), and severe (11-20) [7,13,14].

Statistical Analysis

Statistical Package for Social Sciences (SPSS) software model 22.0 was performed for statistical analyses. Categorical variables were shown as percentages/counts while continuous variables were shown as mean +/-standard deviation. The RT-PCR was evaluated as a reference test to compare the diagnostic performance of chest CT. The CT-SS categories were analyzed to predict the presence of PCR positivity according to Receiver Operating Characteristics (ROC) curve analysis. The 5% type-1 error limit was utilized to approve the test variables' statistically significant predictive value. P<0.05 was considered statistically significant.

Table I. *The yield of chest CT for COVID-19 infection with RT-PCR results as reference*

	Results (n)				Test performance (%)				
	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	Accuracy
Overall	265	213	160	174	60% (265/439)	57% (213/373)	62% (265/425)	55% (213/387)	59% (478/812)
Age									
>50 years	54	21	35	28	65% (54/82)	37% (21/56)	61% (54/89)	43% (21/49)	54% (75/138)
<50 years	211	192	125	146	59% (211/357)	60% (192/317)	62% (211/336)	56% (192/338)	59% (403/674)
Sex									
Male	145	115	95	85	63% (145/230)	55% (115/210)	60% (145/240)	57% (115/200)	59% (260/440)
Female	120	98	65	89	57% (120/209)	60% (98/163)	69% (120/185)	52% (98/187)	58% (218/371)

TP: true positive, TN. true negative, FP: false positive, FN: false negative, PPV: positive predictive value, NPV: negative predictive value, RT-PCR: reverse transcription polymerase chain reaction

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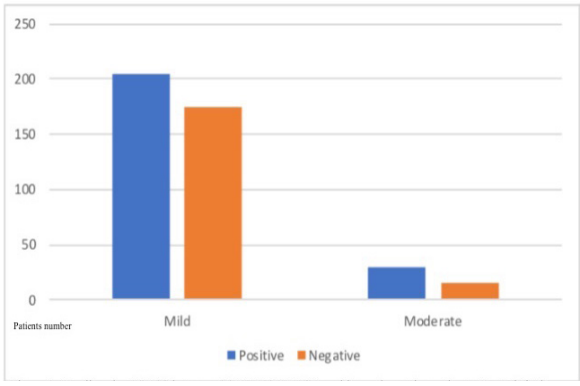

Figure 2. *Baseline chest CT-SS between COVID-19 RT-PCR positive and negative patients. No statistical difference (P:0.75).*

3. RESULTS

Nine hundred outpatients' (mean age 37.8; male 53.9%) data were evaluated at the beginning of the study. Eighty-eight patients were excluded due to a lack of chest CT scans. Eight hundred and twelve patients (mean age, 38; male 54%) were included in the analysis. Figure 1 demonstrates the flowchart of the study. The clinical symptoms were cough (68%), dyspnea (44%), myalgia (14%), and sore throat (13%). One hundred and forty-eight patients had comorbidity; such as 67 patients (7.4%) had hypertension, while 65 patients (7.2%) had asthma and bronchitis and 38 patients (4.2%) had diabetes.

Two hundred and sixty-five patients (mean age: 39,9±12,1 years; 112 males) had positive RT-PCR and positive chest CT while 213 patients (mean age: 38,5±11,9 years, 103 males) had negative RT-PCR and negative chest CT. On the other hand, 174 patients (mean age: 36.8±12.4 years; 85 male) had positive RT-PCR without a pathological finding at the chest CT and 160 patients (mean age: 39.6±11.8 years; 95 males) had suspicious findings for COVID-19 with negative RT-PCR assays.

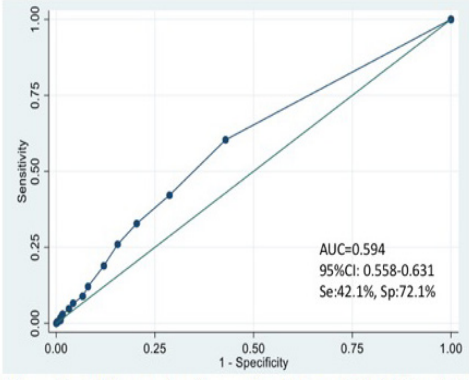

Figure 3. *ROC test for the diagnostic utility of CT-SS in COVID-19. ROC analysis showed the area under the curve (AUC) of CT-SS for diagnosing COVID-19 was 0.594 (95%CI 0.553–0.631). The CT-SS cutoff of 2 had 42.1% sensitivity and 72.1% specificity*

The accuracy, sensitivity, and specificity of chest CT in identifying COVID-19 infection diagnosed by RT-PCR results were 59% (95% CI 55-62%, 478/812), 60%, (95% CI 56-65%, 265/439), 57% (95% CI 52-62%, 213/373) retrospectively. The chest CT performances to diagnose COVID-19 were also assessed according to age and gender (Table I). No significant difference was detected.

Three hundred eighty-seven (47.7%) patients had no pathological CT findings while 425 patients had positive chest CT. CT-SS is categorized into three-part; mild, moderate, and severe. With reference to this score, most of the patients cumulated in the mild group 380/812 (46.8%) while 45/812 patients (5.5%) were in the moderate group, and no patients were in the severe group. According to the CT-SS, the positive RT-PCR rates were 54% in the mild group and 64% in the moderate groups (P=0.75) (Figure 2).

ROC analysis was performed to explore the diagnostic utility of CT-SS to predict RT-PCR positivity (Figure 3). ROC analysis revealed that a cut-off of 2 CT-SS predicted a positive RT-PCR with a sensitivity of 42.1% and a specificity of 71.3% (area under the ROC curve (AUC) was 0.594 (95%CI 0.558–0.631).

4. DISCUSSION

In this study, we investigated whether there was a correlation between RT-PCR results and chest CT images in COVID-19 suspected outpatients and we detected a low sensitivity for chest CT.

Strategies for COVID-19 encircle and management heavily depend on disease diagnosis. RT-PCR test has many limitations such as sampling error, staff experience, quality differences of kits, the detection sensitivity of COVID-19, changing test protocols between countries, and long waiting time to get results. Serial RT-PCR tests should be done to deal with these problems, but it prolongs the diagnostic time. Chest CT imaging is faster and easier than RT-PCR to diagnose COVID-19. Thin-section chest CT imaging is more precise in demonstrating pathological findings even in the early stages of COVID-19 [15,16]. According to two diagnostic accuracy studies, the sensitivity of the RT-PCR test varies between 50% to 83% [17,18]. In a study, 100% of symptomatic COVID-19-infected patients had positive RT-PCR, while 56% of them had no signs of chest CT at the beginning of symptoms [9]. A review evaluating 641 studies has shown that chest CT has limited utility in patients with early disease and those who show no symptoms [19]. Investigators have claimed that chest CT has low sensitivity and specificity and may be used as a supportive tool to diagnose COVID-19, especially for symptomatic patients. Pan et al., showed that chest CT demonstrates high specificity but low sensitivity, in the initial period [20]. Similar to other studies, we found positive lung CT findings in 60% of 439 patients with positive RT-PCR results.

Li et al., showed that among 78 cases, 31% had positive RT-PCR, despite a normal chest CT [13]. The use of lung CT alone as a diagnostic tool in cases with suspected COVID-19 may result in missing positive cases and may also pose a potential infection risk transmission. Still, RT-PCR is a better tool for diagnosing

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COVID-19 infection, while chest CT may be used to support the diagnosis.

In many cases, despite the first RT-PCR test being negative, it has been reported that subsequent tests develop positivity [21]. Repeated RT-PCR tests can be performed to avoid these limitations, but this may prolong the diagnostic process.

In a study by Ai et al., when the RT-PCR test was taken as the reference test, the accuracy, sensitivity, and specificity of lung CT were found to be 68%, 97%, and 25%, respectively [22]. We found chest CT specificity, sensitivity, and accuracy at 57%, 60%, and 59%, respectively. In our study, the sensitivity of chest CT was not high compared to this study.

Our study has certain limitations. First of all, we only analyzed outpatients who were relatively younger with less comorbidities compared to inpatients. This may be a reason of lower CT-SS in our study. Secondly, we did not perform the COVID-19 antibody test in patients with significant findings on lung CT, although, the RT-PCR test was negative. Therefore, we could not check false RT-PCR negativities by testing for COVID-19 antibodies in the blood. Despite this, we tried to emphasize the importance of RT-PCR and lung CT in controlling the COVID-19 pandemic in our study.

Conclusion

We have explored the diagnostic performance between RT-PCR and chest CT in outpatients with clinically suspected COVID-19 infection. We have detected that chest CT does not show high sensitivity for the diagnosis of COVID-19 for outpatients. RT-PCR seems as a first-choice diagnostic tool to be used. In case of high clinical suspicion, despite repeated negative RT-PCR results lung CT may be appropriate. In addition, the over-screening of patients with suspected COVID-19 may also put an unnecessary financial burden on the health system.

In conclusion, our study supports the use of RT-PCR as a first-line test due to the low sensitivity of lung CT in the diagnosis of COVID-19. We recommend RT-PCR as the first choice in the diagnosis of COVID-19 and that chest CT should be used in clinically highly suspicious cases where RT-PCR is negative. It will be important to prioritize RT-PCR testing in primary healthcare centers and outpatient clinics because these centers play an important role in the COVID-19 pandemic.

Compliance with the Ethical Standards

Ethics Committee approval: The study protocol was approved by the Institutional Review Board and the Clinical Research Ethics Committee of Marmara University School of Medicine (approval number: 09.2020.578).

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Conflict of interest: The authors report that they have no conflict of interest.

Authors' contributions: ETT and BB: Study conception and design, ETT, BB, CC and CAK: Data collection, ETT, HPG and VK: Analysis and interpretation of results, ETT, BB, CC and CAK: Draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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Original Article

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Acute ECG changes and post-COVID arrhythmia incidence in patients with acute COVID-19 infection

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ABSTRACT

Objective: During the COVID-19 pandemic, many patients have experienced cardiovascular complications, including a variety of arrhythmias. The aim of our study was to evaluate the acute electrocardiography (ECG) changes and post-COVID arrhythmia incidence in patients with acute COVID-19 infection.

Patients and Methods: One hundred hospitalized COVID-19 patients were consecutively included. Patients were divided into two groups according to their troponin levels. Thirty subjects were included as controls. All patients underwent daily 12-lead ECG during hospitalization and were followed up for at least 12 months, by performing ECG and ambulatory ECG monitoring and questioning their symptoms at 3-month intervals.

Results: Thirty-one patients had elevated high sensitive cardiac troponin I (hs-cTnI). These patients had significantly longer QT dispersion compared to COVID-19 patients with normal troponin levels and controls. Regardless of troponin elevation, COVID-19 patients had significantly longer Tp-e intervals and P wave (PW) durations compared to controls. During the follow-up period; palpitation, beta-blocker usage, and inappropriate sinus tachycardia were more common in the COVID-19 group with hs-cTnI than control group.

Conclusion: COVID-19 causes prolongation in PW durations, Tp-e intervals, and QT dispersion during acute infection, which may lead to arrhythmias in these patients. The higher incidence of inappropriate sinus tachycardia in COVID-19 patients with elevated troponin levels may be a sign of myocardial involvement.

Keywords: Post-COVID, Arrhythmia, Electrocardiography, Troponin

1. INTRODUCTION

Since the beginning of the coronavirus disease 2019 (COVID-19) pandemic, cardiovascular complications have emerged as the predominant extrapulmonary manifestations of COVID-19 infection. Cardiac involvement associated with acute COVID-19 manifests in various patterns, from asymptomatic elevations in cardiac biomarkers to serious conditions such as cardiogenic shock and sudden cardiac death [1,2].

The arrhythmia that develops in acute COVID-19 infection has a broad spectrum. Although, the exact cause of these arrhythmias has not been fully elucidated, direct cell damage by the virus, cardiomyocyte damage due to hyperactivation of the immune system, and direct arrhythmogenic effect of inflammatory cytokines are some of the underlying mechanisms [3]. On the other hand, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus uses the angiotensin converting enzyme-2 (ACE-2) receptor for entry into cells and down-regulates ACE-2 expression [4]. Downregulation of ACE-2, which converts the proinflammatory and prooxidant angiotensin II to angiotensin 1-7, creates a proinflammatory environment that can lead to arrhythmias [5].

Cardiac arrhythmias, usually presenting with palpitations, may endure for several months following acute COVID-19 infection [6]. The range of COVID-19-associated arrhythmias is diverse and likely due to different underlying pathomechanisms. Although, the relationship between COVID-19 and arrhythmia has been demonstrated in the long term after COVID-19, the underlying mechanism has still not been fully explained. Cardiac manifestations are frequently seen in post-COVID syndrome and a new entity has been named as post-COVID

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tachycardia syndrome [7,8]. However, their relationship with cardiac manifestations in acute infection is not clear.

The aim of this study was to evaluate acute electrocardiography (ECG) changes and post-COVID arrhythmia incidence in patients with acute COVID-19 infection.

2. PATIENTS and METHODS

Study population

One hundred consecutive hospitalized COVID-19 patients between March 24, 2020 and May 4, 2020 constituted our study population. And these patients were followed for 12 months. Thirty subjects with no symptoms or signs of active COVID-19 infection were included as a control group. Patients with reduced ejection fraction, left bundle branch blocks were excluded from the study. The usage of beta-blockers, calcium antagonists, or any other antiarrhythmic drugs was also an exclusion criterion of our study. The troponin levels of the patients were noted and patients were divided into two groups according to their troponin levels.

Electrocardiographic Measurements

The standard 12-lead ECG of all patients was obtained using a recorder (Schiller AT-2 plus, Switzerland) set at a 25 mm/sec paper speed and 1 mV/cm standardization. EPCalipers program (Version 2.4 (40) for Windows) was used for online analysis of scanned ECGs. ECGs of the patients were taken before starting the drug treatment. A single physician who was blinded to patient characteristics measured ECG parameters. Basal measurements of all patients such as PR intervals, QRS intervals, and RR intervals were recorded. A mean value of three readings was calculated for each lead and included in the analysis. Only recordings with more than eight leads that can be analyzed were included.

PW dispersion measurements: P wave (PW) duration was measured as between the initial and final deflection of the PW that crossed the isoelectric line. The maximum PW (Pmax.) duration was accepted as the longest PW, that is the longest atrial conduction time, while the minimum PW (Pmin.) duration was accepted as the shortest PW, which is the shortest atrial conduction time. PW dispersion was defined as the difference between Pmax. and Pmin.

QT and QT dispersion measurements: The QT interval was measured from the onset of the QRS complex to the end of the T wave. The end of the T wave was defined as the intersection of the T wave downslope with the isoelectric line when not followed by the U wave. If a U wave was present, this was not included in the measurement of the interval. Corrected QTd (QTcd) was defined as the difference between maximum QT (QT max.) and minimum QT (QT min.) interval and corrected for heart rate with Bazett's (QTc = QT$\sqrt{RR}$) [9], and Framingham formula (QTcFra=QT+0.154 (1−RR)) [10].

The Tp–e interval measurements: The Tp-e interval was measured from the peak of the T wave to the end of the T wave. Measurements of the Tp–e interval were performed by using the standard tangential method from V_5 preferentially [11]. If the T waves were isoelectric or of very low amplitude at V_5, V_2 derivation was used instead. The Tp–e/QT ratio was calculated from these measurements.

Arrhythmia assessment: The ECG was recorded using a Philips Digitrak XT Holter recorder (Philips Healthcare, Andover, Massachusetts) for 24 hours in three channels. During the 12-month follow-up, records were repeated every three months.

This study was approved by the Ethics Committee of the University of Health Sciences, Umraniye Research and Training Hospital (protocol code: 107 and date of approval 4 April 2020). The investigation conformed to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

Statistical Analysis

All statistical tests were performed by a statistical analysis program (SPSS 21.0 for Windows, Chicago, IL). The distribution of data was tested using a one-sample Kolmogorov–Smirnov test. Categorical variables were defined as a percentage, and comparisons were made using the Chi-square test. Continuous data were expressed as mean ± standard deviation and Student's *t* test or ANOVA were used to compare the normally distributed continuous variables while the Mann-Whitney *U* test or Kruskal-Wallis test were used to compare the nonparametric continuous variables. Post hoc analyses were performed using Bonferroni test when an overall statistical significance was determined. A significance level was set at P<0.05.

Table I. *The general characteristics and laboratory parameters of the patients according to high sensitive cardiac troponin I level*

	COVID-19 patients with elevated troponin (n= 31)	COVID-19 patients with normal troponin (n= 69)	Controls (n= 30)	P value
Age (years)	60.9 ± 16.7	53.4 ± 16.3	55.6 ± 17.0	0.118
Male sex (n –%)	20 (64.5%)	39 (56.5)	16 (53.3)	0.649
Hypertension (n–%)	13 (41.9)	18 (26.1)	12(40)	0.195
Diabetes mellitus (n–%)	8 (25.8)	13 (18.8)	6 (20)	0.724
Coronary heart disease (n –%)	9 (29)	15 (21.7)	8 (26.7)	0.704
Heart failure (n –%)	4 (12.9)	3 (4.3)	5 (16.7)	0.108
COPD (n –%)	6 (19.4)	5 (7.2)	4 (13.3)	0.202
hs-cTnI (ng/mL)	0.061 ± 0.049 *,+	0.007 ± 0.018	0.012 ± 0.025	**<0.001**
hs-CRP (mg/L)	13.8 ± 8.7 *,+	7.2 ± 7.8 *	0.5 ± 0.9	**<0.001**
Lymphocyte (/µl)	1465 ± 1022 *	1553 ± 778 *	2463 ± 886	**<0.001**
D-dimer (µg/l)	1467 ± 1699 *,+	890 ± 873*	260 ± 188	**<0.001**

COPD: Chronic obstructive pulmonary disease, hs-cTnI: high sensitive cardiac troponin , hs-CRP: High sensitive C reactive protein, ***PostHoc analysis****: * denotes statistical significance versus control group, + denotes statistical significance versus patients with normal troponin levels*

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3. RESULTS

One hundred patients (mean age: 55 ±16 years, 59 male) with acute COVID-19 infection were consecutively included in the study. Thirty-one patients (31%) had elevated cardiac troponin. The general characteristics and laboratory parameters of the patients according to troponin levels are shown in Table I. Although, there were not any significant differences in the general characteristics of the patients, the COVID-19 patients had significantly higher D-dimer and hs-CRP levels, and lower lymphocyte counts compared to controls, while those patients with high hs-cTnI had also significantly higher D-dimer and hs-CRP levels, compared to COVID-19 patients with normal troponin levels.

Table II. *Electrocardiographic parameters of the study population during acute COVID-19 infection*

	COVID-19 patients with elevated troponin (n= 31)	COVID-19 patients with normal troponin (n= 69)	Controls (n= 30)	P value
AF (n–%)	2 (6.5)	3 (4.3)	1 (3.3)	0.835
RBBB (n%)	4 (12.9)	9 (13)	3 (10)	0.231
ST segment depression (n–%)	4 (12.9)	12 ((17.4)	3 (10)	0.741
T wave changes (n–%)	7 (22.6)	22 (31.9)	3 (10)	0.064
PVC (n–%)	3 (9.7)	3 (4.3)	1 (3.3)	0.469
PAC (n–%)	1 (3.2)	2 (2.9)	1 (3.3)	0.990
PVC ablation (n–%)	0	0	0	
SVT ablation (n–%)	0	0	0	

AF: Atrial fibrillation, RBBB: Right bundle branch block, PVC: Premature ventricular contraction, PAC: Premature atrial contraction, SVT: Supraventricular tachycardia

The conventional electrocardiographic parameters are listed in Table II. There were no significant differences in frequencies of atrial fibrillation, right bundle branch block, ST segment, and T wave changes among patients with different troponin levels.

The arrhythmogenic electrocardiographic parameters are listed in Table III. The COVID-19 patients had significantly higher heart rates, PW max., PW min., QTc max., and Tp-e intervals compared to controls. There were no significant differences in the PW dispersion among groups. The COVID-19 patients with high hs-cTnI had significantly higher QTc dispersion than the COVID-19 patients with normal hs-cTnI and the control group.

During the follow-up period; palpitation, beta-blocker usage, and inappropriate sinus tachycardia were more common in the COVID-19 patients with hs-cTnI than the control group (Table IV). The COVID-19 patients with elevated hs-cTnI had higher frequencies of ventricular extrasystole and three patients had premature ventricular contraction (PVC) ablation procedure at this period. There were no significant differences in frequencies of AF and PAC among patients. Only one COVID-19 patient had an SVT ablation and there was no difference among the groups.

Table III. *The arrhythmogenic electrocardiographic parameters during acute COVID-19 infection*

	COVID-19 patients with elevated troponin (n= 31)	COVID-19 patients with normal troponin (n= 69)	Controls (n= 30)	P value
Heart rate (beats/min)	83 ± 3*	84 ± 8*	76 ± 11	**0.011**
QRS duration (msec)	94 ± 17	94 ± 20	93 ± 24	0.983
PR interval (msec)	154 ± 25	157 ± 29	156 ± 28	0.832
PW max. (msec)	113 ± 16*	112 ± 14*	96 ± 16	**<0.001**
PW min. (msec)	75 ± 13*	76 ± 11*	65 ± 10	**<0.001**
PW dispersion (msec)	38 ± 10	36 ± 12	32 ± 13	0.189
QT max. (msec)	389 ± 43	385 ± 35	381 ± 31	0.688
QT min. (msec)	344 ± 44	349 ± 32	347 ± 29	0.811
QT disp (msec)	45 ± 15*,+	36 ± 16	34 ± 18	**0.015**
QTc max. (Bazett) (msec)	451 ± 34*	452 ± 37*	420 ± 33	**<0.001**
QTc min. (Bazett) (msec)	398 ±37	409 ± 33*	382 ± 34	**0.002**
QTc disp. (Bazett) (msec)	52 ±16*,+	43 ± 20	38 ± 19	**0.012**
QTc max. (Framingham) (msec)	428 ±33*	425 ± 28.5*	407 ± 27	**0.007**
QTc min. (Framingham) (msec)	382 ±35	388 ± 25*	372 ± 28	**0.035**
QTc disp. (Framingham) (msec)	45 ±14*+	37 ±17	35 ± 17	**0.037**
Tp-e interval (msec)	81 ± 16*	78 ± 16*	69 ± 11	**0.006**
Tp-e / QTc ratio	0.17 ±0.3	0.17 ±0.3	0.16 ± 0.2	0.286

*PW: P wave, QTc disp: corrected QT dispersion, PostHoc analysis: * denotes statistical significance versus control group, + denotes statistical significance versus patients with normal troponin levels*

Table IV. *The incidence and types of arrhythmias in patients during the one-year follow-up period*

	COVID-19 patients with elevated troponin (n= 31)	COVID-19 patients with normal troponin (n= 69)	Controls (n= 30)	P value
Palpitations (n–%)	12 (38.7) *	18 (26.1)	3 (10)	**0.036**
Beta blocker usage (n–%)	13 (41.9) *	8 (11.6)	2 (6.7)	**<0.001**
IST (n–%)	7 (22.6) *	4 (5.8)	0	**0.003**
AF (n–%)	1(3.2)	2 (2.9)	1 (3.3)	0.992
PVC (n–%)	5 (16.1) *	1 (1.4)	1 (3.3)	**0.025**
PAC (n–%)	1(3.2)	3 (4.3)	1 (3.3)	0.951
PVC ablation (n–%)	3 (9.6) *	0	0	**0.007**
SVT ablation (n–%)	0	1 (1.4)	0	0.641

*IST: Inappropriate sinus tachycardia, AF: Atrial fibrillation, PVC: Premature ventricular contraction, PAC: Premature atrial contraction, SVT: Supraventricular tachycardia, **PostHoc analysis**: * denotes statistical significance versus control group*

4. DISCUSSION

In our study, we evaluated the acute ECG changes and post-COVID arrhythmia incidence in patients with acute COVID-19 infection We found that during acute infection; all COVID-19 patients had significantly higher PWmax., PWmin., QTc max., and Tp-e intervals compared to controls. The COVID-19 patients with high hs-cTnI had significantly higher QTc dispersion, which is regarded as a marker for succeptibilty for development of cardiac arrhythmias. In parallel, we noted that during the follow-up, inappropriate sinus tachycardia and PVC were more frequent in the COVID-19 patients with elevated troponin levels.

The frequency of arrhythmias in acute COVID-19 infection is observed at a rate of 20% [12]. Although, ECG differences vary according to the clinical status of COVID-19 patients, AF, and all arrhythmias occur more frequently in intensive care patients [13]. In our study, heart rate, PW durations, corrected QT interval and Tp-e interval were significantly higher in the group with high hs-cTnI and there was no difference between the groups in terms of the frequency of AF and PVC. Elevation of troponin, an indirect indicator of myocardial damage, has been observed in approximately 20% of COVID-19 patients in the acute period. High troponin levels were associated with higher in-hospital mortality [14].

We showed at 1-year follow-up, palpitations, beta-blocker usage, and PVC frequency were more common in the troponin-positive group than in the troponin-negative group and the control group. Cardiovascular symptoms may emerge after the acute infection and endure for an extended period [15]. Several large studies have reported persistent cardiac arrhythmias after COVID-19 [16]. The overall prevalence of cardiac arrhythmias ranges from 10 to 20% in post-COVID [17]. Similar to our study, in a study in which COVID-19 patients were followed for 6 months, 154 patients (9.3%) had palpitations [18]. In addition, another study reported that 13.7% of patients had a consistently high heart rate until about 4 months after acute infection [19]. Also, in a study in which patients with a history of hospitalization due to COVID-19 were followed up for 3 months, cardiac arrhythmia was found to be 27%. The most common anomaly detected is PVC, seen in 18% of patients. In our study, PVC was observed in 6 people in the entire COVID-19 patient group during acute infection, while this number increased in the high troponin group and decreased in the troponin-negative group in the follow-up. PVC ablation was applied to 3 patients from the troponin-positive group [20].

We also showed inappropriate sinus tachycardia (IST) in 11% of the entire COVID-19 patients during the follow-up period. IST, a rather common observation in post-COVID patients, is defined by a sinus heart rate of >100 bpm at rest (with an unexplained mean 24-h heart rate> 90 bpm) and is associated with distressing symptoms of palpitations [21]. In a study on post-COVID patients, approximately 20% of them met the criteria of the IST [22]. In our study, IST was significantly more common in the high troponin group.

Atrial fibrillation (AF) occurs in hospitalized acute COVID-19 patients with severe disease progression, often accompanied by hyperinflammatory laboratory indicators [23]. But there are publications stating that the incidence of AF is 1.7 times higher in 6-month follow-up after acute infection in patients who are not hospitalized due to COVID-19 [24]. However, in our study, there was no difference in the incidence of AF between the groups at one-year follow-up.

In a cardiovascular magnetic resonance (CMR) study of 26 people who recovered but still experienced cardiac symptoms, 58% of patients had myocardial edema and scar tissue [25]. In another CMR study, which included 100 patients 3 months after acute infection, it was reported that fibrosis in 78% of the patients and myocardial inflammation in 60% of patients persisted [26]. Fibrosis can potentially disrupt the spread of electrical signals and create pathways for re-entry circuits, thereby playing a role in the development of arrhythmias. The connection between fibrosis and arrhythmias has been observed across various cardiac pathological conditions, with CMR being used to evaluate fibrotic remodeling in nearly all cases [27].

In the long term, the frequency of arrhythmias in the troponin-positive group may have been caused by fibrosis or prolonged myocardial inflammation due to cardiac involvement. There is a need for studies that integrate clinical, laboratory and imaging methods to predict the arrhythmia potential of long-term post – COVID-19 patients.

Limitations

The first limitation was a small sample size, and the study was a single-center study. The absence of previous ambulatory ECG monitoring recordings of the patients was another limitation of the study. Coronary artery disease diagnosis was based on patient-reported medical history in our study which was another limitation of our study. Finally, coronary angiography was not performed in the group with elevated troponin, so ischemia, a possible source of arrhythmia, may have been underdiagnosed.

Conclusions

COVID-19 infection induces prolongation of PW durations, Tp-e intervals, and QT dispersion during the acute infection phase. Relatively increased QT dispersion especially in COVID-19 patients with high troponin levels may serve as an indicator of higher arrhythmia risk in this subgroup during acute infection or follow. The higher frequency of inappropriate sinus tachycardia in COVID-19 patients with elevated troponin levels may be a sign for cardiac involvement in these patients and may bear the potential for arrhythmias. These findings emphasize the importance of closely monitoring cardiac parameters in COVID-19 patients, particularly those with elevated troponin levels, to identify potential arrhythmias and implement appropriate management strategies for improved long-term outcomes. Further research is needed to elucidate the underlying mechanisms of these cardiac changes and their implications for patient care.

Compliance with the Ethical Standards

Ethics approval:. The study was approved by the Ethics Committee of the University of Health Sciences, Umraniye Research and Training Hospital (protocol code: 107 and date of approval 4 April 2020). The investigation conformed to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

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Authors contributions: ZD : Concept, design, data collection and analysis, CI: Supervision, ZD and CI: Literature search and writing, CI: Critical review. Both authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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Social media use in adolescent girls with depression: The relationship between social media use purposes, lack of social support, and cyber victimization

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ABSTRACT

Objective: Our study examined the relationship between purpose of social media use, perceived social support, and cyberbullying among adolescent girls with or without major depressive disorder (MDD).

Patients and Methods: Fifty-two adolescent girls aged 13-18 years with a diagnosis of MDD were recruited. The control group consisted of 51 adolescents who were matched for age and gender. The adolescents completed a sociodemographic form, the Childhood Depression Inventory (CDI), the Social Media Attitude Scale (SMAS), the Cyberbullying Scale (CBS), and the Social Support Appraisal Scale for Children (SSAS).

Results: Social media use was significantly higher among adolescent girls with MDD (P<0.001). They had significantly higher social isolation (P=0.006) and cyberbullying scores (P=0.013). They used more social media for social skills (P<0.001) and their perceptions of social support levels were lower than adolescents without MDD (P<0.001). Cyberbullying was inversely related to perceived social support (P=0.015) and positively related to social media use for social competence (P=0.004) (e.g., satisfying the need for esteem).

Conclusion: This study suggests that increases in social competence problems and decreases in perceived social support were associated with being a victim of cyberbullying. In depressed adolescents, cyberbullying can be prevented by interventions aimed at promoting social competence and obtaining sufficient support.

Keywords: Cyber victimization, Adolescents, Depression, Social media

1. INTRODUCTION

The ease and prevalence of internet access and the high popularity of social media platforms among adolescents provide a new form of interpersonal communication and a different social environment for adolescents [1]. Adolescents' purposes for using social media vary [2]. For instance, adolescents with social anxiety who are shy in face-to-face peer relationships and have difficulty expressing themselves can use social media to communicate more easily with their peers [3]. Depressed adolescents may use social media more because of low self-esteem [4]. Again, depressed adolescents can use social media to cope with their problems, express their depressive feelings, or find social support [5,6]

Unfortunately, social media platforms that adolescents use heavily are environments where bully-victim relationships are also common [7]. Cyberbullying is the name given to all acts of intentional, repetitive aggression through technological communication tools, the internet, and social media [8]. The possibilities for adolescent bullies to hide their real identity on social media, to reach a large number of people in a short time, to interact with them, and to transmit any photo or message to millions per second make social media suitable for bullying [9]. Low self-esteem, excessive time spent on the internet, and playing games with others online are risk factors for cyber victimization [10]. Risky behaviors such as problematic use of social media, trying to communicate with strangers, and making friends also increase the risk of cyber victimization among adolescents [11].

The consequences of cyberbullying on adolescents' psychological health have been investigated in many studies. Cyber victimization is closely related to depression, suicidal thoughts, and anxiety [12,13]. In addition, cyber victimization was found to be associated with low self-esteem, loneliness, decreased academic achievement, and substance abuse [14-16]. Numerous studies have shown that being a cyber-victim can

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lead to depression and the presence of depression poses a risk of cyber victimization among adolescents [13,17].

Protective factors against cyberbullying are self-efficacy, low technology use, parental supervision and monitoring of technology use [18]. Studies examining the factors that prevent the development of depression in adolescents who have been cyberbullied have focused on perceived social support [19,20]. High perceived family support, open and warm communication with parents, high perceived friend support, and positive school climate showed a buffering effect against the development of depression from cyber victimization [18,19].

In light of the literature, we hypothesized that depressed adolescents would be more exposed to cyberbullying. In addition, we thought that the social media usage purposes of depressed adolescents might differ from those of non-depressed adolescents. Finally, we hypothesized that their social media usage purposes and perceived social support might be related to cyberbullying. For this purpose, we compared depressed and non-depressed adolescents regarding cyberbullying, social media usage purposes, and perceived social support.

2. PATIENTS and METHODS

Participants

Between May and August 2018, adolescents who presented to our child psychiatric outpatient clinic and were diagnosed with major depressive disorder (MDD) during face-to-face interviews with the child and adolescent psychiatrist were enrolled in the study. Among 82 patients 18 were excluded from the study due to unwillingness for participation, presence of psychotic symptoms, substance use disorder, or neurological disease. In addition, 10 adolescents were excluded since they were not using social media. Exclusion criteria of the study were being older than 18 years of age, refusing to participate, presence of psychotic symptoms, substance use disorder, and neurological disease. Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS) was applied to 54 adolescents with MDD who consented to participate in the study. Fifty-two of the adolescents were girls, and 2 of them were boys. Male adolescents were excluded from the study for statistical purposes because the number of male MDD adolescents who applied to our clinic and could participate, needed to be higher and would not yield statistically significant results.

An application was made to the Istanbul Provincial Directorate of National Education to select the control group. As a result of the permission obtained, two high schools were selected. Eighty randomly selected adolescent girls and their families were informed about the study. After 20 adolescents and their families declined to participate in the study, K-SADS was applied to 60 age – and sex-matched adolescents. Because nine adolescents had MDD symptoms, they were not included in the control group. Fifty-one adolescent girls formed the control group. Face-to-face interviews were conducted with adolescents and their families separately. The interview took an average of 40 minutes to fill out the questionnaires.

Measures

Parents completed the Sociodemographic Information Form. Adolescents completed the Social Media Attitude Scale [21], Cyber Bullying Scale [22], Depression Scale for Children [23], and Social Support Assessment Scale for Children and Adolescent [24,25]. The psychiatric diagnoses of adolescents were evaluated by applying the K-SADS and using clinical interviews for DSM-5 disorders. This study was approved by Marmara University, School of Medicine Research Ethics Committee on 04.05.2018 with protocol number 09.2018.342.

Sociodemographic Information Form

Parents' age and education level, family income level, psychiatric family history, and children's developmental history were assessed. Socioeconomic Status (SES) was calculated based on both parents' educational level and family income level [26]. Additionally, the adolescents' age, school and class, the time spent on the internet per day, and the time spent on social media were collected.

Child Depression Inventory

The Child Depression Inventory (CDI) was developed by Kovacs for children and adolescents aged 6-17 years [23]. The 27-item scale evaluates school status and relationships with friends concerning child and adolescent depression on a three-point scale. Validity and reliability in the Turkish population have been established [27]. The internal consistency coefficient of the scale is 0.77. The scale has a recommended cutoff point of 19.

Social Media Attitude Scale

The Social Media Attitude Scale (SMAS) was developed by Otrar and Argın to evaluate the social media attitudes of young people between the ages of 13-18 [21]. The 23-item scale has the following subdimensions: sharing need, social competence (e.g., "I think I got rid of loneliness thanks to social media sites", "I use social media to make new friends", "I use social media to meet my esteem needs"), social isolation (e.g., "I cannot spare enough time for my family because of social media"), and relationship with teachers. High scores in the subdimensions indicate that social media is used to meet needs associated sharing, social competence, social isolation, and/or relationship with teachers. For reliability, Cronbach α (0.85) coefficients were calculated for all four sub-dimensions and scales determined as a result of varimax rotation. Item-total and item-remainder correlations calculated by Pearson analysis were significant (P<.001). As a result of the analyzes performed using the t test (27% lower and higher), it was determined that the items and factors were distinctive. In addition, it was determined that the correlations between the factors were significant with Pearson analysis (P<.001).

Social Support Appraisals Scale

The 31-item Social Support Appraisals Scale (SSAS) was developed by Dubow and Ulman based on Cobb's definition

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of social support. It evaluates children's perceptions of the social support they receive from their friends, teachers, and families [24,25]. Ten new items related to support received from classmates and teachers have been added by Dubow [25]. Validity and reliability have been established in the Turkish population [28]. High scores in the subscales indicate definable social support for that subdimension. The Cronbach alpha internal consistency coefficient for the whole scale was found to be 0.93. The internal consistency coefficients obtained for the subdimensions of the scale were calculated as 0.89, 0.86 and 0.88 for the subdimensions of support from friends, support from family and support from teachers, respectively.

Cyberbullying Scale

The Cyberbullying Scale was developed by Stewart et al., to measure cyberbullying behaviors [22]. The validity and reliability of the Turkish version were determined by Küçük [29]. Additionally, 14 items rated on a 5-point Likert scale measure cyber victimization among adolescents. The Cronbach Alpha value of the total internal consistency coefficient of the scale was calculated as 0.87. The scale measures cyber victimization. As the score obtained from the scale increases, it means that being a cyber victim increases.

Statistical Analysis

Data were evaluated using the Statistical Package for the Social Sciences (SPSS – version 21). Descriptive statistics were shown as the mean (M) ± standard deviation (SD) or frequency (%). The chi-square test was used for categorical variables in the prevalence of psychiatric diagnoses determined by K-SADS in the study and control groups. The Fisher Exact test was used if the number was low. While comparing the sociodemographic characteristics of the study and control groups such as age, socioeconomic status, developmental history, data obtained as a result of interviews such as internet use, duration of social media use, Child Depression Inventory, social media attitude, social support, and cyberbullying questionnaires, the chi-square test was used for categorical variables, and multivariate analysis of variance (MANOVA) was applied for continuous variables. Linear regression analysis was used to analyze the variables associated with cyberbullying. We considered the following statistical characteristics when selecting the dependent variable for linear regression analysis: Firstly, the variable should be continuous. Since, the diagnosis of MDD is a categorical variable, we conducted linear regression analysis using the CDI. However, if we had considered the presence or absence of depression diagnosis, we could have used other regression models such as logistic regression or multiple regression. Secondly, dependent variables that exhibit a normal distribution are more likely to yield reliable results. Therefore, we initially assessed the distribution of the variables using the Kolmogorov-Smirnov Test to determine whether they followed a normal distribution. CDI scores, cyberbullying scores, subscale scores of the social media attitude scale, total score of the social support scale, and sub-scale scores were found to be normally distributed. These variables, which exhibited normal distribution, were included in the analysis. Prior to regression analysis, the linear relationship of the variables was evaluated using correlation analysis. Only variables that showed a significant, linear relationship were selected. Additionally, it is important for the variance of the dependent variable to be homogeneous in order to obtain reliable results from regression analysis. To assess the homogeneity of variances, we applied the Tests of Homogeneity of Variances. The variables that met these conditions were included in the regression analysis. Linear regression analysis with the evaluated variables was applied with the forward method. The model with the most significant variables was accepted. In the regression model, the multicollinearity problem was checked in two ways. All predictor variables were checked for correlation to control using correlation coefficients, and only one of the two variables with coefficients of magnitudes of r is 0.8 or higher was used in the analysis. In addition, Variance Inflation Factor (VIF) values were checked. Those below 5.00 were included in the assessment. For all analyses, the significance level was accepted as P <0.05 and the corresponding 95% confidence intervals.

3. RESULTS

Table I shows the demographic data of the adolescent girls in the study and control groups, with a mean age of 15.51 ± 1.15 years. There was no statistically significant difference between the groups in participant, maternal, and paternal age; social status; single-parent households; chronic diseases; or developmental delays (P>0.05). However, participants in the study group had a higher prevalence of psychiatric disorders in family members and low academic performance than those in the control group (P<0.05, Table I).

Table I. *Demographic findings of adolescent girls*

	Depression (52)		Control (51)			
	mean	SD	mean	SD	t	P
Δ Age	15.54	1.18	15.49	1.13	0.25	0.80
Δ Maternal Age	42.00	4.23	42.19	4.40	-0.22	0.82
Δ Paternal Age	45.70	5.05	45.82	5.41	-0.11	0.90
Δ SES	7.84	1.90	8.52	0.98	-1.70	0.09
	n	%	n	%	X2	P
ΔΔ Single Parent	8	15.1	3	5.9	3.40	0.182
ΔΔΔ Psychiatric disorders of family members	16	30.8	6	11.8	5.52	0.019*
ΔΔ Low academic achievement	17	33.3	3	6	12.61	0.002**
ΔΔΔ Chronic illness	14	26.9	6	11.8	3.78	0.052
ΔΔΔ Developmental delay history	7	13.2	6	11.8	0.49	0.824

*SES: Socioeconomic status, Δ is calculated by t-Test, ΔΔ is calculated by Fisher Exact Test, ΔΔΔ is calculated by Chi-square Test, P: *<0.05, P:**<0.01*

Table II shows psychiatric comorbidity in adolescents in both groups. Adolescents with depression had higher psychiatric comorbidity and anxiety disorder rates than adolescents in the control group (P<0.05). No significant difference was found for other psychiatric illnesses (P>0.05, Table II).

Table II. *Psychiatric comorbidities of adolescent girls*

	Depression (52)		Control (51)			
	n	%	n	%	X2	P
ΔΔ Any psychiatric comorbidity	17	41.5	11	21.6	8.67	0.013*
Δ Anxiety Disorders	12	22.6	4	7.8	6.70	0.035*
Δ OCD	3	5.7	2	3.9	0.172	0.67
Δ ODD	3	5.7	1	2	0.96	0.32
Δ ADHD	3	5.7	4	7.8	2.97	0.24
Δ Eating Disorders	2	3.8	0	0	1.96	0.49
Δ PTSD	1	1.9	0	0	0.97	1.00

*OCD: obsessive compulsive disorder, ODD: oppositional defiant disorder, ADHD: attention deficit hyperactivity disorder, PTSD: posttraumatic stress disorder, Δ is calculated by Fisher Exact Test, ΔΔ is calculated by Chi-square Test, P: *<0.05.*

Table III shows that internet and social media use was significantly higher in the study group than in the control group (P<0.05). However, the ratio of time spent on social media to total time spent on internet was similar in both groups (P>0.05, Table III). Internet use for informational purposes and social interaction was significantly higher in the control group (P<0.05, Table III). While, the percentage of girls with depression who intended to make new friends in social media was significantly higher (P<0.05, Table III), there was no significant difference between the two groups in communication with existing friends (P>0.05, Table III).

Table III. *Evaluations of internet usage in adolescent girls*

		Depression		Control			
		n	%	n	%	X2	P
Δ Internet usage time	<1 hour	3	5.8	3	5.9	29.5	<0.001**
	1-3 hours	8	15.4	23	45.1		
	3-5 hours	15	28.8	23	45.1		
	>5 hours	26	50	2	3.9		
ΔΔ Internet usage purpose	Using the internet for information	18	34.6	28	54.9	4.28	0.038*
	Social sharing	36	69.2	47	92.2	8.64	0.003*
	Communication	22	42.3	28	54.9	1.63	0.201
	Entertainment	9	17.3	13	25.5	1.02	0.311
	Gaming	12	23.1	12	25.1	0.00	0.957
		Mean	SD	Mean	SD	t	P ΔΔΔ
Social media usage time		4.1	1.78	1.9	1.09	3.902	<0.001**
Social media ratio		0.74	0.23	0.67	0.19	3.902	0.118
Making new friends		3.71	1.24	2.98	1.28	-2.927	0.004**
Communicate with friends		1.92	1.09	1.87	1.07	0.264	0.792
Problem solving		3.14	1.37	3.33	1.40	0.682	0.497
Self-expression		3.22	1.32	2.63	1.43	2.146	0.034*
Social anxiety		3.62	1.24	3.57	1.46	0.201	0.841
Escape depressive feelings		2.50	1.27	2.08	1.34	1.649	0.102
Interest in depressive contents		3.72	1.29	2.60	1.40	4.199	<0.001**
Not sharing, only follow		3.08	1.32	3.30	1.37	0.836	0.405
Sharing depressive contents		4.08	1.08	3.23	1.41	3.401	0.001**

Social media ratio; social media usage time/internet usage time, Δ is calculated by Fisher Exact Test, ΔΔ is calculated by Chi-square Test, ΔΔΔ is calculated by t-Test, P: *<0.05. P:**<0.01

Table IV. *Comparisons of scales scores between adolescent girls with and without MDD*

	Depression		Control		Mean Square	F	P	Partial Eta Squared
	Mean	SD	Mean	SD				
CDI	28.55	5.66	9.10	3.22	9.453.433	431.134	<0.001**	0.813
SMAS-Social Competence	16.17	6.17	11.55	4.76	551.592	17.914	<0.001**	0.153
SMAS-Social Isolation	13.62	5.56	10.55	4.90	216.544	7.853	0.006**	0.073
SMAS-Sharing Needs	28.55	8.19	30.67	5.70	112.927	2.253	0.137	0.022
SMAS-Total	73.06	14.53	74.14	10.05	22.701	0.143	0.706	0.001
SSAS-Friends	64.96	13.83	82.04	7.33	7.364.693	59.510	<0.001**	0.375
SSAS-Family	40.15	11.15	53.29	6.64	4.440.787	51.558	<0.001**	0.342
SSAS-Teacher	33.59	8.23	38.24	5.76	546.329	10.793	0.001**	0.098
SSAS-Total	138.66	23.63	173.66	14.77	30.916.437	79.218	<0.001**	0.445
Cyberbullying Scale	23.85	7.97	19.84	7.23	380.701	6.405	0.013*	0.061

SSAS: Social Support Appraisals Scale, CDI: Children's Depression Inventory, SMAS: Social Media Attitude Scale, P calculated by MANOV, P: *<0.05, P:**<0.01

Table V. *Linear Regression Analysis of Scales Scores with Cyberbullying Scale Scores in adolescent girls*

	Dependent Variable	Standardized Coefficients B	t	p	95.0% Confidence Interval for B		Collinearity Statistics	
					Lower Bound	Upper Bound	Tolerance	VIF
Adolescents with MDD (52)	SMAS-Social competence	0.387	3.054	0.004**	0.173	0.840	0.994	1.006
	SSAS-Total	– 0.321	-2.533	0.015*	– 0.198	-0.023	0.994	1.006
All Adolescents (103)	SMAS-Social competence	0.415	4.498	<0.001**	0.306	0.790	0.815	1.228
	SSAS-Total	– 0.490	– 3.974	<0.001**	-0.221	– 0.074	0.457	2.189
	CDI	– 0.268	– 2.073	0.041*	– 0.386	-0.008	0.415	2.409

SSAS: Social Support Appraisals Scale, SMAS: Social Media Attitude Scale, VIF: Variance Inflation Factor, P calculated by Lineer Regression Analysis, P: *<0.05, P:**<0.01

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Table IV shows that as expected, depression symptoms were significantly higher in those diagnosed with depression (study group). Adolescents with depression were also significantly more likely to use social media to meet needs of social competence and reported significantly higher social isolation (P<0.05, Table IV). However, no significant difference was found between the study and control groups in overall social media attitudes and sharing (P>0.05, Table IV). There was no significant difference in the social media attitudes of both groups in the relationship with the teachers. The control group also reported significantly higher perceived support from family, friends, and teachers (P<0.05, Table IV). Cyberbullying scores were found to be significantly higher in the depression group (P<0.05, Table IV).

Finally, Table V presents the regression analyses. Linear regression analyses of cyberbullying scales with other scales in adolescents with MDD showed that higher cyberbullying scores were associated with social media use for social competence and lower overall social support (P< 0.05, Table V). Linear regression analyses of cyberbullying scales with other scales in adolescents with or without MDD showed that higher cyberbullying scores were associated with higher depressive scores in addition to social media use for social competence and lower general social support (P< 0.05, Table V).

4. DISCUSSION

This study examined whether internet and social media usage time, social media using purposes; perceived social support from friends, family, and teachers; and cyberbullying scores differed between adolescents with MDD. In addition, this study aimed to evaluate whether existence of MDD, social media usage time, social media using purpose and social support deficiencies were significantly associated with cyber victimization.

The psychiatric diagnoses in the families of the adolescents with MDD in our study were significantly higher than in the families of other adolescents can be explained by their genetic predisposition [30]. The effect of depression on academic functioning and the decrease in the school success of depressed adolescents, as in our study, are consistent with other studies [31,32]. As in many studies, comorbid anxiety disorder was found more frequently in the depression group in our study [33,34]. In our study, while the time spent by adolescents with MDD on the internet and social media increased, the ratio of the two remained constant, indicating the increased time adolescents spent on other platforms besides social media. Therefore, the increased time spent on the internet besides social media may also be a new research topic. Some studies have found that the amount of time adolescents with depression spent on social media has increased [5,35], while others have found that depressed adolescents use social media as much as non-depressed adolescents [36,37]. Meta-analyses examining the relationship between the duration of social media use and depression found a significant but weak relationship. These meta-analyses referred to the heterogeneity of studies in this field [38,39]. A study examining the longitudinal relationship between social media use and depression showed that depressive symptoms predicted small increases in social media use during adolescence [40].

Our study determined that adolescents without MDD use the internet more for information and social sharing than depressed ones. However, adolescents with MDD use social media more for making new friends, dealing and sharing with depressive feelings. In a study conducted in our country, the use of social media for sharing depressive feelings was found to be higher in depressed adolescents, similar to our study [5].

In our study, adolescents with MDD exhibited significantly higher social isolation and social competence needs than their healthy counterparts. The social isolation subscale shows that depressed adolescent girls are more socially isolated in their social relationships than healthy controls. They use social media to overcome this isolation. It is not surprising that, as depressed adolescents spend much more time online, their social isolation also increases through less time spent face-to-face with friends and family. Alternatively, depression symptoms may decrease preference for face-to-face interactions because social skills are inversely related to depression [41,42].

The social competence subscale of the Social Media Attitude Scale refers to adolescents' use of social media to overcome loneliness, make new friends, get noticed by their current friends, and gain prestige [21]. The close relationship between depression and loneliness could lead depressed adolescents to use social media to overcome loneliness [3,43]. Young people who are lonely or have low self-esteem may see social media as a way to build social relationships in a less demanding and perhaps less threatening social context [44]. Another study showed that depressed youth have more strangers on their friend lists than their healthy counterparts [45]. Consistent with the literature, our findings showed that adolescents with MDD who feel socially inadequate might use social media to meet their social needs.

Furthermore, we found that adolescents with MDD perceived less social support from their families, friends, and teachers than healthy controls, suggesting that depression was negatively related with social support perception. This is in line with findings that depression is inversely related to perceived social support [46].

Our study also showed that adolescents with MDD were more exposed to cyberbullying than those in the control group, consistent with previous studies on depression and cyberbullying among adolescents [47,48]. Research on the relationship between depression and cyberbullying emphasizes its bi-directionality, meaning depression may be a consequence of and a risk factor for cyberbullying [49,50]. It is difficult to explain why cyberbullies are more likely to target depressed youth on social media and are aware of their vulnerability in the virtual environment (at the other end of the screen).

The time spent on social media was not related to cyberbullying. In contrast to our findings, research has found an association between time spent on social media and victimization from cyberbullying [51,52]. In a large sample study that included

seven European countries, time spent on social media and cyber victimization were significantly associated [53]. Our contrasting results may be due to our small sample size.

Considering all adolescents with or without MDD, the current study found that overall low level of perceived social support from family, friends, and teachers and using social media for social skills (to overcome loneliness, make new friends, and satisfy the need for prestige) increased the risk of cyberbullying. Perceived social support from family and friends has been shown to be a protective factor against cyberbullying [54] and to buffer against depression when cyberbullying occurs [19]. Although, studies demonstrate the association between loneliness and cyber victimization [8,55], the factors mediating this association are unclear. Adolescents who use social media to overcome their loneliness, make new friends, and satisfy their need for respect may tend to trust people in the virtual environment more, be on the friend list of many people they do not know, and share more personal information with them, and eventually become victims of cyberbullying. Cyber victimization has been found to partially mediate the relationship between self-disclosure on social media and loneliness [56]. However, further studies are needed to examine these potential mediating factors. Through longitudinal studies, we will provide the necessary information to protect adolescents from cyberbullying by clarifying the mediating factors between adolescents' social media use goals and social media behaviors, and the likelihood of being a victim of cyberbullying.

Strengths and Limitations

The important limitations of our study are the small sample size and the analysis of adolescents' social media attitudes – social media usage purposes – and cyber victims with data obtained from self-report scales. In addition, our study consisted of only adolescent girls and was conducted with adolescents in one province. These limitations reduce the generalizability of the findings of the study.

Despite our limitations, our study is valuable because diagnoses were established by face-to-face interviews. In addition, it draws attention to the risk of cyberbullying, primarily based on the social media attitudes – social media usage purposes – of depressed adolescents and those who lack social support. In depressed adolescents, exposure to cyberbullying can be prevented with interventions aimed at helping in social competence and perceived sufficient support from friends and family. Finally, as technology will continue to be an essential aspect of adolescents' lives, a better understanding of the relationship between cyberbullying and depression may promote well-being of developing youth.

Conclusion

According to the findings of the study, adolescent girls with MDD tended to spend more time on social media, were more socially isolated and had less social competence than non-depressed adolescents. In addition, adolescents with MDD were more victims of cyberbullying than non-depressed adolescents. Depressed adolescent girls perceived less social support from their families and friends. The increase in social inadequacy in adolescent girls with MDD and the decrease in their perceived social support were associated with being victims of cyberbullying. Therefore, as a preventive measure, while working with adolescents with depression, clinicians may implement strategies focusing on increasing the social support and the youth may be advised against using social media to compensate for their social inadequacy symptoms, pointing out the risk for possible cyberbullying.

Compliance with the Ethical Standards

Ethics Committee approval: This study was approved by Marmara University, School of Medicine Research Ethics Committee on 04.05.2018 with protocol number 09.2018.342. Written informed consent was obtained from the participants.

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The effect of cardiac rehabilitation on left ventricular diastolic functions assessed by exercise stress echocardiography in patients with acute coronary syndrome

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ABSTRACT

Objective: The aim of our study is to investigate the impact of cardiac rehabilitation on left ventricular (LV) diastolic function in acute coronary syndrome (ACS) patients.

Patients and Methods: Patients were selected consecutively among ACS patients who underwent primary percutaneous intervention and were found eligible for cardiac rehabilitation program from May 2014 to May 2015. Forty-four patients were included in cardiac rehabilitation group and recruited to 30 sessions of Phase 3 cardiac rehabilitation program six weeks after discharge. Twenty consecutive patients were included as control group. LV diastolic functions were assessed by resting and stress echocardiography.

Results: There were not any significant differences in characteristics between the groups. Resting and stress E velocities and resting lateral e' velocity significantly increased after rehabilitation program. Left atrial volume index, resting and stress A velocities and average E/e' ratios were significantly lower while stress lateral e' velocity was significantly higher in rehabilitation group after program compared to controls. The number of patients with diastolic dysfunction decreased after rehabilitation program. Final resting and stress echocardiography revealed significantly lower frequency of diastolic dysfunction in rehabilitation group.

Conclusion: Cardiac rehabilitation improves diastolic functions in ACS patients, which may be detected by stress echocardiography.

Keywords: Acute coronary syndrome, Cardiac rehabilitation, Diastolic function, Diastolic stress echocardiography

1. INTRODUCTION

Acute coronary syndrome (ACS) is the most leading cause of mortality worldwide [1]. ACS affects not only physical but also psychological and social life of the patients, which have highlighted the importance of a multidisciplinary approach to ACS patients.

Cardiac rehabilitation is a structured program which includes exercises, lifestyle modifications, psychosocial support and education compatible with special conditions of each patient. Cardiac rehabilitation is associated with improved clinical outcomes in a broad spectrum of cardiac diseases. Participation in a cardiac rehabilitation program after ACS and coronary artery bypass grafting is associated with reduced mortality [2]. The meta-analysis of exercise training trials in patients with chronic heart failure has provided evidence that exercise training is associated with significant decreases in mortality and hospitalizations [3]. Therefore, current guidelines support cardiac rehabilitation for ACS patients after revascularization either by coronary artery bypass grafting or stent implantations, after valve surgery, and in patients with stable chronic systolic heart failure [4].

Both systolic and diastolic functions may be impaired in patients with ACS. Due to its significant benefits in heart failure with reduced ejection fraction, cardiac rehabilitation is strongly recommended and its reimbursement is approved. On the other hand, for patients with heart failure with preserved ejection fraction is not endorsed by clinical guidelines [4]. A randomized exercise training study of 54 patients with cardiomyopathy demonstrated a reduction in left ventricular (LV) diastolic stiffness in the exercise training group suggesting that cardiac rehabilitation improves diastolic function [5]. Exercise training resulted in a decrease in E/e´ in patients with heart failure with preserved ejection fraction [6].

Echocardiographic examination has a crucial role in diagnosis of ventricular systolic and diastolic functions. Diastolic

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stress testing might be superior to resting echocardiography in showing diastolic dysfunction especially in patients with exertional dyspnea.

The aim of this study was to investigate the effects of cardiac rehabilitation on LV diastolic functions assessed by bicycle stress echocardiography in ACS patients.

2. PATIENTS and METHODS

The study was approved by the Marmara University School of Medicine Research Ethics Committee on 28.06.2013 (approval number: 09.2013.0215). Written informed consent was obtained from all patients before entering the study.

Study Population

In this prospective study, the data were collected in Department of Cardiology, Marmara University School of Medicine, Turkey, from May 2014 to May 2015. Power analysis was performed by an online calculator (https://clincalc.com/stats/samplesize.aspx). Assuming a power of 90 % and α=0.05, a study population including at least 44 individuals was needed. Patients were selected consecutively among patients who were hospitalized in our coronary intensive care unit with the diagnosis of ACS, underwent successful primary percutaneous intervention (PCI) with thrombolysis in myocardial infarction (TIMI) 3 grade flow, discharged with optimal medical therapy (including dual antiplatelet therapy with an angiotensin converting enzyme inhibitor, beta blocker and statin unless there was a contraindication) and were found eligible for cardiac rehabilitation program after discharge. The diagnosis of ACS was based on symptoms, electrocardiography and cardiac markers. ST segment elevation myocardial infarction (STEMI) was defined as the presence of chest pain with persistent ST segment elevation of at least 0.1mV in at least two contiguous leads. Non ST segment elevation myocardial infarction (NSTEMI) was defined as the presence of chest pain during the previous 48 hours with ST segment and/or T wave changes on electrocardiography indicating ischemia and a positive troponin test. Exclusion criteria were presence of multi-vessel disease, PCI in coronary arteries other than infarct-related artery, arrhythmias (atrial fibrillation, atrial flutter, ventricular arrhythmias), bundle branch blocks, significant valvular disease (any stenosis and/or regurgitation greater than mild in severity), severe renal, hepatic or lung disease and malignancy. Seventy-five consecutive patients were invited to the study and were informed about Phase 1 rehabilitation program during hospitalization and Phase 2 rehabilitation program during the 6 weeks following discharge. Twenty patients refused to participate in the cardiac rehabilitation program due to socioeconomic conditions and were included in the study as control group. Exercise test was performed to the remaining 55 patients to assess their eligibility for cardiac rehabilitation program six weeks after discharge. After the exclusion of the patients who developed chest pain (6 patients), syncope/presyncope (2 patients) and significant ST segment depression (3 patients) in the exercise test, the remaining 44 patients who did not have contraindication for Phase 3 cardiac rehabilitation were included as cardiac rehabilitation group.

All patients were evaluated in terms of demographic characteristics, concomitant diseases and cardiovascular risk factors. Hypertension was defined as systolic and/or diastolic blood pressure ≥140/90mmHg, previously diagnosed hypertension, or use of any antihypertensive medications. Diabetes was defined as fasting plasma glucose levels of more than 126mg/dL in ≥3 measurements, previously diagnosed diabetes, or use of antidiabetic medications such as oral anti-diabetic agents or insulin. Hyperlipidemia was defined as serum total cholesterol ≥200mg/dl, serum triglyceride ≥150mg/dl, low-density lipoprotein cholesterol ≥130mg/dl, previously diagnosed hyperlipidemia, or the use of lipid-lowering medication. Smoking status was defined as the history of tobacco use at admission or in the 6 months prior to the visit. Detailed physical examination was performed to all patients. Body mass index (BMI) was calculated from measurements of height and weight. The N-terminal pro brain natriuretic peptide (NT-proBNP) levels of the patients during hospital stay and follow-up were noted [7].

Echocardiographic Examination

All patients underwent transthoracic echocardiography and diastolic stress echocardiography before the cardiac rehabilitation program and within the following five days after the cessation of cardiac rehabilitation program. Control group underwent echocardiographic evaluation 6-8 weeks following discharge and echocardiography was repeated three months later.

Standard transthoracic echocardiography was performed by a single, experienced cardiologist with a Philips iE33 echocardiography device (Philips Medical Systems, Andover, MA, USA). Patients were positioned in the left lateral decubitus position. Data acquisition was performed with a 3.5 MHz transducer at a depth of 16-17 cm in the parasternal and apical views (standard parasternal short-axis from mid-ventricular level; apical long-axis; two, three, and four-chamber images). Standard M-mode, 2D, Doppler and color-coded tissue Doppler images (TDI) were obtained during breath hold, stored in cine loop format from three consecutive beats, and transferred to a workstation for further offline analysis. Gain settings, filters, and pulse repetition frequency were adjusted to optimize color saturation, and a color Doppler frame scanning rate of 100–140 Hz was used for color TDI and a frame rate of 44–82 frames/s for grayscale images. Conventional echocardiographic measurements were performed in accordance with the recommendations of the American Society of Echocardiography guidelines [8,9]. The diagnosis of LV diastolic dysfunction was based on mitral flow velocities (E/A), mitral annular e′ velocity, E/e′ ratio, peak velocity of tricuspid regurgitation jet, and left atrium maximum volume index. The abnormal cutoff values were septal e′ velocity < 7cm/sec, lateral e′ velocity <10cm/sec, average E/e′ ratio >14, left atrial volume index (LAVI) >34mL/m^2, and peak tricuspid regurgitation velocity >2.8m/sec. LV diastolic dysfunction was present if more than half of the available parameters met these cutoff values.

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After baseline echocardiography, all the patients underwent diastolic stress echocardiography using the supine bicycle protocol (General Electric eBike EL tilt-table ergometer). Patients pedaled at constant speed beginning at a workload of 25 Watts (W), with an increment of 25 W every 3 minutes. Subsequently, with continuous electrocardiographic monitoring and interval blood pressure monitoring, patients exercised on the supine bicycle to achieve target heart rate (85% of maximum predicted for age). During the supine bicycle stress, 2D images and Doppler diastolic parameters were acquired in the apical four-chamber views at each stage including peak exercise and recovery. From the apical window, a 1 – to 2-mm pulsed Doppler sample volume was placed at the mitral valve tip, and mitral flow velocities from 5 to 10 cardiac cycles were recorded. The mitral inflow velocities were traced and the following variables were obtained: peak velocity of early (E) and late (A) filling, and deceleration time (DT) of the E velocity. The tricuspid regurgitant jet velocity was also obtained to estimate pulmonary artery systolic pressure using continuous wave Doppler. Mitral annulus velocity was measured by TDI using the pulsed wave Doppler mode. The filter was set to exclude high frequency signals, and the Nyquist limit was adjusted to a range of 15 to 20 cm/s. Gain and sample volume were minimized to allow for a clear tissue signal with minimal background noise. E´ was measured from the apical 4-chamber view with a 2 – to 5-mm sample volume placed at the septal and lateral corner of the mitral annulus. The test was considered definitely abnormal indicating diastolic dysfunction when all of the following three conditions were met: average E/e′ >14 or septal E/e′ ratio >15 with exercise, peak TR velocity >2.8m/sec with exercise and septal e′ velocity <7cm/sec or lateral e′ velocity <10cm/sec at baseline (Figures 1 and 2). The results were normal when average (or septal) E/e′ ratio was <10 with exercise and peak TR velocity was <2.8m/sec with exercise **[9]**.

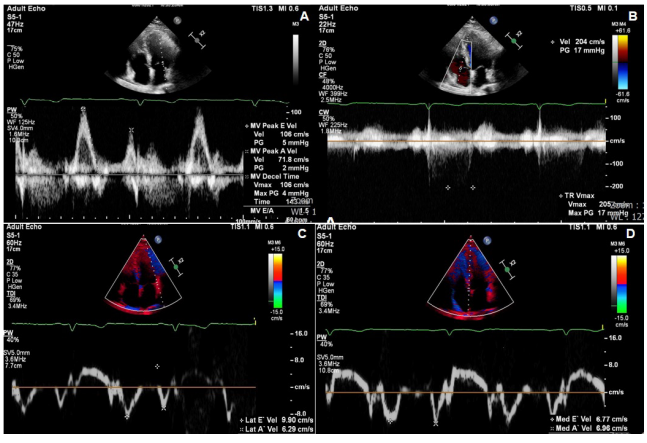

Figure 1. *Baseline Doppler echocardiographic measures of a patient – heart rate is 60/min (A. Mitral inflow velocities (peak velocity of early (E) and late (A) filling and deceleration time of the E velocity); B. tricuspid regurgitant jet velocity; C. mitral annular lateral e′ and a′ velocities; D. mitral annular septal e′ and a′ velocities)*

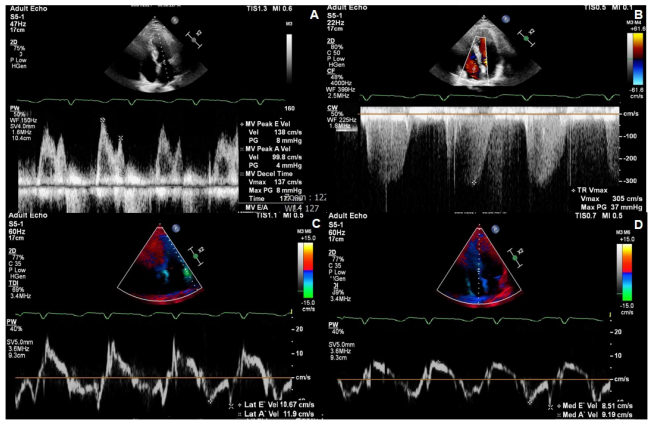

Figure 2. *Exercise Doppler echocardiographic measures of the same patient – heart rate is 104/min (A. Mitral inflow velocities (peak velocity of early (E) and late (A) filling and deceleration time of the E velocity); B. tricuspid regurgitant jet velocity; C. mitral annular lateral e′ and a′ velocities; D. mitral annular septal e′ and a′ velocities)*

Cardiac Rehabilitation Program

Cardiac rehabilitation program included supervised exercise training together with patient education about secondary prevention from cardiovascular diseases [10]. Advices for lifestyle modifications including a healthy, balanced diet with salt restriction, smoking cessation, moderation of alcohol consumption, dealing with stress, regular sleep and maintaining an ideal body weight with regular physical activity were given to each patient and adherence to these advices were checked regularly each week by a trained nurse.

Exercise tolerance test was applied in Cardiac Rehabilitation Unit two days after the first assessment. Contraindications for exercise test were assessed by a cardiologist. Cycle ergometer (Ergoline Ergoselect 2 model 600 and Opticare software program) was used to assess exercise capacity. Patients wore comfortable clothes, stopped eating/drinking three hours before their arrival and did not smoke before the test. Maximum heart rate was calculated according to age and gender. Target heart rate range was calculated according to Karvoneen method [(Maximum heart rate – resting heart rate) × 60-80% + resting heart rate)]. During the test, the patient started cycling with 30 W pedal load and 15 W increment was applied every two minutes with a constant pedaling speed of 55-65 per minute. If target heart rate was reached or the patient wanted to stop due to fatigue, the test was stopped. Maximum pedal load in watts, maximal oxygen consumption in minutes per kg or the metabolic equivalent of task (MET) (ml/kg/minutes) and maximal energy consumption (kcal/minutes) reached were recorded. The data were used to develop individualized rehabilitation program for patients in cardiac rehabilitation group and also used as a follow-up criteria of fitness parameters.

Initially the ratio of maximum load to patient body weight (watt/kg) was calculated. The ratio was used as an indicator of physical fitness. The values lower than 1.4 W/kg indicated

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an untrained person or a patient with moderate to high risk for cardiac complications during physical stress according to the recommendations of the American Heart Association. If the ratio was lower than 1 watt/kg, the patients started the program with low-intensity intermittent training to provide their compliance with the program. When the ratio was 1.4 and over, exercise program was continued with constant heart rate (endurance training) method. Individual exercise programs were revised by weekly assessments according to improvements in physical fitness.

Each training session included reduced load warming and cooling periods for five minutes in the beginning and the end of the training. In the intermittent training program, the patients performed low load for 1 minute and maximum load for 1 minute consecutively for 20 minutes. In constant heart rate method, the maximal load at target heart rate was recorded with the exercise testing. In the program, the patients exercised without exceeding target heart rate. The target heart rate was kept constant with changing the load automatically by cycle ergometer.

After cycle ergometer training, each exercise session was finished with stretching and strengthening exercises. Biceps, triceps, deltoid, quadriceps, hamstrings, abductor muscle groups were trained in the muscle strengthening program. Strengthening exercises were planned with calculation of 1 repetition maximum (RM) method. The maximum load which could be hold by 3 different large muscle groups of lower and upper extremities was determined. Each muscle was strengthened with 3x10 repetitive isotonic contractions with up to 75% resistance of 1 RM. This program was performed 3 days per week for 10 weeks in the hospital (30 sessions lasting approximately 2.5 months). After the program, the patients were encouraged to perform aerobic exercises by themselves in their daily life.

Statistical Analysis

All statistical tests were performed with a commercially available software program (Statistical Package for the Social Science (SPSS) 20.0 for Windows, Chicago, IL, USA). All continuous variables were checked for normal distribution by the Kolmogorov-Smirnov test and presented as mean±standard deviation (SD) while categorical variables were expressed as numbers or percentages. Chi-square test or McNemar test was used to compare categorical variables. Student's *t* test or paired sample test were used to compare continuous variables with normal distribution while Mann-Whitney *U* test or Wilcoxon test were used to compare the continuous parameters without normal distribution. P <0.05 was considered statistically significant.

3. RESULTS

The baseline characteristics, LV size and ejection fraction of the 44 patients in cardiac rehabilitation group and the 20 patients in control group are shown in Table I. There were not any significant differences in age, sex and comorbidities between the cardiac rehabilitation group and controls. In the cardiac rehabilitation group, 20 patients had anterior STEMI while 2 patients had lateral STEMI and 10 patients inferior STEMI. In the control group, 8 patients had anterior STEMI and 6 patients had inferior STEMI. LV size and ejection fraction of the rehabilitation group and controls were similar. Nine patients in the cardiac rehabilitation group and 4 control patients had LV ejection fraction <50%. None of the patients had dilated LV. There were not any significant changes in the LV diameters (for LV end diastolic diameter 47.2 ± 4.7mm to 46.4 ± 4.9mm, P=0.286 in cardiac rehabilitation group and 48.8 ± 5.9mm to 48.6 ± 5.1mm, P=0.242 in controls; for LV end systolic diameter 32.5 ± 5.4mm to 31.8 ± 4.9mm, P=0.314 in cardiac rehabilitation group and 35.6 ± 5.8mm to 35.1 ± 5.4mm, P=0.342 in controls) and EF (56.5 ± 8.5% vs 56.9 ± 8.1%, P=0.614 in cardiac rehabilitation group and 53.8 ± 5.4% vs 55.2 ± 6.8%, P=0.156 in controls) in control echocardiographies. The NT-proBNP values of the cardiac rehabilitation group and controls were similar before the rehabilitation program (410.9±529.4pg/mL vs 445.2±601.8pg/mL P= 0.142). The NT-proBNP values decreased significantly in cardiac rehabilitation group to 218.8±274.1pg/mL (P<0.001) after rehabilitation program whereas the decrease in NT-proBNP levels was not significant in controls (396.7±456.8pg/mL, P= 0.112).

Table I. *Patient characteristics and baseline left ventricular size and ejection fraction*

	Cardiac Rehabilitation Group (n= 44)	Control Group (n= 20)	P
Age (year)	53.2 ± 9.9	57.1 ± 14.1	0.274
Male gender (n-%)	40 (90.9%)	15 (75%)	0.124
Hypertension (n-%)	29 (65.9%)	9 (45%)	0.114
Diabetes (n-%)	13 (29.5%)	7 (35%)	0.663
Hyperlipidemia (n-%)	33 (75.0%)	14 (70%)	0.675
Smoking (n-%)	26 (59.1%)	15 (75%)	0.219
Body mass index (kg/m^2)	27.6 ± 3.7	26.5 ± 2.9	0.300
Left atrium (mm)	37.4 ± 4.0	40.3 ± 6.5	0.127
Left ventricular end diastolic diameter (mm)	47.2 ± 4.7	48.8 ± 5.9	0.399
Left ventricular end systolic diameter (mm)	32.5 ± 5.4	35.6 ± 5.8	**0.014**
Interventricular septum thickness (mm)	10.5 ± 1.8	11.4 ± 1.9	**0.033**
Posterior wall thickness (mm)	10.0 ± 1.3	9.4 ± 1.3	0.054
Left ventricular ejection fraction (%)	56.5 ± 8.5	53.8 ± 5.4	0.191

The resting diastolic parameters and diastolic stress echocardiographic parameters of the cardiac rehabilitation group and controls are shown in Table II. Among the baseline resting and stress echocardiographic parameters; only LAVI was significantly different between the cardiac rehabilitation group and controls. Resting and stress E velocities and resting

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lateral e' velocity increased significantly in cardiac rehabilitation group after rehabilitation program while comparison of control group's baseline and third month echocardiography revealed no significant change in resting and stress echocardiographic diastolic parameters except from resting A velocity; which increased significantly. LAVI, resting and stress A velocities were significantly lower while stress lateral e' velocity was significantly higher in cardiac rehabilitation group after rehabilitation program compared to control. Although, there were not any significant differences in resting and stress E/e' parameters between cardiac rehabilitation group and controls, cardiac rehabilitation group had significantly lower resting and stress E/e' measures after rehabilitation program compared to third month echocardiography measures of control group (Figure 3).

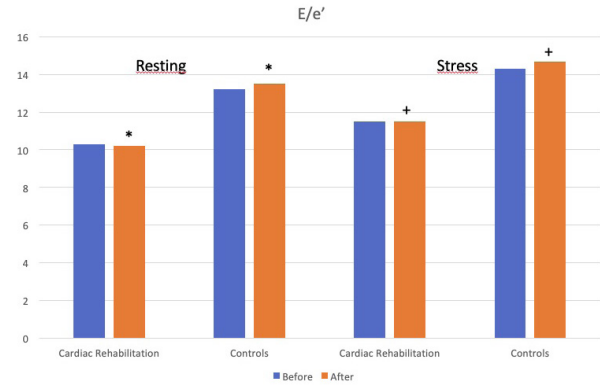

Figure 3. *Bar graph showing resting and stress E/e' measures of the cardiac rehabilitation group and controls before and after (* and + denote statistically significant differences in resting and stress E/e' between cardiac rehabilitation group after rehabilitation program and controls after three months, respectively).*

Table II. *The resting and diastolic stress echocardiography parameters of the cardiac rehabilitation group and controls*

	Cardiac Rehabilitation Group (n= 44)			Control Group (n= 20)				
	Before rehabilitation	After rehabilitation	P1	Baseline	At 3rd month	P2	P3	P4
LAVI (mL/m²)	25.4±6.4	24.3±6.6	0.165	33.5±12.5	33.0±12.1	0.586	**0.005**	**0.002**
Rest E (m/s)	0.85 ± 0.15	0.92 ± 0.15	**0.007**	0.93 ± 0.19	0.98 ± 0.17	0.225	0.101	0.137
Stress E (m/s)	1.06 ± 0.18	1.15 ± 0.14	**0.002**	1.14 ± 0.21	1.21 ± 0.17	0.135	0.088	0.166
Rest A (m/s)	0.68 ± 0.15	0.71 ± 0.15	0.200	0.73 ± 0.16	0.81 ± 0.17	**0.019**	0.246	**0.039**
Stress A (m/s)	0.79 ± 0.17	0.77 ± 0.16	0.436	0.82 ± 0.18	0.91 ± 0.25	0.103	0.323	**0.038**
Rest DT (ms)	192 ± 31	178 ± 27	**0.003**	180 ± 28	184 ± 34	0.636	0.183	0.727
Stress DT (ms)	162 ± 35	175 ± 26	**0.011**	166 ± 23	170 ± 38	0.840	0.506	0.190
Rest Septal e′ (cm/s)	7.8 ± 2.4	8.4 ± 2.1	0.074	7.4 ± 2.8	7.3 ± 2.5	0.879	0.445	0.102
Stress Septal e′ (cm/s)	8.4 ± 2.8	9.1 ± 2.2	0.118	7.4 ± 3.1	7.9 ± 2.5	0.453	0.103	0.070
Rest Lateral e′ (cm/s)	9.5 ± 2.3	10.7 ± 3.1	**0.005**	9.1 ± 3.6	9.3 ± 3.4	0.717	0.326	0.115
Stress Lateral e′ (cm/s)	11.5 ± 3.2	11.9 ± 2.7	0.406	10.6 ± 3.9	10.3 ± 3.4	0.720	0.209	**0.044**
Rest Average E/e′	10.3 ± 2.7	10.2 ± 2.6	0.963	13.2 ± 6.7	13.5 ± 5.2	0.363	0.151	**0.041**
Stress Average E/e′	11.5 ± 4.0	11.5 ± 3.0	0.815	14.3 ± 6.0	14.7 ± 4.9	0.820	0.063	**0.041**
Rest TR velocity (m/sec)	2.2 ± 0.4	2.2 ± 0.3	0.054	2.4 ± 0.6	2.5 ± 0.5	0.058	0.092	0.054
Stress TR velocity (m/sec)	2.5 ± 0.4	2.5 ± 0.4	0.646	2.7 ± 0.6	2.8 ± 0.5	0.119	0.237	**0.030**
Patients with diastolic dysfunction by resting echocardiography (n – %)	5 (11.4%)	3 (6.8%)	0.625	5 (25%)	7 (35%)	0.500	0.263	**0.008**
Patients with diastolic dysfunction by stress echocardiography (n – %)	12 (27.3%)	8 (18.2%)	0.344	10 (50%)	9 (45%)	1.0	0.076	**0.024**

LAVI: left atrial volume index; E= early diastolic mitral flow velocity; A= late diastolic mitral flow velocity; DT: deceleration time; e´= early diastolic velocity of the lateral mitral annulus; TR: tricuspid regurgitation
P1: comparison of cardiac rehabilitation group before and after rehabilitation program
P2: comparison of control group's baseline and third month echocardiography parameters
P3: comparison of baseline echocardiographic parameters between cardiac rehabilitation group and controls
P4: comparison of final echocardiographic parameters between cardiac rehabilitation group and controls

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Although, in resting echocardiography, diastolic dysfunction was detected in 5 patients in cardiac rehabilitation group and in 5 patients in controls; stress echocardiography revealed diastolic dysfunction in 12 patients in cardiac rehabilitation group and in 10 control patients. Diastolic dysfunction assessed by stress echocardiography improved in 7 patients after rehabilitation program while 5 patients remained to have diastolic dysfunction and 3 new patients had worsened diastolic function. On the other hand, only 3 patients had improved diastolic function while 7 patients remained to have diastolic dysfunction and 2 new patients had worsened diastolic function in the control group. The number of the patients with diastolic dysfunction decreased after cardiac rehabilitation program. And the frequency of diastolic dysfunction assessed by either resting or stress echocardiography was lower in cardiac rehabilitation group after the rehabilitation program compared to controls (6.8% vs 35% P=0.008 and 18.2% vs 45% P=0.024).

4. DISCUSSION

Diastolic dysfunction and elevated LV filling pressures are associated with poor prognosis in patients with acute myocardial infarction even in patients with relatively preserved systolic function [11]. Cardiac rehabilitation is associated with reduced mortality after myocardial infarction, after percutaneous coronary intervention and in patients with LV systolic dysfunction [3,12-14]. However, the effects of exercise training on diastolic function are less definite. In our study, we showed that cardiac rehabilitation program improved diastolic parameters in ACS patients with relatively preserved LV systolic function. The novelty of our study was the demonstration of the improvement in diastolic parameters by also stress echocardiography.

High intensity aerobic treadmill exercise has been shown to improve early diastolic relaxation in patients with stable coronary artery disease, measured by the mean LV early diastolic strain rate [15]. Similarly, in our study, resting and stress E velocities and resting lateral e' velocity increased significantly after cardiac rehabilitation program. However, another study did not show any improvement in LV diastolic indices including E, A, E/A ratio and deceleration time after an 8-week cardiac rehabilitation program in post-myocardial infarction patients revascularized with coronary artery bypass grafting or percutaneous coronary intervention although exercise capacity improved [16].

E/e' ratio can be used to predict LV filling pressures and is a powerful predictor of survival after acute myocardial infarction [17]. In our study, although the baseline E/e' were not significantly different between the groups, after cardiac rehabilitation program the difference became statistically significant. Cardiac rehabilitation program is associated with improved LV diastolic function. Similar to our study, Acar et al., explored the effect of cardiac rehabilitation on LV diastolic function in ACS patients revascularized by percutaneous coronary intervention and found that although E/e' decreased with cardiac rehabilitation, the difference was not statistically different **[18]**. They showed better E/A and septal e' in the cardiac rehabilitation group after the rehabilitation program compared to the controls and concluded that cardiac rehabilitation partially improved LV diastolic function in these patients. In another study conducted prospectively in 24 patients, a 3-month exercise-based cardiac rehabilitation program improved E/e' ratio and diastolic function in 12 patients while 9 patients remained at the same grade of diastolic dysfunction and one patient had worsened diastolic function [19]. Similarly, in our study among the 12 patients who has diastolic dysfunction assessed by stress echocardiography; diastolic function improved in 7 patients after rehabilitation program.

The improvement of diastolic dysfunction may also be explained by the revascularization procedure and the effect of optimal medical therapy initiated in these patients. However; a study exploring the effect of cardiac rehabilitation in 146 patients undergoing percutaneous coronary intervention revealed that cardiac rehabilitation improved diastolic functions and the distribution of diastolic dysfunction was changed significantly only in the cardiac rehabilitation group [20]. The most prominent improvement was observed in the patients with grade I diastolic dysfunction.

Diastolic stress echocardiography is superior to resting echocardiography in evaluation of patients with concealed diastolic dysfunction. Although, diastolic parameters are normal with resting echocardiography, augmentation of myocardial relaxation may be limited and E/e′ ratio increases with exercise in these patients. Diastolic stress echocardiography is recommended in patients with indeterminate or grade 1 diastolic dysfunction [9]. In our study, most of the patients had normal LV ejection fraction with normal or indeterminate LV diastolic function. We evaluated diastolic functions of the patients also with stress echocardiography and found more patients to have diastolic dysfunction with stress echocardiography. Although, there was not any significant decrease in the stress E/e′ ratio or tricuspid regurgitation velocity in cardiac rehabilitation group, the final E/e′ ratio and tricuspid regurgitation velocity of the cardiac rehabilitation group were significantly lower than that of controls.

Study Limitations

The major limitations of our study were the relatively small sample size and being a single center study. Most of the patients had preserved LV function and the results might be different in a population with more pronounced LV systolic dysfunction. Non-randomized design of the study and the inclusion of the patients who refused cardiac rehabilitation as control group were also major limitations. Adherence to medication and lifestyle modifications were not evaluated in patients who refused cardiac rehabilitation. Adherence might be poor in these patients which might affect study results. The follow period was short and many patients discontinued cardiac rehabilitation program due to socioeconomic status and various other reasons which limited the analysis of prolonged effect of cardiac rehabilitation in these patients.

Conclusion

Cardiac rehabilitation improves diastolic parameters in ACS patients. Although, resting echocardiographic parameters are useful in assessing the response in diastolic functions; diastolic stress echocardiography may reveal concealed LV diastolic dysfunction and its response to cardiac rehabilitation in these patients. Larger studies with longer follow up periods are necessary to elucidate the temporal changes in diastolic parameters in these patients.

Compliance with the Ethical Standards

Ethics Approval: The study was approved by the Marmara University School of Medicine Research Ethics Committee on 28.06.2013 (approval number: 09.2013.0215). Written informed consent was obtained from all patients before entering the study.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

Authors contributions: MS, OY and NS: Concept and design, YB, KT and BO: Supervision, FB and JM: Data collection and/or processing, FB, IY and AC Analysis and interpretation. All authors read and approved the final version of the manuscript.

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The association of serum 25-hydroxyvitamin D levels with early neonatal morbidity and mortality in late preterm infants monitored in the neonatal intensive care unit

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ABSTRACT

Objective: We aimed to determine the association of serum vitamin D levels with early neonatal mortality/morbidity in late-preterm (LP) infants (born between 34 0/7 and 36 6/7 weeks of gestational age) monitored in the neonatal intensive care unit (NICU).

Patients and Methods: This retrospective study was conducted by reviewing live-born LP neonates from singleton pregnancies. The infants were monitored and treated in our hospital's NICU between June 2016 and June 2019.

Results: Maternal age at delivery was higher among infants with adequate vitamin D levels than among those with deficient vitamin D levels (P=0.007). A weak positive correlation was found between maternal age at childbirth and neonates' vitamin D levels (r=0.296). The rate of low-birth-weight deliveries was greater in babies with sufficient vitamin D levels than in those with deficient levels. There was a weak negative association between the number of stools on the day that the infants' serum 25-hydroxyvitamin D (25-OHD) levels were taken and their vitamin D level (P=0.027, r=-0.244).

Conclusions: A significant correlation was shown between serum 25-OHD levels and maternal age and low birth weight in LP neonates. Upon examining the influence of vitamin D levels on the number of defecations per day, no significant difference was detected; however, a weak negative association was identified between them.

Keywords: Late preterm, Mortality, Early morbidity, Vitamin D deficiency

1. INTRODUCTION

Infants with a gestational week (GW) of 34 0/7–36 6/7 or who were born between the 239th-259th postconceptional days are called late preterm (LP) infants [1]. For babies delivered without enduring the last 6 weeks of pregnancy, which is an important period related to the completion of foetal maturation, their major morbidity and mortality rates in the early neonatal period are higher than their rates in the term period. In contrast to term newborns, the resuscitation requirements at birth, respiratory problems during the neonatal period (respiratory distress syndrome (RDS), transient tachypnoea of the newborn (TTN), pneumonia), apnoea, hypoglycaemia, hyperbilirubinemia, sepsis, feeding intolerance, various neurological problems, and frequency of the need for rehospitalization are increased for LP neonates [2-4].

Vitamin D, being one of the fat-soluble vitamins, can be described as a group of sterols that have important roles in the bone-skeletal metabolism of the body and are also hormones and hormone precursors that are produced in the body [5]. The production of vitamin D occurs after its sterol derivative precursors in the skin are under the influence of sunlight, and the precursors transform into the active substance form by being converted in the liver and kidney. Vitamin D is regarded as a vitamin with hormonal properties due to its involvement in many important pathways, such as calcium (Ca) phosphorus (P) metabolism, cell growth, proliferation, development, anti-inflammatory processes, and insulin synthesis [6,7].

The vitamin D levels of premature infants are particularly low, as vitamin D has a short intrauterine half-life. Therefore, these infants are at risk for vitamin D deficiency. The third trimester is especially critical for the placental transfer of vitamin D [8].

Although, there are numerous publications in the literature that have investigated the relationship between 25-hydroxyvitamin D (25-OHD) levels of term and preterm infants and their different morbidities (newborn respiratory distress, bronchopulmonary

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dysplasia (BPD), acute lower respiratory tract infections, RDS, necrotizing enterocolitis (NEC), late-onset neonatal sepsis, retinopathy of prematurity), we were unable to find a study involving only LP infants [9-19]. Therefore, we aimed to determine the association of serum vitamin D levels with early neonatal mortality/morbidity in LP infants.

2. PATIENTS and METHODS

After the approval for this study was obtained from University of Health Sciences Istanbul Bakirkoy Dr. Sadi Konuk Training and Research Hospital Clinical Trials Ethics Committee dated 22.04.2019, and the protocol number was 2019/178; we retrospectively evaluated the medical records of LP neonates treated in the neonatal intensive care (NICU) between June 2016 and June 2019.

Live-born LP infants from singleton pregnancies who were between 34 0/7 and 36 6/7 weeks of gestational age admitted to the NICU were included in this study. Multiple birth babies, those with chromosomal anomalies determined via genetic tests and those diagnosed with perinatal asphyxia were excluded. A total of 83 newborns were included in the study.

The demographic characteristics of the patients, maternal data, anthropometrical and the newborns' clinical parameters that were recorded in the delivery room, clinical data from the NICU, and laboratory data were obtained from our hospital's electronic health record system, the NICU physician and nurse clinical record files, and the delivery room newborn close follow-up baby registration forms.

Serum concentrations of 25-OHD, Ca, P, Mg, and alkaline phosphatase (ALP) were obtained from the data collected in the biochemistry laboratory of our hospital by reviewing the electronic records retrospectively.

When calculating the cumulative vitamin D replacement (parenteral/enteral) dose (IU) that was administered to the baby, on the day the serum 25-OHD level was measured, the doses of any oral multivitamin supplements, along with any vitamin D supplementation that was given intravenously to the neonate through total parenteral nutrition (TPN), were taken as the basis. The amount of vitamin D contained in these drugs was recorded from the original package insert information.

By dividing the LP infants included in the study into 3 groups according to their serum 25-OHD level, the infants were categorized as vitamin D deficient (< 20 ng/ml), insufficient (20-30 ng/ml), and adequate (30-100 ng/ml), and the data were compared statistically.

Statistical Analysis

Data were analysed with SPSS for Windows version 21.0. The distribution of the variables was evaluated with the coefficient of skewness, kurtosis coefficient and the Kolmogorov–Smirnov test. As the continuous variables did not follow a normal distribution in at least one of the groups, nonparametric tests were utilized. For descriptive statistics, the median (min-max) was used to describe continuous variables, and the categorical variables were described with numbers and percentages. The significance of the difference between more than two independent groups was examined using the Kruskal–Wallis test. In the pairwise comparisons between groups, the Mann–Whitney U test was performed to evaluate the variables after the application of the Bonferroni correction. Regarding evaluating the categorical variables, the differences between the groups were assessed via the Pearson's chi-square test and Fisher's exact test. For relationships between nonnormally distributed variables, the correlation coefficients and statistical significance were determined using Spearman's test. All calculations were two-tailed, and a p value of <0.05 was considered statistically significant.

3. RESULTS

Of the 83 infants included in the study, 37 (44.6%) were girls, and 46 (55.4%) were boys. The mean length of gestation was 34.9±0.83 weeks. Twenty-five (30.1%) LP infants were born via spontaneous vaginal birth, and 58 (69.9%) were born by c-section. The average age of the mother at childbirth was 29.9±6.8 years. The mean birth weight of the LP infants was 2471±530.1 g, their mean length was 45.2±2.4 cm, and their average head circumference was 32.5±1.6 cm on average.

The mean APGAR scores of the babies at 1 minute and 5 minutes were 7.1±1.2 and 8.4±0.95, respectively. The indications for admission to the NICU were subclassified as respiratory system diseases, infections, nutritional problems, neonatal jaundice, infant of a diabetic mother and other indications. The most commonly identified indication for hospitalization was respiratory system diseases (congenital pneumonia, TTN, RDS, pneumothorax), involving 68.7% of the infants (n=57).

The average postnatal age of the newborns on admission to the NICU was 15.29±44.9 hours. The mean length of their NICU stay was 17±8.5 days. Noninvasive respiratory support (continuous positive airway pressure (CPAP)/intermittent mandatory ventilation (nasal IMV)) (44.6%) was the method of respiratory support that was most frequently applied. The mean time duration of receiving TPN support was 6.1±4.3 days, that of antibiotic administration was 12±6.7 days and that of phototherapy was 4.1±2.7 days on average. Blood samples from the babies for serum 25-OHD levels were collected on approximately the 9.5±4.3th day of hospitalization. The mean daily urinary frequency of the infants on the day of the serum 25-OHD level measurement was detected was 7.6±1.2, and the daily average number of stools was 3.6±1.7. Upon discharge from the NICU, all of the neonates could feed on their mother's breast.

The mean serum 25-OHD level of the babies was 18.8±12.1 ng/ml, and the simultaneous obtained values of Ca, P, Mg, and ALP on average were 9.7±1 mg/dl, 5.8±1.3 mg/dl, 1.9±0.2 mg/dl, and 250.4±107 U/L, respectively.

Vitamin D was shown to be deficient (group 1) (n=54) in 65.1% of the infants, insufficient (group 2) (n=17) in 20.5%, and sufficient (group 3) (n=12) in 14.5%.

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The maternal demographic, clinical and obstetric data were compared according to the infants' vitamin D levels (Table I). The age of the mother at childbirth was revealed to be significantly different in the 3 groups based on the vitamin D levels (P=0.025). The maternal age at the time of delivery was found to be higher in the infants with sufficient vitamin D levels than in the infants with deficient levels, and this difference was statistically significant (P=0.007). A weak positive correlation was detected between the age of the mothers at childbirth and the vitamin D level (P=0.007 and r=0.296). The vitamin D levels increased with increasing maternal age. There was no statistically meaningful difference between the maternal clinical and obstetric data and the vitamin D levels (P>0.05) (Table I).

Table I. *Comparison of the maternal demographic, clinical and obstetric data of late preterm infants based on serum 25-hydroxyvitamin D levels*

Characteristics	Group 1 (n = 54)	Group 2 (n = 17)	Group 3 (n = 12)	P value
Age of the mother at childbirth (mean ± SD)	28.6 ± 6.5	30.5 ± 7.2	34.5 ± 5.8	0.025*
Mode of delivery				0.83**
NSD	16 (29.6)	6 (35.3)	3 (25)	
C-Section	38 (70.4)	11 (64.7)	9 (75)	
Presence of maternal illness prior to pregnancy[1]				0.09***
No	41 (85.4)	11 (73.3)	7 (58.3)	
Yes	7 (14.6)	4 (26.7)	5 (41.7)	
Presence of maternal gestational diseases and pregnancy-related complications[2]				0.76**
No	28 (58.3)	8 (50)	7 (63.6)	
Yes	20 (41.7)	8 (50)	4 (36.4)	
Oral multivitamin replacement during pregnancy				0.75**
No	25 (47.2)	9 (52.9)	7 (58.3)	
Yes	28 (52.8)	8 (47.1)	5 (41.7)	
Smoking during pregnancy				0.1***
Absent	41 (85.4)	15 (100)	9 (75)	
Present	7 (14.6)	0 (0)	3 (25)	

Values are given as n (%).
**Kruskal–Wallis test, **Pearson's chi-square test, ***Fisher's exact test*
[1]Presence of maternal illness prior to pregnancy: Diabetes, hypertension, thyroid dysfunction (hypothyroidism), kidney diseases, digestive conditions (gastritis and gastroesophageal reflux disease (GERD)), rheumatic disorders, haematological findings (thrombocytopenia), neurological conditions (migraine, epilepsy), respiratory disorders (asthma), cardiovascular diseases.
[2]Maternal gestational diseases and pregnancy-related complications: Preeclampsia, eclampsia, placental abruption, placenta previa, gestational hypertension, gestational diabetes, gestational cholestasis, urinary tract infection during pregnancy, anaemia during pregnancy, hypothyroidism during pregnancy
Group 1: Vitamin D deficiency of the infants, Group 2: Vitamin D insufficiency of the infants, Group 3: Vitamin D sufficiency of the infants

The birth weights of the babies were divided into 2 groups: normal (2500-3999 g) and low birth weight (<2500 g). In group 3, 91.7% of the infants with adequate vitamin D had a low-birth-weight rate, 64% of them in group 2 had a low-birth-weight rate and insufficient levels, 51.9% of the infants in group 1 had a low birth weight rate and vitamin D deficiency, and a statistically significant difference was observed between the groups (P=0.036). The percentage of low-birth-weight infants in group 3 was greater than that in group 1. A weak negative correlation was identified between the vitamin D level and birth weight (P=0.01 and r=-0.280).

The sex, gestational age, birth weight (g), birth length (cm), birth head circumference (cm) and subclasses of these measurements in accordance with the Fenton curve, such as appropriate for gestational age (AGA), large for gestational age (LGA), and small for gestational age (SGA), were compared between the groups, and no statistically remarkable difference was detected (P>0.05).

No statistically significant difference was detected in the comparison of the infants' APGAR scores at 1 and 5 minutes after delivery (P>0.05).

The indications for admission to the NICU (respiratory diseases (congenital pneumonia, TTN, RDS, pneumothorax), infections (early-onset neonatal sepsis, late-onset neonatal sepsis, omphalitis, urinary tract infection), nutritional issues (feeding intolerance), neonatal jaundice (indirect hyperbilirubinemia), baby of a diabetic mother, others (intrauterine growth restriction (IUGR), major congenital anomaly, polycythaemia, congenital metabolic disease, neonatal convulsion, early neonatal transient hypoglycaemia) were contrasted across the groups based on their vitamin D levels, and no statistically meaningful difference was demonstrated (p> 0.05).

Comparisons of the number of daily bowel movements based on the day babies' serum 25-OHD levels were taken between the groups with respect to the vitamin D status, and no remarkable difference was detected (P=0.06) (Table II). However, a weak negative correlation was noted between the number of stools on the day of serum 25-OHD level measurement and the vitamin D level (P=0.027 and r=-0.244).

While reporting the type of hospital discharge from the NICU, most infants in the groups with deficient and insufficient vitamin D levels were discharged with improvement/full recovery, and the medical support requirement at the time of discharge persisted for 1 patient in the group with sufficient vitamin D levels (Table II).

There was no considerable difference identified across the groups when the clinical and laboratory data of the babies in the NICU were compared according to the serum 25-OHD levels (P>0.05) (Table II).

Table II. *Comparison of the neonatal intensive care unit clinical and laboratory data of late preterm infants according to the serum 25-hydroxyvitamin D levels*

Characteristic	Group 1 (n = 54)	Group 2 (n = 17)	Group 3 (n = 12)	P value
Age on admission to the NICU (hours)	13 ± 37	28 ± 73	8 ± 19	0.61*
Weight on admission to the NICU (g)	2500.6 ± 503.7	2325.3 ± 503.1	2249.6 ± 482.4	0.1*
Length of NICU stay (days)	17 ± 9.5	18.3 ± 7.1	15.3 ± 4.7	0.50*
Recurrent NICU admissions status, n (%)				0.15**
Present	1 (1.9)	2 (11.8)	0 (0)	
Absent	53 (98.1)	15 (88.2)	12 (100)	
Respiratory support requirement, n (%)				0.17*
None	11 (20.4)	6 (35.3)	2 (16.7)	
Free-flow blended oxygen	5 (9.3)	2 (11.8)	1 (8.3)	
CPAP/NIV	23 (42.6)	5 (29.4)	9 (75)	
Invasive mechanical ventilation	15 (27.8)	4 (23.5)	0 (0)	
TPN support (day)	6 ± 4.5	6.5 ± 4.8	6.1 ± 3	0.84*
Age at initiation of enteral nutrition (day)	2.6 ± 2.4	2.8 ± 2.9	2.2 ± 0.8	0.96*
Transition time to full enteral feeding (150–160 ml/kg/day)	8.9 ± 5.3	8.4 ± 6.1	8.3 ± 3.3	0.99*
Transition time to full at-breast feedings	9 ± 7.9	9.2 ± 7.4	8.3 ± 6.9	0.98*
Duration of antibiotic therapy (day)	12.5 ± 7.6	11.9 ± 5.7	10 ± 2	0.71*
Duration of phototherapy (day)	3.6 ± 2.2	5.5 ± 3.1	4.1 ± 3.3	0.08*
Mean arterial blood pressure value on test day (at 9 am) (mmHg)	52.9 ± 7.7	52.2 ± 9.2	47.3 ± 9.2	0.46*
Oxygen saturation on test day (SpO2) (%)	98.2 ± 1.9	98.3 ± 1.8	97.9 ± 3.2	0.89*
Heart rate on test day (beats/min)	137.2 ± 12.7	141.6 ± 14	136.5 ± 13.7	0.35*
Daily urinary frequency on test day	7.6 ± 1.1	7.2 ± 1.7	7.9 ± 0.3	0.54*
Number of daily bowel movements on test day	3.9 ± 1.6	2.9 ± 1.8	3.2 ± 1.5	0.06*
Age on test day (day)	9 ± 4.1	11.4 ± 5.7	8.7 ± 1.9	0.21*
Total vitamin D supplementation on test day (IU)	1521.6 ± 1742.2	1731.8 ± 1525.4	1165 ± 735	0.71*
Concurrent laboratory tests on test day				
Ca (mg/dl)	9.6 ± 1	9.7 ± 1	10.1 ± 0.8	0.33*
P (mg/dl)	5.6 ± 1.2	6.2 ± 1.2	6.1 ± 1.6	0.08*
Mg (mg/dl)	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	0.80*
ALP (U/L)	263.4 ± 116.3	221.8 ± 86.6	234 ± 84.5	0.36*

*Values were given as the mean ± standard deviation. Test day refers to the day of the serum 25-OHD level measurement. *Kruskal–Wallis test, **Fisher's exact test*

4. DISCUSSION

The age of the mother at childbirth was shown to be significantly different in the 3 groups that were based on the vitamin D levels. It was established that the age of the mother at the time of delivery was higher in those with sufficient vitamin D levels than in those with vitamin D deficiency. A weak positive correlation was demonstrated between the age of mothers at childbirth and their vitamin D levels. Consistent with our results, it has been reported in the literature that vitamin D deficiency, as measured using cord blood, is more common in babies who are born to younger mothers [20, 21]. In a study published in 2019, unlike our findings, mothers of newborns with low vitamin D levels were revealed to be older at the time of delivery [10]. There are also studies that did not identify a significant relationship between maternal age at childbirth and the serum 25-OHD levels of the mothers and the 25-OHD levels in the cord blood of their infants [8, 9, 15, 22-26].

In our study, we allocated neonates into two groups: normal (2500-3999 g) and low (<2500 g) birth weights. The rate of low birth weight was demonstrated to be 91.7% in group 3, where the patients had sufficient vitamin D, 64.7% in group 2 with insufficient levels, and 51.9% in group 1 with vitamin D deficiency, and a statistically significant difference was observed across the groups. The percentage of low birth weight was found to be higher in those who had sufficient vitamin D than in those with deficient levels. With regard to our study, a weak negative correlation was detected between vitamin D level and birth weight. Thus, it was shown that birth weight decreased as the

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vitamin D levels increased. In contrast to our findings, studies that have revealed a positive correlation between birth weight and serum vitamin D levels exist in the literature, and it is stated in these studies that maternal vitamin D deficiency during pregnancy elevates the risk of low birth weight and increases the number of SGA newborns [23, 24, 27-29]. However, there are also studies that have also identified no relationship between the serum 25-OHD levels of the mother during pregnancy or the serum 25-OHD levels in their infant's cord blood and the presence of normal or low birth weight [8, 15, 22]. Nevertheless, in a large-scale retrospective cohort study published in Southern China in 2021, it was established that the risk of macrosomic (birth weight ≥4000 g) births increases in babies whose mothers have low vitamin D levels during the second trimester of pregnancy [30]. This study supports our results.

When investigating the association of bowel movements per day, as a marker of intestinal motility [31], with serum 25-OHD levels, a weak negative correlation was detected between the number of stools on the day that the vitamin D level was measured and the serum vitamin D concentration. As the vitamin D level increased, the number of stools decreased. There was no study in the literature that has explored the relationship between serum 25-OHD levels and bowel movements of newborns, but the data indicating that VDR plays a role in cell proliferation, differentiation, and induction of apoptosis within the intestinal system endorse our findings [32].

On reviewing the relationship between the discharge from the NICU and the serum 25-OHD levels of infants in our study, discharge with improvement/full recovery was noted to be 100% in the groups with vitamin D deficiency and insufficiency. For the group with sufficient serum vitamin D concentrations, 91.7% were discharged with improvement/full recovery and 8.3% had other types of discharge (Orogastric tube, MV support, oxygen support). In our study, the mortality rate of the LP babies in the NICU was detected as 0%. That is, we have discharged all of our infants in the groups with different vitamin D levels home from the hospital; consequently, we cannot comment on the association of vitamin D levels with mortality. Upon examination of the literature, in a study conducted on very low birth weight (1250 g and below) newborns, discharge home with oxygen support was observed to be statistically higher among those with deficient 25-OHD vitamin levels. In the same study, no correlation was identified between the serum 25-OHD level and mortality rate at the hospital [9]. Another study, in which babies with a birth weight of less than 1500 g were included, reported that the death rate before discharge increased as the vitamin D level decreased [10].

In the comparisons of the maternal clinical characteristics, obstetric data and mode of delivery across the groups according to the serum 25-OHD level of the LP babies, no statistically remarkable difference was revealed in our study. Additionally, there are studies in the world literature demonstrating results similar to our data [8-10, 13, 15, 20, 21]. However, among different studies contrasting identical characteristics based on the 25-OHD vitamin levels, there are also studies that have presented significant results [24, 27, 33]. In our study, no statistically meaningful difference was noted between the maternal use of multivitamin supplements during pregnancy and the serum 25-OHD levels of LP infants. In a study investigating the relationship between vitamin D supplementation during pregnancy and the cord blood 25-OHD levels of newborns, no difference was identified, which is consistent with our study [21]. Contrary to our findings, in a study conducted with women who took low levels of vitamin D supplementation during pregnancy, the serum 25-OHD levels were found to be deficient in both the mothers and in the cord blood of their babies [26]. With respect to another study, a negative correlation was established between maternal vitamin D supplement intake during pregnancy and the serum 25-OHD level of the baby [10]. No statistically significant difference was found in our study between smoking during pregnancy and the serum 25-OHD levels of LP newborns, and these results are compatible with the literature [21].

In our study, no statistically meaningful difference was established comparing the sex of LP babies and their gestational age at delivery based on the serum 25-OHD levels. According to neonatal studies conducted at different gestational weeks, no correlation was detected between sex and the gestational age of the infant and maternal and newborn cord blood 25-OHD levels [8-10, 13, 15, 20-23, 26, 34]. Unlike our data, there are also studies in the world literature revealing significant results [35, 36].

When comparing the birth weight (g), birth length (cm), birth head circumference (cm) of LP babies and the subcategorized groups of these measurements between the groups and according to the Fenton curve using the AGA, LGA, SGA with serum 25-OHD levels of LP infants, there was no statistically significant difference observed across the groups in our study. In the literature, other studies have had similar [9, 10, 21, 26, 34] and dissimilar [10, 20, 27, 28] results compared to our study.

In our study, when the 1 – and 5-minute APGAR scores were compared based on the serum 25-OHD levels of LP babies, no statistically significant difference was found between the groups. Similarly, in the literature, no relationship was demonstrated between the APGAR scores at 1 and 5 minutes after birth and the serum 25-OHD levels of newborns [9, 15, 21]. In contrast, in one study, the 1-minute APGAR score diminished as the vitamin D level decreased, and the 5-minute Apgar score did not change [10].

In our study, when the indications for admission to the NICU were compared between the groups according to the serum 25-OHD levels of LP babies, no statistically significant difference was found. There are studies in the world literature similarly showing no association [8, 10, 19, 21, 34, 37, 38]. However, significant statistical results were also established in many studies that have investigated the relationship between different disease groups (RDS, BPD, pneumonia, sepsis, NEC, IUGR) and the serum 25-OHD levels of newborns, worldwide and in our country, in different age groups [9-13, 15, 16, 18, 34].

On review of the association between the NICU clinical data of LP babies and serum 25-OHD levels, different results were noted for each parameter. First, there was no statistically significant

difference detected between the groups when comparing LP infants in terms of age on admission to the NICU, weight on admission, recurrent admissions status, and length of NICU stay according to the serum 25-OHD levels. In local and international studies, similar results to our study [8, 9, 21] and other studies [10, 13, 39] have been reported.

In our study, comparing the respiratory support requirement of LP babies in NICU, duration of receiving TPN support, time at initiation of enteral nutrition, transition time to full enteral feeding, transition time to full breast feedings, durations of antibiotic treatment and phototherapy between the groups based on the 25-OHD levels, no statistically remarkable difference was observed. In many studies, such as our study, no relationship was found between the need for respiratory support and the serum 25-OHD levels of newborns [8, 9, 13, 39]. In another study, it was demonstrated that with decreasing serum 25-OHD levels of infants, the length of stay on mechanical ventilation as well as the durations of receiving noninvasive ventilation and total supplemental oxygen support were increased [10]. Additionally, no correlation was identified between the 25-OHD level of cord blood and the rate of phototherapy in the literature [21].

Serum creatinine, blood urea nitrogen and urinary frequency are indicators of kidney function [40]. In our study, we also reviewed the relationship between the number of times that the LP infants urinated on the day that the serum 25-OHD levels were taken and the vitamin D levels, and we did not find a statistically significant difference. There was no study identified in the literature that has investigated the relationship between the serum 25-OHD levels of newborns and urinary frequency.

On examination of the association between discharge from the NICU and the serum 25-OHD levels of LP babies, 100% of the patients in the group with vitamin D deficiency had discharge with improvement/full recovery, as for the group with sufficient serum vitamin D levels, 91.7% were discharged with improvement/full recovery and 8.3% had other forms of hospital discharge (Orogastric tube, MV support, oxygen support). In our study, the mortality rate of LP newborns in the NICU was detected as 0%. When the infants with very low birth weight (1250 g and below) were compared according to the serum 25-OHD levels, those with vitamin D deficiency were found to be discharged home more often with supplemental oxygen. Moreover, in this study, no correlation was detected between the serum 25-OHD levels and mortality at the hospital [9]. Contrary to our data, in a study conducted with babies with a birth weight of <1500 g, it was demonstrated that the mortality rate prior to discharge increased as the vitamin D levels decreased [10].

In our study, when the NICU clinical and laboratory data of LP babies were compared between the groups according to the 25-OHD levels, no statistically significant difference was found, and no study that has investigated this relationship was found in the literature.

There was no statistically remarkable variation detected in our study. When the LP babies' postnatal age on the day that the serum 25-OHD level was taken, the total vitamin D supplementation (IU) and simultaneous laboratory tests (Ca, P, Mg, ALP) were compared with respect to the vitamin D levels. In line with our study, no correlation was noted between the vitamin D levels and serum concentrations of Ca, P, Mg, and ALP in two studies [20, 26].

Conclusion

We examined the association of maternal and neonatal demographic and clinical data with the serum 25-OHD levels in LP infants. By reviewing the parameters, we determined that the serum vitamin D levels of the babies rose with increasing maternal age. Higher vitamin D levels were observed in infants born with low birth weight in comparison to those delivered with a normal birth weight. We demonstrated a weak negative correlation between the number of stools per day and the vitamin D level. A limitation of our study is that the vitamin D levels of the neonates were not measured from cord blood because of the retrospective nature of the study.

Compliance with Ethical Standards

Ethical approval: Ethical approval was obtained from University of Health Sciences Istanbul Bakirkoy Dr. Sadi Konuk Training and Research Hospital Clinical Trials Ethics Committee dated 22.04.2019, and the protocol number was 2019/178. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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The effect of laparoscopic sleeve gastrectomy on metabolic syndrome parameters during one year of follow-up

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ABSTRACT

Objective: We aimed to evaluate the effectiveness of laparoscopic sleeve gastrectomy (LSG) as a treatment method for morbid obesity and its impact on reducing the incidence of metabolic syndrome and its components.

Patients and Methods: This retrospective and a single-center study included patients with obesity who underwent LSG and were followed up at an endocrinology and metabolism outpatient clinic for at least one year. Anthropometric measurements, blood pressure, and blood examinations including fasting plasma glucose and lipid profile were assessed before the surgery and one year after the surgery. The presence of metabolic syndrome and related comorbidities was documented.

Results: The study included 62 patients, with a mean age of 38.2 ± 8 years and a female predominance (88.7%). At one year post-surgery, significant improvements were observed in body weight, waist circumference, blood pressure, and metabolic parameters ($P<0.001$ for all). The prevalence of metabolic syndrome decreased from 66.1% to 6.5% ($P<0.001$). The prevalence of diabetes, hypertension, and hepatosteatosis also decreased significantly ($P<0.05$).

Conclusion: Laparoscopic sleeve gastrectomy demonstrates substantial weight loss and positive effects on metabolic syndrome components. The procedure appears to be an effective intervention for obese patients with obesity-related comorbidities. Longer-term prospective studies are needed to further validate these promising results.

Keywords: Obesity, Bariatric surgery, Metabolic syndrome

1. INTRODUCTION

Obesity, with its multifactorial nature and complex pathogenesis, is notably recognized as a major risk factor for cardiometabolic diseases [1,2].

The widespread adoption of technological advancements and Western lifestyles, the shift towards high-calorie diets combined with a decrease in physical activity and the transition from rural to modern urban living have all contributed to a global rise in obesity prevalance across all age groups [3].

The most recent epidemiological findings reveal concerning patterns, with over 30% of the global population currently classified as overweight or obese. Based on the observed increase in obesity rates, projections suggest that approximately 38% of the population will be overweight and an additional 20% will be obese by the year 2030 [4].

Contemporary medical understanding views obesity not merely as a standalone condition but also as a component and a catalyst of metabolic syndrome, as outlined in the American Heart Association and the National Heart, Lung, and Blood Institute (AHA-NHLBI) joint consensus report of 2009 [5]. Metabolic syndrome is considered a pandemic affecting approximately 20% to 30% of the adult population in many countries, and it is frequently associated with obesity [6]. In our country, the prevalence of metabolic syndrome in the general population has been reported to be 32.9% [7].

Obesity treatment involves employing diverse methods, including diet, exercise, behavioral therapy, medical interventions, and bariatric/metabolic surgery. As obesity is a significant risk factor for cardiometabolic comorbidities such

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as diabetes, hypertension, and coronary artery disease, the methods used in obesity treatment should not only lead to anthropometric improvements but also provide benefits for these comorbidities. One of the bariatric surgery techniques, laparoscopic sleeve gastrectomy (LSG) has shown to be effective in achieving lasting weight loss and improving obesity-related comorbidities [8]. In this study, we aimed to compare the anthropometric measurements and metabolic parameters of obese patients who underwent LSG before the surgery and one year after the procedure.

2. PATIENTS and METHODS

Study population

This is a single-center, cross-sectional, and retrospective study conducted on patients who were under follow-up in the endocrinology and metabolism department of our hospital and underwent LSG between January 2018 and January 2022 with a body mass index (BMI) over 40 kg/m², or a BMI between 35 and 40 kg/m² with serious comorbidities, according to the International Federation for the Surgery of Obesity (IFSO) criterion [9].

Patients between the ages of 18 and 65 years who attended regular appointments at our endocrinology and metabolism outpatient clinic for at least 6 months before the surgery and at the 12th months after the surgery were included in the study.

Patients under the age of 18 and over the age of 65, those who did not attend regular appointments at the endocrinology and metabolism outpatient clinic beyond the postoperative 1-year follow-up period, individuals using medications that could affect body weight and metabolic parameters during this time, patients with newly diagnoed endocrinological diseases, and those with incomplete anthropometric measurements or missing information about metabolic parameters in their medical records were excluded from the study.

The study was conducted in accordance with ethical principles outlined in the Helsinki Declaration with the approval of the local ethics committee (Number: 2022/0617). The study protocol ensured the confidentiality of patient information.

Clinical assessment

The data of the participants included in the study were collected through a retrospective examination of the hospital's central information system, e-nabız (electronic health records system), and patient files.

During the pre – and post-operative appointments at the endocrinology and metabolism clinic, each participant responded to a standardized questionnaire, providing details about their sociodemographic background, personal and family medical history (including hypertension, diabetes, coronary artery disease, and hyperlipidemia), and smoking habits.

The participants' height (in centimeters) and weight (in kilograms) were measured using a height scale and weight machine (SECA 799+220, seca GmbH & Co, Germany). The BMI was calculated using the formula: weight in kilograms divided by height in meters squared. Waist circumference was measured with a non-flexible measuring tape at the narrowest point of the abdomen between the lower part of the last rib and the top of the hip at the end of expiration, while hip circumference was measured at the widest part of the hips.

Laboratory examinations were performed after 8-12 hours of overnight fasting, including triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, hemoglobin A1C (HbA1C), and thyroid function tests, using the American AU480 automatic biochemical analyzer.

Blood pressure was measured using an electronic sphygmomanometer (HEM-7155, Omron, Japan), after the patients rested for 5-10 minutes.

Individuals with and without metabolic syndrome were identified based on the criteria outlined in The US National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATP III) [10]. Diagnoses of hypertension, diabetes mellitus, hyperlipidemia, and hepatosteatosis were also documented.

Liver imaging

Before the surgical procedure and at the 12 months post-surgery, an abdominal ultrasound (US) was performed. Hepatosteatosis in the US was classified using the following grading system:

Grade 1 (mild): A slight and diffuse increase in liver echogenicity with normal visualization of the diaphragm and portal vein wall.

Grade 2 (moderate): A moderate increase in liver echogenicity with slightly impaired appearance of the portal vein wall and the diaphragm.

Grade 3 (severe): Marked increase in liver echogenicity with poor or no visualization of the portal vein wall, diaphragm, and posterior part of the right liver lobe.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) 28.0 software was used for all the analyses. Descriptive statistics of the characteristics measured in the study participants were tabulated as mean, standard deviation (SD), number and % frequencies. The compatibility of the numerical characteristics with the normal distribution was examined with the Kolmogorov-Smirnov test. For the analysis of dependent quantitative data, paired sample t-tests and Wilcoxon tests were used. The McNemar test was used for the analysis of dependent qualitative data. $P<0.05$ was considered statistically significant.

3. RESULTS

In this study, we conducted a retrospective review of 144 patients with obesity who underwent LSG surgery at our hospital. Among these patients, 22 did not attend regular endocrinology and metabolism outpatient clinic appointments after the surgery, and 50 could not be reached by phone to obtain their consent, leading to their exclusion from the study. Additionally,

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8 patients were excluded from the study due to the diagnosis of hypothyroidism during follow-up, and 2 died after the surgery.

Finally, the study included 62 patients, with a mean age of 38.2 ± 8 years and a predominance of females (88.7%). During the preoperative period, the patients had a mean BMI of 45.7 ± 4.9 kg/m^2, a mean waist circumference of 136.6 ± 14.4, and 66.1% of them were diagnosed with metabolic syndrome. Additionally, 22.6% had hypertension, and 12.9% were being followed up for diabetes mellitus. Hepatosteatosis was observed in 77.4% of patients based on US imaging.

When examining Table I, there is a significant decrease in BMI, waist circumference, systolic, and diastolic blood pressure at postoperative 1 year compared with the preoperative period (P<0.001). The frequency of obesity-related comorbidities before the surgery and one year after the surgery is presented in Figure 1. According to the data, the frequency of metabolic syndrome decreased from 66.1% to 6.5% (P<0.001). The prevalence of diabetes (P=0.039), hypertension (P=0.039), and hepatosteatosis (P<0.001) also significantly decreased one year after the surgery compared with the preoperative period.

Table I. *Comparison of anthropometric measurements and blood pressure measurements before and 1 year after LSG*

Variable	Preoperative (Mean ± sd)	Postoperative (Mean ± sd)	P
Systolic blood pressure (mmHg)	129.4 ± 18.9	117.6 ± 16.8	<0.001
Diastolic blood pressure (mmHg)	81.6 ± 12.10	72.8 ± 10.4	<0.001
Weight (kg)	122.6 ± 18.2	79.0 ± 14.7	<0.001
Body mass index (kg/ m^2)	45.7 ± 4.9	29.6 ± 5.3	<0.001
Waist circumference (cm)	136.6 ± 14.4	95.4 ± 13.4	<0.001

Table II. *Comparison of laboratory values before and 1 year after LSG surgery*

Variable	Preoperative (Mean ± sd)	Postoperative (Mean ± sd)	P
Fasting glucose (mg/dl)	99.2 ± 16.7	86.5 ± 10.6	<0.001
HOMA-IR	4.3 ± 2.3	1.6 ± 1.5	<0.001
Triglyceride (mg/dl)	166.2 ± 77	103.8 ± 42.5	<0.001
HDL-cholesterol (mg/dl)	44.3 ± 10.5	62.3 ± 10.5	<0.001
LDL-cholesterol (mg/dl)	110.8 ± 32.8	106.2 ± 35.2	<0.001
AST(U/L)	24.7 ± 15.8	15.2 ± 3.8	<0.001
ALT (U/L)	31.2 ± 24.1	12.3 ± 7.8	<0.001
GGT (U/L)	26.5 ± 25.9	11.4 ± 6.3	<0.001
ALP (U/L)	74.2 ± 18.7	65.4 ± 20.0	<0.001
Creatinine (mg/dl)	0.8 ± 0.7	0.7 ± 0.5	0.002
Uric acid (mg/dl)	5.0 ± 1.6	4.0 ± 1.1	<0.001
Sodium (mmol/L)	139.3 ± 1.8	140.6 ± 2	0.001

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma-Glutamyl Transferase

Laboratory values before the surgery and one year after the surgery are presented in Table II. When comparing the laboratory findings related to metabolic syndrome, there was a significant decrease in fasting blood glucose and triglycerides, as well as a significant increase in HDL-cholesterol (P<0.001). Further biochemical test results are provided in Table II for comparative analysis.

When evaluating postoperative complications, 1 (1.6%) patient had anastomotic leakage, 1 (1.6%) had fever, and 1 (1.6%) had haemorrhage. There was no detection of stenosis in any of the patients.

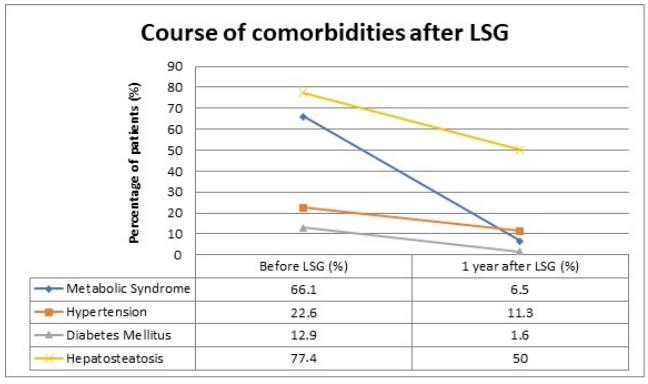

Figure 1. *Comparison of comorbidity prevalence before and after laparoscopic sleeve gastrectomy (LSG)*

4. DISCUSSION

In this study, patients with obesity who underwent LSG at our hospital and followed up by the endocrinology and metabolism clinic were retrospectively evaluated. After a 1-year follow-up period, improvements were observed in anthropometric measurements, blood pressure, and metabolic parameters compared with the preoperative period. Additionally, the frequency of patients being followed up for hepatosteatosis, metabolic syndrome, diabetes, and hypertension decreased.

Obesity is a chronic, systemic disease that requires a multidisciplinary approach for both diagnosis and treatment. It rarely resolves spontaneously and is associated with increased morbidity, mortality, and decreased quality of life [11].

The initial treatment typically includes diet modifications, behavioral adjustments, regular exercise, and, if necessary, medical interventions [12]. However, a significant number of patients who lose weight through these methods tend to regain weight over time. A meta-analysis of 29 long-term weight loss studies found that more than half of the lost weight was regained within two years, and by five years, more than 80% of the lost weight was regained [13].

Bariatric surgery is widely recognized as the most effective method for the sustainable weight loss today. Among the various bariatric surgical techniques, LSG is considered the most commonly used approach due to its ease of application from a

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surgeon's perspective, minimal impact on nutrient absorption, and absence of dumping syndrome induction [14].

In our study, a significant reduction in body weight was observed after 1 year postsurgery, consistent with previous studies [15,16]. However, the aim of bariatric surgery is not only to achieve weight loss but also to reduce obesity-related cardiometabolic risks. Therefore, in our study, we examined the effects of LSG on metabolic syndrome components. Before the surgery 41 patients (66.1%) had metabolic syndrome, while in our postoperative 1-year evaluation, only 4 patients (6.5%) were found to have metabolic syndrome (P<0.001). In a study by Péquignot et al., where they evaluated 241 patients with a preoperative BMI mean of 47.2 kg/m^2, they observed metabolic syndrome in 36 patients before LSG, and at postoperative 1 year, 17 patients had metabolic syndrome, showing a significant difference [15]. Similarly, in the retrospective study conducted by Wojciak et al., involving 211 patients, it was observed that one year after LSG, there was improvement in all metabolic syndrome parameter [17].

In our study, the preoperative prevalence of diabetes mellitus was 12.9%, which dropped to 1.6% (P=0.039). In a study conducted by Smeu et al., on early glycemic control after LSG, they found a significant improvement in glycemic control from 6 months postoperatively compared with the preoperative period [18]. These findings provide support for the therapeutic effect of LSG on type 2 diabetes.

Systolic and diastolic hypertension are components of metabolic syndrome. According to the NCEP/ATP-III criteria, having a blood pressure of ≥130/85 mmHg is a diagnostic criterion for metabolic syndrome. Therefore, a decrease in blood pressure is expected as an outcome of obesity treatment. In our study, it was observed that the prevalence of hypertension significantly decreased one year after the surgery compared with the preoperative period. In a review by Graham et al., involving 14 studies and a total of 3550 patients, the prevalence of hypertension among patients in the preoperative period was 36.5%, which decreased to 14.79% in the 5th postoperative year [19]. In the study conducted by Samson et al. with 870 patients who underwent LSG, they observed a significant decrease (P<0.001) in both diastolic and systolic blood pressure levels during the postoperative period compared with the preoperative period, which is consistent with the findings of our study [20]. Considering these results together, it becomes evident that LSG is an effective intervention for obese people with hypertension.

Dislipidemia is an atherosclerotic risk factor, making it an important component of metabolic syndrome. In our study, a significant decrease in triglycerides and a significant increase in HDL-cholesterol were observed, but no significant changes were found in LDL-cholesterol. Sharma et al., conducted a study involving 134 patients with similar characteristics, out of which 71 underwent LSG, while the remaining 63 were followed up as the control group. After a 3-year follow-up, they found a significant (P<0.01) decrease in plasma triglyceride levels and a significant (P<0.001) increase in HDL-cholesterol levels among those who underwent LSG compared to the control group [21]. In a systematic review involving 3997 patients, Shabbir et al., demonstrated that LSG had a significant effect on HDL-cholesterol and triglyceride levels, while it did not show a significant impact on LDL-cholesterol levels [22].

Hepatosteatosis is frequently associated with obesity and is evaluated using USG in clinical practice. In our study, hepatosteatosis was detected at various stages in 48 patients (77.4%) during the preoperative period. At the end of the first year postoperatively, hepatosteatosis was not observed in 17 of these patients. Additionally, none of the patients who had normal US imaging during the preoperative period were found to have hepatosteatosis in the postoperative period. In a study involving 67 morbidly obese patients, Salman et al., found a significant reduction in the degrees of hepatic steatosis, hepatocellular ballooning, and lobular inflammatory changes following LSG [23]. In a study conducted by Elyasinia et al., it was demonstrated that in 50% of the patients who underwent LSG, the levels of hepatosteatosis significantly decreased [24]. These findings support the therapeutic effect of LSG on hepatosteatosis.

The Fourth International Consensus Summit on Sleeve Gastrectomy, involved a survey of 46133 cases, which revealed favorable complication rates, including a 1.1% incidence of high leaks, 1.8% for hemorrhages, and 0.9% for stenosis [25]. In our study, among the 62 patients who underwent LSG, leakage was recorded at a rate of 1.6%, bleeding at a rate of 1.6%, and fever at a rate of 1.6%. There were no cases of stenosis observed as a postoperative complication. When compared to the literature, our complication rates were found to be at an acceptable level. While two patients excluded from the study due to mortality were known to have experienced postoperative complications, further detailed information could not be obtained.

The main limitation of our study is the relatively short follow-up period, which was limited to 1 year after the LSG procedure. However, data collection is still ongoing for many patients who participated in this study and newly recruited patients, which will be assessed in future studies. Another limitation of our study is the retrospective design, which resulted in the inability to access the data of some patients who underwent surgery at the 1-year follow-up. As a consequence, the number of eligible patients included in the study was relatively small.

In conclusion, LSG proves to be an effective treatment for morbid obesity. Our study results suggest that LSG is promising also in reducing the incidence of metabolic syndrome and its components. Nevertheless, further studies with a longer follow-up period and prospective design are required to validate the findings of this study.

Compliance with the Ethical Standards

Ethics Committee approval: The study was conducted in accordance with ethical principles outlined in the Helsinki Declaration with the approval of the local ethics committee (approval number: 2022/0617). The study protocol ensured the confidentiality of patient information.

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Conflict of interest: The authors have no potential conflicts of interest to disclose.

Authors contributions: MB: Study design, literature review, MT: Literature review, supervision, CT: Data analysis, writing, MS: Research, study design. All authors approved the final version of the manuscript.

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Effects of virtual reality usage on kappa angle, accommodation, pupil, depth perception, and examination of the relationship of these parameters with discomfort perception

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ABSTRACT

Objective: This study aims to examine the effects of virtual reality (VR) usage on the eyes and investigate the parameters responsible for the subsequent discomfort sensation.

Materials and Methods: This prospective study enrolled 20 healthy volunteers who were engaged in a 10-minute VR game session. Refractive errors, kappa angles, phoria presence, accommodative responses, and scotopic, mesopic, and photopic pupillometry values were recorded before and after using VR. A Virtual Reality Sickness Questionnaire (VRSQ) was applied to assess discomfort, and the relation with evaluated parameters was investigated.

Results: Twenty volunteers (mean age 29.80±0.57 years) included 11 females (55%) and 9 males (45%). The mean spherical equivalent refractive error was – 1.94±0.28 diopters and 5 (25%) volunteers had phoria. Average kappa angles were 0.23±0.02 mm (x-axis) and 0.11±0.01 mm (y-axis). Post-VR, the median [(interquartile range (IQR)] stereopsis decreased from 30 (30-60) to 60 (60-60) arc seconds (P<0.001). Pupil sizes increased significantly across all lighting conditions (P<0.001). Accommodation did not significantly change post-VR (P>0.05). VRSQ scores correlated positively with phoria and kappa-x angle (r=0.458, P=0.003 and r=0.330, P=0.038) while negatively with stereopsis and kappa-y angle (r=-0.375, P=0.017 and r=-0.326, P=0.04).

Conclusion: Virtual reality use reduces depth perception and induces significant mydriasis across lighting conditions. Post-VR discomfort feeling may be related to phoria, kappa angle, and stereopsis.

Keywords: Virtual reality, Accommodation, Kappa, Stereopsis, Pupillometry

1. INTRODUCTION

As technology and internet usage continue to advance, the time spent looking at screens close has steadily increased. In parallel, utilizing virtual reality (VR) headsets has emerged as a promising trend. These headsets provide users with a more immersive experience by creating a sense of three-dimensional vision that separates the view of each eye. Consequently, the adoption of VR technology has increased, offering users an enhanced and realistic experience.

Virtual reality headsets position the screen only 15 cm away from the eyes. This proximity triggers various ocular responses, including pupillary constriction, ciliary muscle contraction, lens adjustment, and extraocular muscle convergence [1]. Accommodation, the mechanism by which the eyes adapt to focus on a near object's image onto the retina, is a complex reflex involving processes like convergence of the eyes inward, increased lens refractive power, and pupil constriction. Failure to achieve proper accommodation results in blurred vision, leading to a condition known as accommodative dysfunction. Individuals experiencing this dysfunction may report symptoms like blurred near vision, headaches, visual fatigue, and other asthenopic discomforts [2].

The angle between the anatomical axis of the eye and the visual axis is referred to as the kappa angle. A normal kappa angle indicates a slight outward deviation of the eyes, allowing each eye to slightly diverge outward [3]. However, this angle varies between individuals and can affect convergence and accommodation mechanisms. Increased kappa angles are associated with more significant outward eye divergence and potentially increased accommodation for convergence.

Virtual reality usage involves continuously changing focus due to dynamic visual stimuli. As a result, users need to constantly adjust their focus through near convergence and accommodation, leading to symptoms like discomfort, fatigue, headaches, blurred vision, and temporary dizziness when

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using VR. Scoring systems such as the Virtual Reality Sickness Questionnaire (VRSQ) have been developed to quantify this subjective discomfort [4].

Previous studies have investigated the effects of VR usage on visual parameters such as accommodation, vergence, stereopsis, refractive errors, pupil size, intraocular pressure, and choroidal thickness [5-8]. The central inquiry has been whether the increasing adoption of VR is linked to increased myopia progression and its implications for eye health. Additionally, the discomfort associated with VR usage and its correlation with these parameters remains an important research focus.

This study aims to assess the discomfort that can arise from a short-duration gameplay using VR headsets and investigate the relationship between this discomfort and various ocular parameters that could trigger it. To achieve this goal, volunteers engaged in a 10-minute VR game session, and parameters such as refractive errors, accommodation levels, horizontal and vertical kappa angles, stereopsis (depth perception) ratios, as well as static and dynamic pupillometry readings under scotopic, mesopic, and photopic conditions were evaluated both before and after gameplay. The primary objective is to examine the correlation between these parameters and the volunteers' VRSQ scores.

2. MATERIALS and METHODS

Study Design

This prospective study was conducted between January 2023 and February 2023 at the Neuro-Ophthalmology Division, Ophthalmology Department of Marmara University, School of Medicine. The study was designed in accordance with the principles of the Helsinki Declaration and received approval from the Marmara University Faculty of Medicine Clinical Research Ethics Committee under approval number 03.02.2023.336. Written informed consent was obtained from all the participants.

Participants

Residents attending the Ophthalmology Residency Program, aged 18 and above who volunteered for the study and consented to play a 10-minute game using a VR headset, were included. Participants with arrhythmias, tachycardia, bradycardia, hypertension, individuals over 45 years of age due to potential impact on accommodation, those with amblyopia, absence of stereopsis, history of corneal, uveitic, glaucomatous, retinal, or neuro-ophthalmological diseases, dry eye syndrome, epilepsy, migraine, history of motion sickness (car, ferry, airplane, etc.), vertigo, or vestibular dysfunction were excluded.

Procedure

All participants played the "Beat Blaster for Oculus" game for 10 minutes using the Oculus Quest 2 (Meta, USA) device. Prior to gameplay, demographic data, including age and gender, were collected. Additionally, pre-game measurements included refractive error measurements (spherical equivalent, diopters) using an auto-refractometer (TonoRef III, Nidek Co., Japan), accommodation measurements (diopters) using the same device, presence of esophoria or exophoria using the cover test, stereopsis values (arc seconds ["]) using the TNO Stereo Test, static and dynamic pupillometry readings (millimeters) under scotopic, mesopic, and photopic conditions using the Sirius corneal topography device CSO (Costruzione Strumenti Oftalmici, Florence, Italy), horizontal and vertical kappa angle values based on the pupil center using optical biometry device (Lenstar, Haag-Streit, USA). The participants who had refraction errors used contact lenses during VR gaming.

Post-Game Assessments

After the 10-minute gameplay, participants were surveyed using the VRSQ to assess discomfort levels with nine questions. This questionnaire included questions about general discomfort, fatigue, eyestrain, and difficulty focusing, which evaluated the "Oculomotor discomfort – C score". Additionally, it had questions about the level of headache, fullness of head, blurred vision, dizziness with eyes closed, and vertigo, which evaluated "Disorientation discomfort – D score" [4]. Participants' accommodation levels, stereopsis degrees, and static and dynamic pupillometry readings under scotopic, mesopic, and photopic conditions were measured again to assess changes in these parameters. Lastly, the relationships between initial and post-game parameter measurements, their differences, and VRSQ scores were examined.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 24.0 was used for data analysis. Data distribution was assessed using the Shapiro-Wilks test. Categorical data were presented as numbers (percentages), parametric data as mean ± standard deviation, and non-parametric data as median and interquartile range (IQR). The Mann-Whitney *U* test was used for comparing non-parametric data. The Spearman correlation test was used to assess correlations between parameters. $P<0.05$ was considered statistically significant.

3. RESULTS

A total of 20 volunteers, contributing 40 eyes, were included in the study. The volunteers had a mean age of 29.80±0.57 years, with a female-to-male ratio of 11/9 (55%/45%). Exophoria was present in 5 individuals. The median spherical equivalent refractive error was – 1.50 D (IQR: – 2.44 to – 0.75 D). The median IQR horizontal and vertical kappa angle values, measured using Lenstar, were 0.25 mm (0.15 – 0.34 mm) and 0.10 mm (0.04 – 0.16 mm), respectively.

There was no significant difference observed between participants' accommodation levels measured before and after playing the game (3.39 D [IQR: 2.19 – 5.10 D] and 4.03 D [IQR: 1.82 – 5.87 D], $P=0.121$), respectively. However, the median IQR stereopsis value decreased significantly from 30 arc seconds (30 – 60 arc seconds) before gameplay to 60 arc seconds (60 – 60 arc seconds) after a 10-minute gameplay session ($P<0.001$) (Table I).

Post-game measurements revealed a significant increase in median IQR pupil diameter compared to pre-game measurements under scotopic (6.30 mm [5.91 – 6.77 mm] and 5.99 mm [5.53 – 6.36 mm], P<0.001), mesopic (5.01 mm [4.56 – 5.38 mm] and 4.28 mm [3.90 – 4.79 mm], P<0.001), and photopic (4.08 mm [3.78 – 4.44 mm] and 3.67 mm [3.21 – 3.93 mm], P<0.001) conditions, respectively (Table I).

Table I. *Changes of evaluated parameters and statistical comparisons before and after 10 minutes of playing games using virtual reality (Oculus Quest 2)*

	Pre-VR usage Median (IQR)	Post-VR usage Median (IQR)	P value*
Accommodation, diopter	3.39 (2.19 – 5.10)	4.03 (1.82 – 5.87)	0.121
Stereopsis, arc/sec	30 (30 – 60)	60 (60 – 60)	**<0.001**
Scotopic Pupillometry, mm	5.99 (5.53 – 6.36)	6.30 (5.91 – 6.77)	**<0.001**
Mesopic Pupillometry, mm	4.28 (3.90 – 4.79)	5.01 (4.56 – 5.38)	**<0.001**
Photopic Pupillometry, mm	3.67 (3.21 – 3.93)	4.08 (3.78 – 4.44)	**<0.001**

VR: virtual reality, IQR: 25-75% Interquartile Range, Wilcoxon test was used for P values. P<0.05 values were considered significant and marked in bold.

The median IQR VRSQ score after gameplay was 51.67 (44.38 – 69.38) points. The "C score", assessing oculomotor discomfort, had a median (IQR) of 58.33 (43.75 – 66.67) points, and the "D score", evaluating disorientation discomfort, had a median (IQR) of 46.67 (40 – 71.67) points. A negative correlation was observed between C score and pre-game stereopsis (r=-0.438, P=0.005), and a positive correlation with pre-game accommodation (r=0.325, P=0.040) and vertical kappa angle (r=-0.316, P=0.047). D score correlated positively with the presence of exophoria (r=0.510, P=0.001) and post-game scotopic pupil measurement (r=-0.412, P=0.008). Total VRSQ score demonstrated positive correlations between exophoria and kappa-x angle (r=0.458, P=0.003 and r=0.330, P=0.038, respectively), and negative correlations between stereopsis and kappa-y angle (r=-0.375, P=0.017 and r=-0.326, P=0.04, respectively).

4. DISCUSSION

In this study, it was observed that there was no significant change in the accommodation following 10 minutes of VR gameplay, but pupil diameter increased, and depth perception decreased. Additionally, the VRSQ questionnaire revealed that participants experienced some level of discomfort, especially related to oculomotor and disorientation issues. This discomfort was found to be associated with horizontal and vertical kappa angles, as well as the presence of exophoria and stereopsis levels.

Virtual reality technology has rapidly advanced in recent years and is utilized in various fields. VR headsets offer users the opportunity to experience interactive 3D virtual worlds. However, prolonged VR usage can induce discomfort in some users, a phenomenon called "cybersickness." Cybersickness is believed to arise from a mismatch between what an individual perceives and feels. It has been proposed that discomfort occurs when there is a discrepancy between what one feels without seeing it or when there is a difference between what is seen and what is felt [9]. This sensation of discomfort following VR use is thought to stem from the perception of motion in the absence of corresponding physical action [10]. Researchers have been actively investigating factors that could contribute to this discomfort, aiming to reduce it and enhance the VR experience.

A recent study found that discomfort in individuals performing specific tasks without playing VR games decreased when anisometropia was induced to suppress stereopsis [11]. However, this reduction in discomfort was accompanied by worsened motion and spatial perception [11]. Another study showed that discomfort perception remained unchanged despite reducing stereopsis or motion parallax [12]. In the current study, it has been observed that when participants were engaged in a 10-minute gaming session, there was a significant reduction in the measured post-game stereopsis values. Additionally, contrary to the studies mentioned in the present work, it has been demonstrated that the introduction of gaming, in alignment with the literature, is associated with an inverse relationship between stereopsis and both the total scores on the VRSQ as well as the C score assessing oculomotor discomfort.

Previous studies have investigated the visual parameters and effects of 30 minutes of VR usage. In their study, Yoon et al. examined changes in refractive errors and accommodation after a 30-minute VR gaming session with 23 volunteers. While no significant alterations were observed in refractive errors following VR usage, they reported a noteworthy increase in near-point accommodation, near-point convergence, and subjective complaints [5]. Munsamy et al. investigated binocular accommodative and vergence changes in 42 individuals after playing VR games for 25 minutes while the control group watched a television film projected on a screen at 1 m. The study's findings indicated that binocular accommodative changes were significantly greater in the group using VR [8]. In the current study, no significant differences were observed in accommodation power following a shorter duration of gameplay (10 minutes). It was observed that significant pupil dilation occurred after gaming sessions across scotopic, mesopic, and photopic lighting conditions. Additionally, it has been demonstrated that VR usage is associated with decreased intraocular pressure [7] and increased choroidal thickness [5]. This increase in choroidal thickness has led to the suggestion that VR usage may not act as a myopogenic stimulus [5].

In the current study, in addition to the existing literature, the impact of horizontal and vertical kappa angles on the discomfort sensation following VR usage has been examined. Given that VR devices require the user to look at a close distance, such as 15 cm, during use, the eyes need to be converged. However, a larger horizontal kappa angle and a smaller vertical kappa angle define a position where the eyes are more divergent. Consistent with this proposition, the study has concluded that the magnitude of the horizontal kappa angle and the smallness of the vertical kappa angle are associated with increased discomfort sensation measured by the VRSQ. Building upon this outcome, it can be suggested that discomfort sensation can be reduced by

measuring individualized kappa angles during device usage through simple techniques and implementing necessary automatic lens adjustments on the device. This approach could potentially contribute to the development of devices that offer an improved VR experience by addressing the unique kappa angle variations among different individuals.

One of the significant limitations of the study is the timing of measurements taken immediately after gameplay and the absence of consecutive measurements. As a result, the study's findings do not allow for an assessment of how long it takes for the parameters that exhibited significant changes to return to their baseline state. Another limitation of the study is the uniformity in both the duration of gameplay and the specific device used. The study did not investigate the effects of different lens and optic models on VR devices or varying durations of gameplay. In the future, addressing these limitations through more comprehensive research could lead to a better understanding of the impacts of VR goggles on the eyes and the evolving discomfort sensation. This might involve investigating the effects of different devices with varying lenses and durations of gameplay, thus providing a more nuanced perspective on the subject.

In conclusion, this study demonstrated that despite no changes in accommodation following a 10-minute motion-based gameplay session with VR, significant alterations in pupil size occurred across all lighting conditions. This change in pupil size could be particularly noteworthy for individuals who have undergone corneal refractive surgery. Moreover, the study indicated a potential relationship between stereopsis, fusion presence, and the horizontal and vertical kappa angles with the discomfort sensation experienced after VR usage. By evaluating individual differences in kappa angles prior to device usage and implementing necessary adjustments, devices that offer a more comfortable experience could be designed. Overall, these findings shed light on the multifaceted impacts of VR devices and provide insights for potential improvements in user comfort and experience.

Compliance with the Ethical Standards

Ethics Committee approval: This study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (approval number 03.02.2023.336).

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Combined Mustardé and Furnas type otoplasty with minimal conchal cartilage excision

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ABSTRACT

Objective: We aimed to evaluate the complication rate and satisfaction status of patients who underwent otoplasty with the combined Mustardé and Furnas sutures with minimal medial conchal cartilage excision.

Materials and Methods: Forty-four ears of 22 patients, operated for prominent ear deformity were retrospectively included in the study. Patients were called for follow-up at various time intervals. At the sixth month, the patient and parents were asked to rate their satisfaction with the operation on a scale of 1-10 and noted.

Results: The mean age of the patients was 8.09±2.72 years. The antihelix was created with Mustardé technique, minimal medial conchal excisions were performed, two conchamastoid sutures were placed with Furnas technique. The mean follow-up period was 23.86±11.97 months. Complications developed in 4 of 44 ears (9%). Major complication rate was 4.5%. Two patients (4.5%) had suture extrusion in one ear. None of the patients scored lower than 7 in the satisfaction questionnaire.

Conclusion: Otoplasty with a combination of cartilage-sparing techniques has low complication, revision, and high satisfaction rates. Minimal medial conchal cartilage excision in addition to this technique is beneficial in reducing the conchamastoid tension and does not increase the risk of complications.

Keywords: Prominent ears, Otoplasty, Antihelix, Mustardé, Furnas

1. INTRODUCTION

Prominent ear deformity is a condition of excessive visibility of the ear, which occurs most commonly due to absence or underdevelopment of the antihelix, less frequently due to overdevelopment of the conchal cartilage, and more rarely due to overdevelopment of the lobule [1]. Deformity occurs when these anatomical changes occur alone or together or less frequently with the addition of conditions such as excessive concha and mastoid angle, excessive protrusion of the mastoid process, overdevelopment of the helix tail, and outward bending of the upper part of the auricle [2]. Prominent ear deformity is observed with a frequency of 5% in the population and similar rates have been reported in men and women [1,2]. Prominent ear deformity is inherited as autosomal dominant [2]. Although, it is not worried much by families, it may cause social and psychological problems during adolescence when the child starts school, participates in social life, and becomes more aware of the changes in his/her body. Especially, in the early years of primary school, these children may often be exposed to peer bullying. Parents who are disturbed by this situation apply for the treatment of their children. Prominent ear correction surgery is called otoplasty. The most appropriate age for otoplasty is after the age of 5, which is the preschool period when the growth and shape of the ear cartilage is largely completed [2]. Completion of the treatment in the preschool period prevents psychological trauma to the child and facilitates adaptation to social life.

Although, hundreds of surgical techniques have been proposed in the treatment of prominent ear deformity, there is not a single universally accepted technique yet [1,2]. Surgical techniques are categorized under two main headings: 1 – Cartilage cutting techniques in which the cartilage is shaped by cutting the cartilage and removing parts, 2 – Cartilage sparing techniques in which the cartilage is shaped by suturing without making an incision in the cartilage. It is accepted that shaping the ear only with sutures without making an incision in the cartilage

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reduces the complication rate. In 1963, Mustardé proposed a technique to create a natural-looking antihelix using horizontal matrix sutures without incision to the cartilage [3]. In 1968, Furnas published a technique in which he sutured the concha to the mastoid periosteum with conchamastoid sutures without making an incision in the concha [4]. Today, the combined use of these two techniques is accepted by many surgeons and has become widespread worldwide.

In this study, we aimed to retrospectively evaluate the complication rate and satisfaction status of patients who underwent otoplasty with the combined Mustardé and Furnas sutures with minimal medial conchal cartilage excision and to discuss the results.

2. MATERIALS and METHODS

Forty-four ears of 22 patients who were admitted to tertiary care city hospital between 2017 and 2022 for prominent ear deformity, who were operated by the same surgeon, with absent or underdeveloped antihelix and excessive conchal depth were retrospectively included in the study. The study protocol was approved by the Istanbul Goztepe Prof. Dr. Suleyman Yalcin City Hospital Clinical Studies Ethics Committee (approval number: 2023/0576). The ages of the patients ranged from 6 to 16 years. Patients with incomplete clinical data, who underwent revision surgery or in whom antihelix was created with cartilage incisions were excluded. When the patients presented for surgery, detailed anamnesis was taken and a complete otolaryngologic examination was performed. Patients were questioned about comorbidities, tendency to bleeding and hypertrophic scar formation. After detailed preoperative blood tests and anesthesia examination, written surgical consent was obtained from the patients' parents.

Surgical Technique

Before surgery, anterior, posterior, and lateral photographs of the patients were taken and the anatomical features of the auricle such as the absence of antihelix and excessive conchal depth were evaluated and surgical planning was made. All patients were operated by the same surgeon under general anesthesia. During surgery, the patient was covered by following the rules of asepsis-antisepsis. The auricle was noted by measuring the distances between the auricular rim and mastoid from four points. The auricle posterior and anterior skin was infiltrated with 20 mg/ml lidocaine hydrochloride and 0.0125 mg/ml epinephrine. An antihelix was created anteriorly with two 2/0 silk guide sutures. Posteriorly, an hourglass-shaped incision of 4-4.5 cm in length was made without approaching the edges more than 1 cm and the skin and subcutaneous tissue were removed. The skin was elevated towards the anterior edge of the auricle. An antihelix was created with 3 horizontal matrix sutures using 4/0 polypropylene monofilament suture with Mustardé technique. Care was taken to ensure that the sutures remained anteriorly under the skin and above the perichondrium to be strong and not cut the cartilage. No filing, weakening, or incision was applied to the cartilage during antihelix formation. After antihelix formation, the anterior silk guide sutures were cut and removed. Minimal medial conchal cartilage excision was performed in a half-moon shape from the upper and lower parts of the conchal cartilage, away from the external auditory canal, to reduce the tension between the auricle and the mastoid bone. Two conchamastoid sutures were placed between the concha and mastoid periosteum with 4/0 polypropylene monofilament suture using Furnas technique. Bleeding was controlled with bipolar cautery and the skin was sutured intradermally with 5/0 absorbable monofilament polyglecaprone 25 sutures. Before the end of the operation, the points measured preoperatively were measured again and the amount of correction was noted, and if there was asymmetry between the two ears, intervention was performed. Nitrofurazone-impregnated, saline-soaked sterile cottons were placed on the anterior surface of the auricle, conchal cavity, antitragus edges and postauricular sulcus to prevent hematoma formation and to support the newly created auricula shape. The ear was covered with sterile gases and tightly closed with the mastoid dressing that we use in tympanoplasty operations. On the first postoperative day, the dressings were opened to check for hematoma or ischemia at the wound site and the patient was discharged if there was no problem. The patient was called for follow-up every 2 days and the dressing was renewed for one week. Amoxicillin clavulanic acid as antibiotic and paracetamol as painkiller were given for one week. At the end of the first week, the dressing was removed, and the patient was advised to wear an elastic ear bandage or a tennis headband as much as possible during the day and all night long for one month. Patients were called for follow-up at the first, third, sixth, and twelfth postoperative months. To evaluate the patient's satisfaction at the sixth month, the patient and his/her parents were asked to rate their satisfaction with the operation on a scale of 1-10, with 1-2=very bad, 3-4=bad, 5-6= acceptable, 7-8=good, 9-10=very good, and noted.

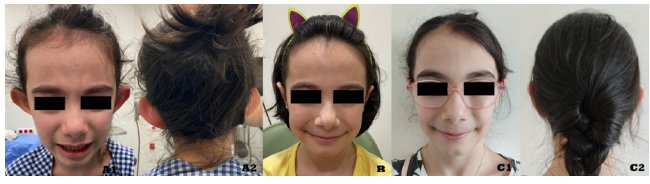

Figure 1. *Preoperative (A1-2), 6th month (B) and 24th month (C1-2) postoperative photographs of our six-year-old girl patient (with the kind permission of her family).*

3. RESULTS

Of the patients included in the study, 10 were male and 12 were female. The mean age of the patients was 8.09±2.72 (range, 6-16) years. All patients had absence or weakness of antihelix and overdevelopment of the conchal cartilage. Under general anesthesia, for both ears of all patients, the antihelix was created with 3 horizontal matrix sutures using the Mustardé technique and minimal medial conchal excision was performed to reduce conchamastoid tension. In combination, two conchamastoid

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sutures were placed with Furnas technique. The mean follow-up period was 23.86±11.97 (range, 8-48) months. Complications developed in 4 of 44 ears (9%). As a major complication, keloid developed on the upper edge of both auricles in one patient in the late period (10th month). Major complication rate was 4.5%. This patient underwent revision surgery due to lack of response to intralesional steroid administration. Two patients (4.5%) had protruding polypropylene suture ends in one ear, one in the 13th month and the other in the 19th month. The sutures were removed under local anesthesia. One patient was found to have mild asymmetry in the sixth month and did not accept revision surgery because the patient' parents were satisfied. None of the patients scored lower than 7 in the satisfaction questionnaire performed at the sixth month. 13 patients and their parents scored 7-8 (good) and 9 patients and their parents scored 9-10 (very good). Considering these findings, patient satisfaction was evaluated as 100%.

4. DISCUSSION

Prominent ear deformity is the most common congenital anomaly in the head and neck region and although it does not cause functional loss, it may cause psychological trauma especially in children and thus lead to impaired adaptation to social life [1,2]. The more prominent appearance of the ears may cause children with prominent ears to attract more attention and be disturbed by other children. These children may be nicknamed because of prominent ears and may be exposed to peer bullying. The aim of otoplasty surgery is to make these prominent ears less visible and give them a natural appearance. Therefore, it is important that otoplasty surgery is performed before starting school. It has been reported in the literature that children and adults benefit psychologically and emotionally from otoplasty surgery [5,6].

Although, many surgical techniques have been used in otoplasty surgery, there is still no single, widely accepted effective technique [1,2]. Each ear should be analyzed individually, and treatment should be planned with the most appropriate technique or combination of techniques. It has been found that 95% of patients with prominent ear deformity have absent or underdeveloped antihelix or overdeveloped conchal cartilage [2]. In this study, cases in which antihelix formation was achieved with the Mustardé technique, minimal medial conchal excisions were performed and conchamastoid sutures were placed in combination with the Furnas technique were retrospectively reviewed in terms of patient satisfaction and complications. Major complications in otoplasty have been reported as large hematoma requiring drainage, wound dehiscence, tissue necrosis, wound infection requiring intravenous antibiotics and drainage, and major cosmetic deformity [2,7,8]. In this series, none of these complications were observed, only one patient developed keloids in two ears (4.5%), which did not respond to intralesional steroid treatment and excision was performed with revision surgery, and intraoperative corticosteroids were applied to the wound edges. Calder et al., reported the rate of keloid development as 2.1% in their series of 93 patients [8]. The reason for the high rate of keloid in our series was thought to be due to the small number of cases. In order to reduce the risk of keloid development, it has been recommended that excessive skin excision should be avoided, sutures should not be too taut, and the incision line should not be extended to the upper part of the auricle, which is the area where keloid development occurs most frequently [8-10]. In our case, keloid was thought to be caused by the incision line being close to the upper level of the auricle.

Minor complications in otoplasty have been reported as suture extrusion, hypertrophic scar, hypesthesia, irritation, pruritus, and suture abscess [2,7,8]. In two patients (4.5%), one in the 13th and the other in the 19th postoperative month, the sutures were removed under local anesthesia due to protrusion of the polypropylene suture ends in one ear, and no deformity occurred in these patients. The wound healed in one week with topical antibiotic ointment and no additional treatment was needed. With the increase in cartilage sparing suture techniques, the problem of suture extrusion has been a topic of discussion in the literature. The rate of suture extrusion has been reported as 0-22% in the literature [11]. Permanent sutures such as polypropylene and mercylene are used in suture techniques; extrusion of sutures may occur due to reasons such as foreign body reaction, infection, and skin thinness. Postauricular adipofascial flaps have been described to cover Furnas and Mustardé sutures with soft tissue to prevent suture extrusion. Horlock et al., reported no suture extrusion following the use of postauricular adipofascial flaps in a series of 51 cases [12] and Irkoren et al., reported no suture extrusion in a series of 100 cases [13]. Sinha and Richard [14] reported a suture extrusion rate of 2.64% in their series of 227 patients using adipofascial flaps. Boroditsky et al., reported a suture extrusion rate of 16.8%, a revision rate of 1.7% and a success rate of 97% in a series of 119 ears in which they did not use adipofascial flaps and performed otoplasty with the Mustardé technique with a follow-up period of 104 weeks [11]. They claimed that the suture extrusion rate was not high and was due to the long follow-up period. The suture extrusion rate of 4.5% in our series was considered low and reasonable.

In the literature, if antihelix and conchal correction is to be performed, it is recommended to perform conchal excision first, followed by helix surgery. Since, we think that it is more effective to create an antihelix to evaluate the conchamastoid tension without conchamastoid suturing, minimal medial excision of the concha was performed after the antihelix was created and conchamastoid sutures were placed. Cartilage-sparing suture techniques are thought to reduce the formation of major complications such as hematoma and necrosis compared to cartilage-cutting techniques [2,8,11]. In our series, suture techniques were combined with minimal medial conchal cartilage excision and no major complications occurred such as hematoma and necrosis. In this study, it was thought that the addition of small cartilage incisions and minimal cartilage excisions to reduce tension in addition to suture techniques would not increase the complication rate.

Cartilage sparing techniques and cartilage cutting techniques have been compared in the literature in various aspects and similar results have been reported with a complication rate of around 20% and a revision rate of 6-7% [11]. Cartilage sparing techniques are increasing in popularity due to their prevention of major early complications (postoperative hematoma and infection), no irreversible cartilage replacement and high patient/family satisfaction. In this series, the major complication rate was 4.5% and the total complication rate was 9%. Complication rates were low and consistent with the literature. One patient required major intervention due to keloid and 2 patients required minor intervention due to suture extrusion. All our patients reported that they were satisfied with the operation, and there was no need for revision surgery due to recurrence of the deformity or patient dissatisfaction. The results of our otoplasty series, which combined Mustardé and Furnas techniques with cartilage-sparing suture techniques and minimal medial conchal cartilage excision, were considered satisfactory. In the surgical series, symmetry between the ears was achieved in all but one patient (95.5%) and natural looking antihelix formation was achieved. The helix was visible behind the antihelix, the distance between the mastoid and the edge of the auricle was below 2 cm, and a natural ear appearance was achieved without obliterating the auricular sulcus. In the satisfaction scoring questionnaire we conducted at the sixth month, all our patients scored their satisfaction with the operation as good or very good and did not report any recurrence of deformity or residual deformity requiring revision.

Conclusion

Otoplasty with a combination of cartilage-sparing suture techniques is a technique with low complications, low revision, and high satisfaction rates. Long-term complications such as suture extrusion can be corrected under local anesthesia in office conditions in a very short time and do not cause additional deformity. Minimal medial conchal cartilage excision in addition to this technique is beneficial in reducing the conchamastoid tension, does not increase the risk of complications and can be applied successfully. Large-scale studies are needed to support the findings of this study.

Compliance with Ethical Standards

Ethical Committee approval: The study protocol was approved by the Istanbul Goztepe Prof. Dr. Suleyman Yalcin City Hospital Clinical Studies Ethics Committee (approval number: 2023/0576). Written surgical consent was obtained from the patients' parents. Consent for medical photography: The parent of one of the patients gave written consent for the photographs to be used in medical publication and he agreed for the images to be shown for teaching purposes.

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Authors' contributions: The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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Serum fibroblast growth factor-21 levels and its relationship with carotid intima-media thickness in type 1 diabetes mellitus patients

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ABSTRACT

Objective: The study aimed to evaluate fibroblast growth factor-21 levels in type 1 diabetes patients and its relationship with carotid intima-media thickness which is a marker of atherosclerosis.

Patients and Methods: We recruited 39 patients with type 1 diabetes mellitus and 39 healthy controls. Blood samples for fibroblast growth factor-21, adiponectin and carboxymethyllysine were drawn from subjects after 8 hours fasting. Fasting blood glucose and hemoglobinA1c levels were obtained from patient records. Carotid intima media-thickness was measured via B-mode ultrasound by the same physician.

Results: Median fibroblast growth factor-21 levels were 0.54 (0.10-10.69) ng/ml in type 1 diabetes patients, 0.42 (0.09-1.57) ng/ml in healthy controls (P=0.13). There was no correlation between serum fibroblast growth factor-21 levels and carotid intima-media thickness. Carboxymethyllysine levels were similar in both groups (P=0.86). Adiponectin level was 16336.7 ± 7338.7 ng/ml in type 1 diabetes patients, 13343.1 ± 5318.7 ng/ml in control group (P=0.04).

Conclusion: Our study did not find any relation between serum fibroblast growth factor-21 levels and carotid intima-media thickness. Further researches with wider study population are needed.

Keywords: fibroblast growth factor-21, carotid intima-media thickness, type 1 diabetes mellitus

1. INTRODUCTION

Type 1 diabetes mellitus (DM) is a chronic disease which is characterized by insulin deficiency and usually appears at early ages. Deficiency of insulin leads to hyperglycemia which results in microvascular and macrovascular complications [1].

Fibroblast growth factor-21 (FGF-21) is a regulatory hormone belonging to fibroblast growth factor family. It is synthesized by liver, pancreas and white adipose tissue. FGF-21 level is increased at obesity [2], type 2 DM [3], dyslipidemia [2,3], impaired glucose tolerance [3], nonalcoholic fatty liver disease [4] and coronary artery disease [5]. While serum FGF-21 levels have been shown to increase in type 2 DM, there is limited data in type 1 DM patients. It was demonstrated that FGF-21 levels were decreased in type 1 DM and latent autoimmune diabetes of adult patients [6]. In a study published in 2015, fasting and postprandial FGF-21 levels have been compared between type 1 diabetes and healthy controls. Fasting FGF-21 levels were found to be lower in the diabetic group than controls. In these two studies, the relationship between FGF-21 levels and diabetic complications were not evaluated [6,7].

Type 1 DM is a major risk factor for atherosclerosis. Carotid intima-media thickness (CIMT) which is measured by ultrasonography is initial sign of atherosclerosis and cardiovascular diseases in type 1 diabetes patients [8,9].

Adiponectin synthesized by adipose tissue has an insulin sensitizing effect. While, the level of adiponectin is low in obesity, type 2 DM and coronary artery disease [10], it is increased in type 1 DM [11]. Adiponectin plays an antiatherogenic role by acting directly on endothelial cells and its levels are decreased in coronary artery disease. Determining plasma adiponectin level is considered to be an important parameter in assessing the risk of coronary artery disease [12].

The prolonged exposure to hyperglycemia leads to development of microvascular and macrovascular complications such as nephropathy, neuropathy, retinopathy and atherosclerosis. Advanced glycation end-products (AGE) play a key role in these complications [13]. Carboxymethyllysine is a member of non-crosslinked AGE group and it is a new marker in monitoring the

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development of late complications due to glycation in diabetic patients [14].

Our study aimed to evaluate serum FGF-21 levels in type 1 diabetes patients and to determine its relationship with HbA1c, lipids, adiponectin, AGE (carboxymethyllysine) and carotid intima-media thickness. We hypothesized that FGF-21 levels change in type 1 diabetes mellitus patients and are exhibit positive correlations with CIMT, adiponectin, carboxymethyllysine, LDL, total cholesterol and HbA1c levels.

2. PATIENTS and METHODS

Study Design and Subjects

The study was approved by Marmara University Faculty of Medicine Ethics Committee (Protocol number: 09.2013.0357, date: 20.12.2013). An informed consent was obtained from all study participants.

Type 1 diabetes patients were recruited from outpatient clinics of Endocrinology and Metabolism at Marmara University Hospital. The control group was recruited from individuals applied for general health check-up in Internal edicine outpatient clinics at the same hospital. Type 1 DM patients, having the diagnosis for at least 6 months, aged between 18 and 65 years and having a body mass index (BMI) between 18.5 and 25 kg/cm2, were included. Smoking, chronic inflammatory disease, pregnancy and breastfeeding were the exclusion criteria. Type 1 diabetes patients who have microvascular complications were excluded.

Body mass index was calculated according to weight and height measurements of participants (kg/cm2). Waist circumference was measured. All measurements were obtained by the same physician.

Laboratory Assays

Fasting blood glucose levels, HbA1c, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride and total cholesterol levels which were measured in the last 6 months, have been obtained from the patient's records. After 8 hours fasting, blood samples were obtained to determine the serum FGF-21, adiponectin and carboxymethyllysine levels from all participants. The blood samples were kept at – 80°C refrigerator. All the samples were analyzed together at the end of the study. The FGF-21, carboxymethyllysine and adiponectin kits were based on the sandwich ELISA principle. Kits for FGF-21 and adiponectin were obtained from Assaypro lab (®Assaypro, St Louis, USA). Carboxymethyllysine kits were obtained from Sunred Biotechnology Company (®Sunred Biotechnology Company, Shanghai, China). All steps applied during the test were made according to kit instructions. The intra – and inter-assay variations were 4.7% and 7.2% for FGF-21, 3% and 8.3% for adiponectin, less than 10% and less than 12% for carboxymethillysine, respectively.

Measurement of Carotid Intima-Media Thickness

Carotid intima-media thickness was measured with a B-mode ultrasonography device on the right and left side by the same physician, with the head turned to the opposite side while lying on the supine position. According to the Rotterdam study ultrasonography protocol [9], measurements were made in 3 different regions from the main carotid artery, bifurcation region and proximal internal carotid artery for both sides. Mean values of CIMT measurements were calculated.

Statistical Analysis

Data were analyzed in Statistical Package for Social Sciences (SPSS) 17.0 (Chicago, IL, USA). Continuous variables were defined as mean ± standard deviation and median (minimum-maximum). Variables were compared by independent sample t-test and Mann Whitney-U test. All data were analyzed by Pearson and Spearman correlation analysis. The p value lower than 0.05 was the threshold for the statistical significance.

3. RESULTS

The study included 39 type 1 diabetic patients (13 [33.3%] males, 26 [66.6%] females) and 39 healthy volunteers (12 [30%] males, 27 [70%] females). Both groups were similar regarding gender, age (in patient group 27±8 years, in control group 29 ± 5 years) and BMI (in patient group 22.1 ± 2.1 kg/cm2, in control group 22.7 ± 2.09 kg/cm2).

Variables were compared between type 1 diabetic patients and controls (Table I).

Table I. *Comparison of variables between type 1 diabetes patients and healthy controls*

		Patient (n=39)	Control (n=39)	P
Age		27±8	29 ± 5	0.28
Gender	Female	26	27	0.80
	Male	13	12	
Body Mass Index (kg/cm2)		22.1 ± 2.1	22.7 ± 2.09	0.20
Waist Circumference (cm)		76.4 ± 8.4	74.7 ± 9.1	0.30
CIMT-right side (mm)		0.53 ± 0.05	0.52 ± 0.05	0.30
CIMT-left side (mm)		0.54 ± 0.60	0.51 ± 0.50	0.01
Mean CIMT (mm)		0.54 ± 0.05	0.51 ± 0.05	0.04
Adiponectin (ng/ml)		16336.7 ± 7338.7	13343.1 ± 5318.7	0.04
Fasting Blood Glucose (mg/dl)		193.8 ± 99.8	85.8 ± 9.6	<0.01
Hemoglobin A1c (%)		9.1 ± 2.8	4.9 ± 0.3	<0.01
Total Cholesterol (mg/dl)		180 ± 35	187.6 ± 39	0.37
LDL (mg/dl)		106.5 ± 26.6	114.5 ± 32.5	0.24
HDL (mg/dl)		55 ± 13.9	55 ± 14.3	0.95
Triglyceride (mg/dl)		89.4 ± 42	93.7 ± 60.9	0.71
FGF-21 (ng/ml)		0.54 (0.1-10.6)	0.42 (0.09-1.57)	0.13
Carboxymethyllysine (ng/ml)		577.5 (337.9-2405)	759.3 (170.7-2979.6)	0.86

The data were expressed as mean ± standard deviation or median (minimum-maximum). CIMT: Carotid Intima-Media Thickness, LDL: Low-density Lipoprotein, HDL: High-density Lipoprotein, FGF-21: Fibroblast Growth Factor-21

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There was no correlation between serum FGF-21 levels and other parameters except BMI (Table II). When the diabetic group was separately analyzed, there was a significant correlation between serum FGF-21 levels and BMI (r=0.35, P=0.02). There was no relation between FGF-21 and other variables in the diabetic group.

Serum carboxymethyllysine levels were not correlated with other parameters.

Table II. *Correlation analysis between FGF-21 and other parameters*

	FGF-21	
	r	P
Age	-0.03	0.76
BMI	0.24	0.02
Waist Circumference	0.02	0.85
Mean CIMT	-0.01	0.87
Carboxymethyllysine	0.02	0.84
Adiponectin	0.10	0.34
Fasting Blood Glucose	0.00	0.97
Hemoglobin A1c	0.13	0.22
Total Cholesterol	0.07	0.51
LDL	0.08	0.47
HDL	0.01	0.90
Triglyceride	0.03	0.77

FGF-21: Fibroblast Growth Factor-21, BMI: Body Mass Index, CIMT: Carotid Intima-Media Thickness, LDL: Low-density Lipoprotein, HDL: High-density Lipoprotein.

Adiponectin levels were correlated positively with HbA1c (r =0.2, P=0.01), HDL (r =0.3, P=0.03) negatively with LDL (r =-0.2, P=0.02), triglyceride (P=0.02), waist circumference (r =-0.25, P=0.02) and BMI (r =-0.27, P=0.01).

4. DISCUSSION

Fibroblast growth factor-21 levels were found to be relatively higher in patients with type 1 DM, but this result was not significant. On the other hand, the mean and left-sided CIMT and adiponectin levels were increased in patients with type 1 DM. Carboxymethyllysine levels were found to be similar in both groups. Furthermore, there was no significant correlation between FGF-21 levels and other variables except BMI.

In a study by Xiao et al., association of FGF-21 with different diabetic subtypes has been analyzed and as a result, it was found that FGF-21 levels were increased in type 2 diabetes mellitus, modestly decreased in LADA subtype, and decreased in type 1 DM [6]. Zibar et al., have shown that basal FGF-21 levels of type 1 diabetic patients were lower than controls [7]. Our study found that FGF-21 levels were relatively, but not significantly higher in type 1 diabetic patients compared to the controls. Nevertheless, we cannot conclude that serum FGF-21 levels were increased in type 1 diabetes patients. The age of our study population was higher than age of study population conducted by Xiao et al., and lower than age of study population conducted by Zibar et al. [7]. The difference in serum FGF-21 levels from other studies might have been resulted from age differences in study populations. In a study investigating serum FGF-21 levels and the microvascular complications in type 1 DM showed that FGF-21 levels were decreased in type 1 diabetic patients but there was no relation between its levels and microvascular complications [15]. Association of FGF-21 with macrovascular complications in type 1 diabetic patients has not been evaluated in previous studies [16]. This is the first study to evaluate the association of FGF-21 levels with carotid intima-media thickness which is a marker for atherosclerosis in type 1 diabetics. The measurement of carotid intima-media thickness which is a good indicator of atherosclerosis as a macrovascular complication of diabetes was increased in type 1 diabetic children [17-19]. The results of our study were similar to earlier studies; left-sided and mean CIMT measurements of type 1 diabetic patients were higher than controls but there was no difference between right-sided CIMT measurements. Given the body of existing evidence, we hypothesized that FGF-21 levels might exhibit a positive correlation with CIMT measurements, indicative of early atherosclerosis and a macrovascular diabetic complication [20,21]. However, our study did not find a correlation between CIMT measurements and FGF-21 levels in both the patient and control groups. Unfortunately, we were unable to assess other complications as our patients did not exhibit any additional complications.

In the previous studies, adiponectin levels were increased in patients with type 1 diabetics and patients with retinopathy, nephropathy, cardiovascular complications [22-24]. Our study found that adiponectin levels were increased in type 1 diabetics. However, there was no correlation between adiponectin levels and carotid intima-media thickness. The results of our study confirmed the association of HDL and adiponectin as demonstrated in previous studies [25]. Our study showed that triglyceride, LDL, waist circumference and BMI values decreased as adiponectin levels increased. This data supports the results of previous studies [25,26].

In a study, high serum carboxymethyllysine levels in type 1 diabetic adolescents play a role in diabetic complications and serum carboxymethyllysine levels indicate the long-term control of diabetes [27]. However, in our study, there was no difference in carboxymethyllysine levels between patients with type 1 diabetes and healthy volunteers. Additionally, there was no correlation between carboxymethyllysine and FGF-21, glucose, HbA1c and carotid intima-media thickness.

This is the first and unique study that evaluates association of FGF-21 levels with carotid intima-media thickness in type 1 diabetic patients and controls. It is the strength of our study.

Limitations

There were several limitations in our study, patients with type 1 DM were relatively young and had no known history of diabetic complications; further studies which include type 1 diabetes patients with advanced complications would provide more comprehensive assessment of the association of FGF-21, adiponectin and carboxymethyllysine levels with complications. The primary limitation of our study is the small number of patients in the population. The serum FGF-21 levels may have

shown significant differences if a larger number of patients had been enrolled.

Conclusion

Our study revealed relatively elevated serum FGF-21 levels in individuals with type 1 diabetes, although, the statistical significance was not achieved due to the limited size of our study population. The larger-scale studies are necessary to provide more conclusive results.

Compliance with ethical Standards

Ethics Committee Approval: The study was approved by Marmara University Faculty of Medicine Ethics Committee (Protocol number: 09.2013.0357, date: 20.12.2013). An informed consent was obtained from all study participants.

Conflicts of Interest: The authors declare that they have no conflict of interest relevant to this article.

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Effects of upadacitinib and PD29 on oxidative damage and inflammation in bleomycin-induced scleroderma model kidney tissues

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ABSTRACT

Objective: Scleroderma (SSc) is a rare autoimmune tissue disease. There is currently no effective treatment for SSc. The aim of this study was to investigate the antioxidant and anti-inflammatory effects of upadacitinib and PD29 on total oxidant status (TOS), total antioxidant status (TAS), *malondialdehyde* (MDA), catalase (CAT), glutathione (GSH) peroxidase levels, and interleukin-6 (IL-6) and interleukin-13 (IL-13) in kidney tissues of an experimental SSc model.

Materials and Methods: The experimental design was established with five groups of eight mice: Control, bleomycin (BLM) (5 µg/kg), BLM + upadacitinib (3mg/kg), BLM + PD29 (5 mg/kg) and BLM + PD29 + upadacitinib group. BLM was administered subcutaneously once a day for 21 days. PD29 was administered subcutaneously and upadacitinib (gavage) were injected for 21 days. Renal tissues were collected at the end of the experiment. Renal TOS, TAS, MDA, CAT, GSH levels, and IL-6 and IL-13 gene expressions were evaluated.

Results: Upadacitinib and PD29 affected oxidant status and TOS. MDA levels decreased, and GSH, CAT, and TAS levels increased. Also, upadacitinib and PD29 decreased inflammation via IL-6 and IL-13 cytokines.

Conclusion: Upadacitinib and PD29 may have therapeutic roles for SSc renal crisis.

Keywords: Scleroderma, Upadacitinib, PD29, Renal tissue

1. INTRODUCTION

Scleroderma (SSc) is an autoimmune disease characterized by fibrosis involving internal organs and the skin. The pathogenesis of SSc has not been fully elucidated, yet, dermis thickening, inflammation, and uncontrollable extracellular matrix (ECM) increase are observed [1, 2]. Clinically, systemic sclerosis is a heterogeneous disease that is measured by the presence of different antibodies, progresses with internal organ involvement, and is divided into different subgroups according to the severity of the disease [3-5]. SSc is rare, with a prevalence ranging from 50-to-300 per million. As many other autoimmune diseases, women are at higher risk than men [6, 7] and SSc is not genetically transmitted [8]. SSc pathogenesis is complex and is associated with dysregulation of type I interferon (IFN), type II IFN, interleukin-6 (IL-6), IL-13 IL-2, and IL-23 regulated by JAK-STAT pathways [9]. Increased activation of Janus-kinase-2 (JAK-2) is detected in the skin of patients with SSc, especially in fibroblasts [10], due to increased levels of transforming growth factor-β (TGF-β). In this study, it is emphasized that JAK signaling pathways can be intracellular targets for the treatment of SSc [10]. The distribution of SSc patients also shows heterogeneity in clinics [10, 11].

In this study, the *Balb/c* mouse strain was used. The substance bleomycin (BLM) was used for the SSc model. BLM is an anti-tumor antibiotic isolated from the fungus *Streptomyces verticillus* [12-14]. This model is still assumed to be the best experimental model. In the previous work of the project team, the SSc model was successfully applied [15, 16].

Today, there is no approved drug used in the treatment of SSc [17-19]. Further studies, molecular and clinical data are needed primarily for the treatment of the disease [18].

Janus-kinase/signal transducer and its activators of the transcription pathway (JAK / STAT) is a conserved pleiotropic cascade involved in development and homeostasis from humans to flies [19]. JAKs are tyrosine kinases that play a key role in many cytokines, especially in IL-6 and IL13 and cellular signaling pathways (type 1 IFNs), type I/II cytokines such as IL-12 and

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IL-23, etc. [20]. Dysregulation of the JAK-STAT pathways is associated with various immune disorders, diseases, and inflammation [21]. JAK inhibitors (Jakinibs) act on competitive ATP binding and block phosphorylation of cytokine receptors. Thus, it leads to decreased production of cytokines and impaired differentiation of Th1, Th2 and Th17 cells [22]. First generation pan-JAK inhibitors (tofacitinib, baricitinib, ruxolitinib, peficitinib) and second-generation selective JAK inhibitors (decernotinib, filgotinib, upadacitinib) have been developed for the treatment of various autoimmune and malignant diseases [23]. Upadacitinib is the third selective JAK1 inhibitor approved for the rheumatoid arthritis (RA). Upadacitinib is effective in the treatment of RA and has been approved by the FDA (2019, August) and EMA (2019, December) for use in the treatment of moderate to severe RA in adults, if other treatments fail [24].

Molecular mechanisms of SSc renal involvement are not fully known, the role of oxidative stress has been demonstrated in many studies [25-29]. Increased reactive oxygen species (ROS) production leads to lipid peroxidation *malondialdehyde* (MDA) and oxidative damage in important cellular macromolecules such as proteins and even DNA. Also, increased ROS production, decreases the activity of cellular antioxidant enzymes, such as catalase (CAT), and glutathione (GSH) peroxidase.

SSc is characterized by accumulation of ROS with microvascular and immune dysregulation that causes multi-organ fibrosis [30-33]. Activation of the TGF-β pathway is known by transcriptome analysis in SSc skin biopsies [34]. TGF-β plays a central role in the pathogenesis of SSc by regulating fibrotic responses, including myofibroblast differentiation, ROS production, ECM synthesis, and hardening [33]. In addition, the role of oxidative stress in SSc has been emphasized in many researches conducted on patients and animal models [35-37]. High ROS rate in SSc has been found to be directly proportional to increased activation of dermal fibroblasts as well as collagen synthesis [37]. In short, tissue fibrosis and oxidative stress in SSc feed each other [38-40]. In SSc dermal fibroblasts, high levels of intracellular and mitochondrial ROS have been shown to induce oxidative stress and the expression of genes involved in the fibrotic process [41]. There is a significant imbalance between high oxidative stress level and insufficient antioxidant defense in SSc [42]. Reduction of ROS production in fibroblast, immune and endothelial cells is necessary for clinical recovery of the disease [43, 44]. In SSc, antioxidant defense is important in regulating ROS production and plays an important role in the pathogenesis of the disease [45].

The PD29 peptide, with its 29 amino acid sequence, is designed to target pulmonary fibrosis (PF). It has been shown that PD29 is responsible for anti-angiogenesis, inhibition of matrix metalloproteinase activities and inhibition of integrins in PF [46]. Again, in the same study, it is suggested that PD29 can treat PF by partially regulating the expression of TGF-ß1, Smad3 and Smad7 [46].

In this study, the effects of upadacitinib and PD29 on oxidative damage and inflammation in BLM-induced SSc model kidney tissues were investigated. TOS, TAS, MDA, CAT and GSH parameters and IL-6 and IL-13 gene expressions were examined in scleroderma kidney tissues.

2. MATERIALS and METHODS

SSc animal model

SSc animal model studies were approved by Kutahya Health Sciences University (KSBU), Faculty of Medicine, Experimental Animals Local Ethics Committee (KSBU-DEHYUB) (23.03.2022, 46959). The SSc experimental animal model was used in our previous projects [15, 16].

Experimental design:

Mice were randomly divided into 5 groups:

Group 1: Control group (n = 8): Mice in this group were given 0.9% saline subcutaneously and as oral gavage for 21 days.

Group 2: Bleomycin group (SSc group, n=8): Mice in this group were given 5 mg/kg BLM (Onko, Koçsel, Turkey) subcutaneously for 21 days and 0.9% saline by oral gavage [17, 18].

Group 3: Bleomycin + upadacitinib (ABT-494) group (n=8): Mice in this group were given 5 mg/kg BLM subcutaneously and 3mg/kg [47] upadacitinib (ABT-494) as oral gavage for 21 days.

Group 4: Bleomycin + peptide PD29 group (n=8): Mice in this group were given 5 mg/kg BLM (Onko, Koçsel, Turkey) and 5 mg/kg peptide PD29 subcutaneously for 21 days [48].

Group 5: Bleomycin + peptide PD29 + upadacitinib (ABT-494) group (n=8): Mice in this group were given 5 mg/kg BLM subcutaneously for 21 days, 3mg/kg [35] gavage upadacitinib (ABT-494) and 5 mg/kg peptide PD29 were administered subcutaneously [48].

Mice were sacrificed at the end of day 21 under 90 mg/kg ketamine and 10 mg/kg Xylazine anesthesia. Kidney samples were stored at – 150^0 C until the relevant experimental step.

Homogenization method

All tissue samples were washed twice with cold saline and homogenized using a Tissue-Lyser (TissueLyser, Qiagen, West Sussex, UK). 0.1 g of each tissue was homogenized at a ratio of 1:8 (w/v) with 50 mM Tris-HCl, pH 7.0, that included 0.15 M NaCl, 10 mM CaCl 2 and 0.05% Brij 35, 20 µ g/ml pepstatin and 20 µ g/ml leupeptin. The homogenates then were centrifuged at 15000 g for 15 min. The supernatants were used for analysis. All procedures were performed at +4 ° C. All homogenates were stored at – 150 ° C until the experimental step.

TOS and TAS determination

TAS and TOS were determined with kits (Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey) developed by Erel. Measurement of the TAS level was determined using an Erel method. In this method, the antioxidative effect of the sample against the potent free radical reactions, which are initiated by the produced hydroxyl radical, is measured [49]. The results are expressed as µmol Trolox Eq/L.

Measurement of the TOS value was determined using a novel method, such as TAS, developed by Erel [50]. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the experimental

sample. The assay was calibrated with hydrogen peroxide (H2O2) and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H2O2 Eq/L).

Oxidative stress index

The OSI is defined as the ratio of the TOS to TAS level, expressed as a percentage. For this calculation, TAS units were changed to mmol/L, and the OSI value was calculated according to the following formula:

OSI (arbitrary unit) = TOS (μmol H2O2 Eq/L)/TAS (μmol Trolox Eq/L).

MDA level assay

MDA, one of the peroxidation products formed by the reaction of fatty acids with free radicals, was measured as the formation of its colored form with thio-barbituric acid. For each renal tissue, 200 μl homogenate was transferred to a tube, and 800 μl phosphate buffer, 25 μl butylhydroxytoluene solution, and 500 μl 30% trichloroacetic acid were added. The tubes were mixed and incubated on ice for 2 h. They were then centrifuged at 2000 rpm for 15 min, and 1 ml of each supernatant was transferred to a new tube, after which 75 μl ethylene-diamine-tetraacetic acid and 25 μl thio-barbituric acid was added. The tubes were mixed and incubated in a hot water bath for 15 min. They were then brought to room temperature, and the absorbance at 532 nm was read on a UV/Vis spectrophotometer.

CAT activity assay

CAT activity in renal tissue was determined using the method described by Aebi et al., previously [51]. First of all, 2.8 mL 30 mM H2O2 was placed in a blind tube, and 0.2 mL phosphate buffer was added to it. Then 2.8 mL 30 mM H2O2 was added to the sample tube. For both tubes, 0.2 ml enzyme was added. The absorbances at 240 nm were read twice at 30 s intervals to determine the catalase activity.

GSH assay

The GSH level was determined using the method described by Beutler et al. [52]. First, 200 μl renal tissue homogenate was diluted in an 800 μl phosphate buffer, and the first absorbance (OD1) was measured at 412 nm. Then 100 μl Ellman's reagent was added to the same tube, and the second absorbance value (OD2) was recorded.

The levels of MDA and activities of CAT were standardized according to the protein level. Protein levels determination of inappropriately prepared tissue homogenates was spectrophotometrically performed according to the Bradford method.

IL-6 and IL-13 gene expression experiments (qPCR)

cDNA synthesis was achieved from isolated RNA by using a reverse transcription kit (Prime Script RT, Qiagen, USA). qPCR was established with SYBR Green PCR master mix (RT2 SYBR Green qPCR Mastermix, Qiagen, USA) using the qPCR instrument (Rotor-Gene, Qiagen, USA). The used primer sequences (Oligomer Biotechnology, Turkey) are presented in Table I. ß-actin, which is a housekeeping gene, was used for the normalization of the results. Gene expression fold changes were calculated according to the 2ΔΔ Ct method.

Statistical Analysis

All statistical analyses were done by Prism version 9 (GraphPad Software, California, US). The Kruskal–Wallis test was performed to determine the differences between multiple independent groups. The Mann–Whitney *U* test was used to analyze the difference between two independent groups. For correlation analysis, Pearson's test was used. All data were presented as mean ± SD. P< 0.05 was considered statistically significant.

3. RESULTS

TOS and TAS determination

SSc renal tissue TOS level was detected to be significantly higher in the BLM group (426.0 ±40.35 μmol/L) compared to the control group (189.6 ± 27.73 μmol/L) (P = 0.02). However, upadacitinib (227.7± 42.30 μmol/L) (P= 0.03), PD29 (332.8±40.97 μmol/L) (P = 0.04) and upadacitinib + PD29 (319.50±1.78 μmol/L) (P = 0.04) administration significantly decreased tissue TOS level compared to BLM group (Figure 1(a)). In addition, SSc renal tissue TAS level was detected to be significantly lower in the BLM group (0.07 ± 0.05 μmol/L) compared to that of the control group (0.23 ± 0.03 μmol/L) (P= 0.02). However, upadacitinib (0.22 ± 0.06 μmol/L) (P = 0.02), PD29 (0.217 ± 0.10 μmol/L) (P = 0.02) and upadacitinib + PD29 (0.18± 0.07 μmol/L) (P = 0.02) administration significantly increased tissue TAS level (P< 0.05) compared to BLM group (Figure 1(b)).

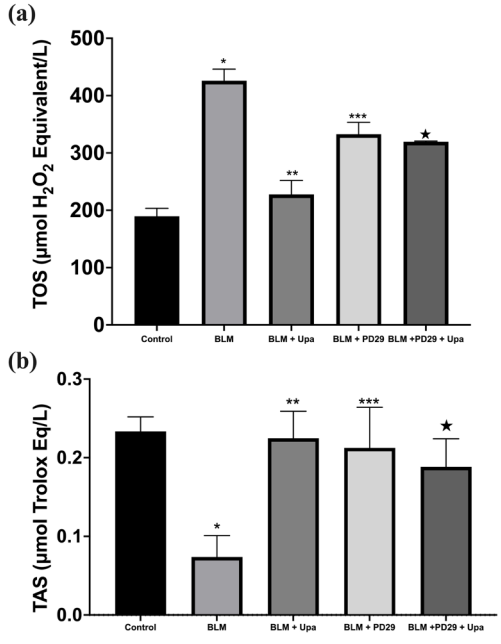

Figure 1. *(a) TOS and (b) TAS levels in SSc renal tissue. Upadacitinib, PD29 and PD29 + Upadacitinib decreased TOS and increased TAS statistically *. Control vs BLM, ** BLM vs BLM + Upa, *** BLM vs BLM + PD29, ★ BLM vs BLM + PD29 + Upa*

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OSI level of BLM group was found to be significantly higher in the BLM group (10.262 ± 8.126) compared to control group (816.8 ± 92.64) (P = 0.02). Upadacitinib (1162±287.3) (P = 0.02), and PD29 (1727 ± 449.3) (P= 0.02) administration significantly decreased compared to BLM group (Figure 2).

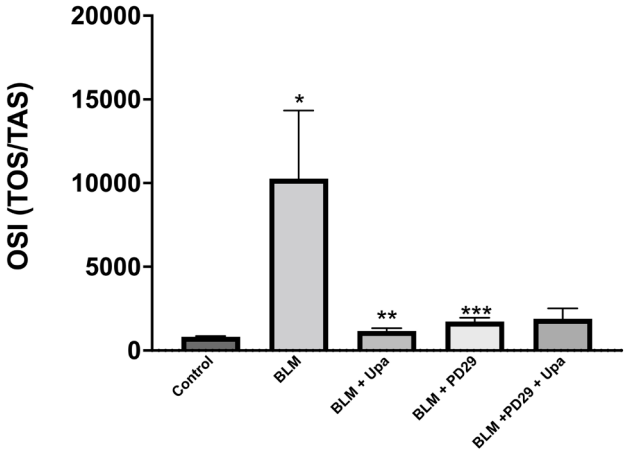

Figure 2. *OSI levels on SSc renal tissue. * Control vs BLM, ** BLM vs BLM + Upadacitinib, *** BLM vs BLM + PD29*

MDA level assay

SSc renal tissue MDA levels were detected to be significantly higher in the BLM group (0.244± 0.02 nmol/mg protein) compared to that of the control group (0.10 ± 0.009 nmol/mg protein) (P= 0.02). However, upadacitinib (0.11 ± 0.03 nmol/mg protein) (P= 0.03), PD29 (0.08 ± 0.009 nmol/mg protein) (P = 0.01) and upadacitinib + PD29 (0.08 ± 0.01 nmol/mg protein) (p = 0.02) administration significantly decreased tissue MDA level compared to BLM group (Figure 3).

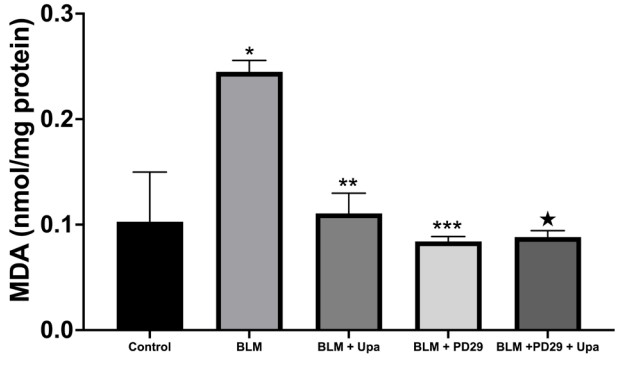

Figure 3. *MDA levels in SSc renal tissue. Upadacitinib, PD29 and PD29 + Upadacitinib, decreased MDA levels statistically. * Control vs BLM, ** BLM vs BLM + Upadacitinib,, *** BLM vs BLM+PD29, ★ BLM vs BLM + PD29 + Upadacitinib*

CAT activity assay

SSc renal tissue CAT levels were detected to be significantly lower in the BLM group (2.34 ± 0.94 U/ mg protein) compared to that of the control group (4.65± 0.99 U/ mg protein) (P = 0.02). However, just upadacitinib administration significantly increased tissue CAT levels compared to levels of BLM group (5.32 ± 1.62 U/ mg protein) (P= 0.04) (Figure 4).

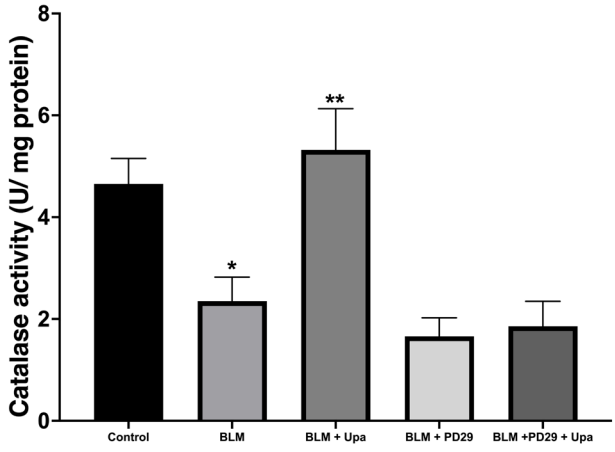

Figure 4. *CAT activity in SSc renal tissue. Upadacitinib, increased CAT activity statistically. * Control vs BLM, ** BLM vs BLM + Upadacitinib*

GSH assay

SSc renal tissue GSH levels were detected to be significantly lower in the BLM group (0.22 ± 0.003 nmol/g protein) compared to that of the control group (0.27 ± 0.06 nmol/g protein) (P = 0.00). However, just PD29 administration significantly increased tissue GSH level (0.38 ± 0.11 nmol/g protein) (P= 0.01) compared to BLM group (Figure 5).

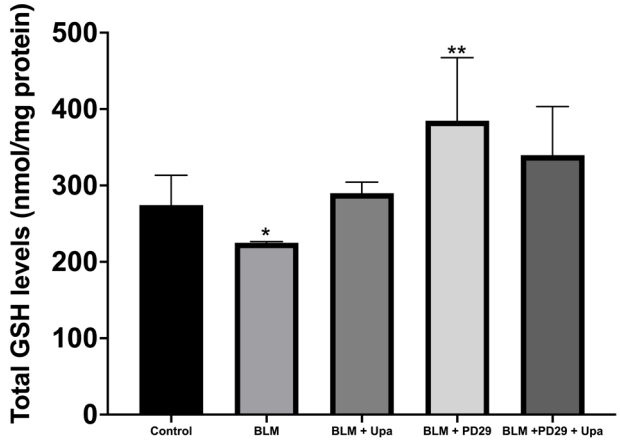

Figure 5. *Total GSH levels in SSc renal tissue. PD29 increased GSH levels statistically. * Control vs BLM, ** BLM vs BLM + PD29*

IL-6 and IL-13 gene expression (qPCR)

IL-6 and IL-13 gene expression in the renal tissue were found to be significantly higher in the BLM group compared to the control group (P= 0.02). However, upadacitinib and PD29 led to a significant decline in the IL-6 and IL-13 gene expression in the BLM + upadacitinib and BLM + PD29 group (P= 0.02) compared to BLM group (Figure 6).

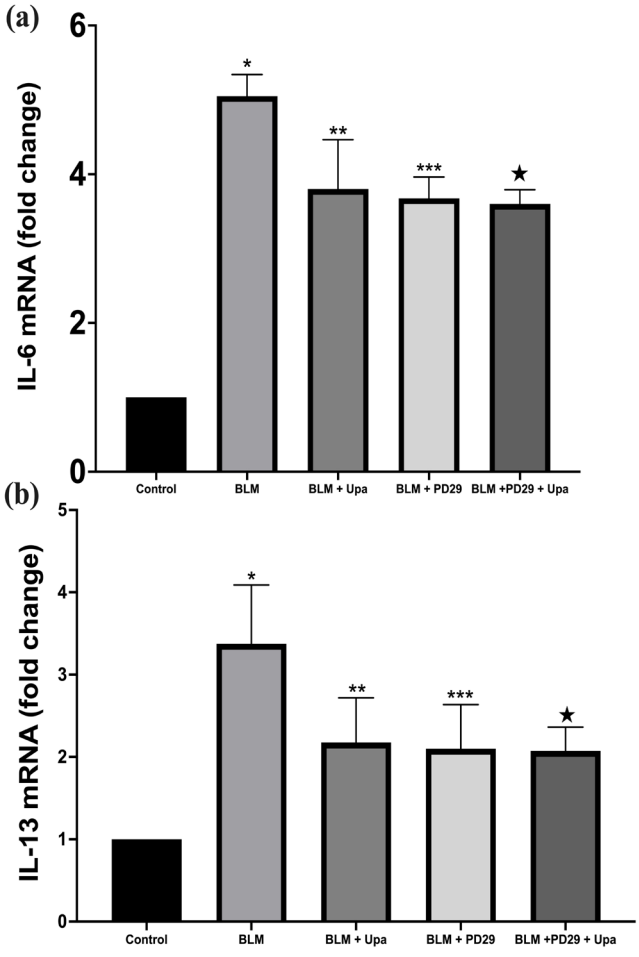

Figure 6. *(a) IL-6 and (b) IL-13 gene expression levels in SSc renal tissue. Upadacitinib,, PD29 and PD29 + Upadacitinib, decreased IL-6 and IL-13 statistically. * Control vs BLM, ** BLM vs BLM + Upa, *** BLM vs BLM + PD29, ★ BLM vs BLM + PD29 + Upadacitinib,Also, in correlation analyses, MDA and TAS, IL-13 and TOS, IL-13 and IL-6 pairs were significant and they had positive correlations (P= 0.024, 0.048 and 0.011 respectively).*

4. DISCUSSION

The present study showed that upadacitinib and PD29 decreased oxidative stress and inflammation in SSc. This is the first study to reveal that upadacitinib and PD29 decreased MDA, TOS levels and IL-6 and IL-13 gene expression and increased GSH, CAT and TAS levels in SSc model renal tissue.

Li et al., in 2018 noted that JAK signal transducers and activators of the transcription (JAK-STAT) signaling pathway contribute to injury as well as inflammation in liver cells [53]. This may explain the potential beneficial effects of upadacitinib to reverse the oxidative stress in tissue TAS, TOS, MDA, CAT activation levels. In addition, upadacitinib significantly improved IL-6 and IL-13 expression levels which were elevated due to JAK-1 inhibition effects.

In SSc, the molecular mechanisms of ROS stimulation of the fibrotic process are highly complex and involve many molecular pathways. The newly produced myofibroblasts are highly active mesenchymal cells capable of producing large quantities of interstitial fibrillar collagens and other fibrotic proteins. The high production of these proteins by activated myofibroblasts and their exaggerated accumulation in the interstitial space of the affected organs result in the severe and often progressive fibrotic changes that are characteristic of SSc.

Animal models are critical for several disease pathogenesis, disease duration research and drug exploration. BLM induced mice are the most widely used for SSc. In 1993, oxidative stress was proposed as the etiology of SSc [54]. Many subsequent studies confirmed this hypothesis. High levels of oxidative stress markers and decreased antioxidant components were found in SSc [55]. Some of them were correlated with disease duration, modified Rodnan skin score (mRSS), cardiovascular events, renal vascular damage, the severity of pulmonary fibrosis and immunological abnormalities [56-59]. We confirmed that OSI and TOS levels are increased in the SSc group as BLM group. Also, we showed that there is a correlation between oxidative stress and inflammation parameters in the renal tissue. Regarding to our results, especially JAK-STAT signaling leads to inflammation and oxidative stress. The important role of oxidative stress was also confirmed in other experimental SSc mouse models [14, 15].

The peptide PD29 is new and 29 amino acid peptide that can interfere with the pathogenesis of PF through three mechanisms that are anti-angiogenesis, inhibition of collagen degradation, and integrin inhibition. In addition, PD29 has high biological activity, relatively low toxicity, and does not easily accumulate in the body [46]. In the same research, PD29 reduced oxidative damage in the lungs reduced the release of inflammatory and profibrotic factors in BLM affected mice. In our results, we showed that, PD29 reduced TOS, TAS, MDA, IL-6 and IL-1.

PD29 may be by downregulating the TGF-β/Smad signaling pathway, also its mechanism directly inhibited TGF-β downstream proteins such as smad2/3 and promoting smad7 expression [46]. In SSc, TGF-β/ Smad signaling has a pivotal role in the disease progression.

ROS and TGF-β have important roles in cell metabolism. TGF-β can stimulate ROS production in cells [60] and several studies have shown that TGF-β can induce ROS production in different cellular compartments. TGF-β reduces ROS production in mitochondria [61, 62]. A mitochondrial thioredoxin (TXN2)-responsive mechanism that regulates TGF-β-induced ROS production in mammary epithelial cells has been recently

described. These data suggest that a cysteine thiol-disulfide exchange reaction in mitochondria may be involved in TGF-β-mediated regulation of ROS and gene expression [63]. ROS production in SSc also increases due to TGF-β, but as we have shown in our study, oxidative stress and markers are reduced with PD29 application.

Qingbo et al., showed that in PD29 group, IL-6 and MDA were decreased. Also, they showed that GSH was increased in PD29 dose dependent application compared to BLM stimulation [46]. We showed that GSH levels were increased in the PD29 group. Researches related to ROS/RNS production during metabolic processes, open another branch of research focusing on the role of GSH ranging from antioxidant/radical scavenger to redox signal modulator [64]. GSH effectively scavenges free radicals and other ROS and RNS (e.g., hydroxyl radical, lipid peroxyl radical, superoxide anion and hydrogen peroxide) directly and indirectly through enzymatic reactions. The chemical structure of GSH determines its functions, and its wide distribution among all living organisms reflects its important biological role [64]. The PD29 may direct stimulating GSH levels in metabolism because we showed just significant effect on PD29 group.

The expression levels of protective antioxidants were reduced in the skin of the tight-skin (TSK-1/+) mouse fibrosis model [65]. It is another mouse model of SSc. Increased total protein excretion was detected in 17.5% of SSc patients and albuminuria was identified in 25% [66].

The effect of the synergistic effect of upadacitinib and PD292 together on oxidative stress should be repeated with more detailed studies. This research shows that upadacitinib and PD29 may effectively be used for the treatment of SSc.

Conclusion

In conclusion, our findings show that upadacitinib and PD29 have notably protective antioxidative effects on SSc renal tissue induced by BLM.

Compliance with the Ethical Standards

Ethics Committee approval: This study was approved by Kutahya Health Sciences University, Faculty of Medicine, Experimental Animals Local Ethics Committee (approval number: 23.03.2022, 46959).

Conflicts of interest: Authors declare no conflicts of interest.

Funding: Authors received no specific funding for this work.

Authors contributions: AK: Designed the research and also conceived and planned the experiments, AK, MKG, G.K., and EA: Carried out the experiments. AK, MKG, GK, EA: Contributed to the interpretation of the results, AK: Took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Evaluation of differential effects of CDP-choline and choline on parasympathetic activity and changes in choline levels with heart rate variability

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ABSTRACT

Objective: Heart rate variability (HRV) is used to evaluate the autonomic activity of heartbeat. This study aimed to investigate the effects of cholinomimetic drugs cytidine diphosphate-choline (CDP-choline) and choline, on short-term HRV parameters.

Materials and Methods: Animals were randomized into three groups; control (0.9% NaCl), choline (100 mg/kg), CDP-choline (400 mg/kg). Electrocardiography recordings were obtained for 45-minutes after treatments with 15-minutes intervals. HRV analyses and total choline level measurements in serum and heart tissues were performed.

Results: High frequency power and total power increased in treatment groups, while heart rates were decreased. Low frequency was decreased with choline while very low frequency power decreased with CDP-choline. Choline affected most of the HRV parameters in the first 15 minutes, while the effect of CDP-choline started within 30 minutes. Total choline levels were higher in both treatment groups than in the control while the levels were also higher in the choline group compared to CDP-choline group.

Conclusion: This study showed that CDP-choline and choline treatments produced a rapid response to short-term HRV parameters, while increasing tissue choline levels. Moreover, the differences in effects and onset time between the drugs on HRV might be related to tissue choline concentration.

Keywords: Parasympathomimetics, Autonomic nervous system, Cytidine diphosphate choline, Choline

1. INTRODUCTION

Complicated patterns of variability in biological systems have been widely investigated in various areas of medical research. Multifarious oscillations in time differences between successive beats and heart rhythm cause rapid reactions in the cardiovascular system resulting from different stressors that affect homeostasis [1]. Heart rate variability (HRV) is defined as the differences in duration between successive heartbeats in electrocardiographic (ECG) recording [2]. HRV measures the function of the autonomic nervous system (ANS) as well as the individual contributions of the sympathetic (SNS) and parasympathetic nervous systems (PNS). Since, the nervous vagus is the key contributor of the PNS, evaluating vagal activity with HRV analyses is used for assessment of changes in parasympathetic activity [3].

Apart from the normal function, pharmacological interventions affecting the autonomic nervous system and their effect on cardiac dynamics can also be analyzed by using HRV analysis. Choline, a precursor of the main neurotransmitter in parasympathetic nervous system acetylcholine (ACh) also directly interacts with cholinergic receptors to induce cholinergic neurotransmission [4]–[6]high mobility group box 1 (HMGB1. CDP-choline is an intermediary endogenous molecule that is produced during phosphatidylcholine (PC) synthesis via Kennedy pathway [7]. Phosphodiesterases (PDEs) in the cell membrane split CDP-Choline into Choline and cytidine, which raises the total choline levels in the brain and circulation [8], [9]. Choline rapidly diffuses into the blood circulation, cross the blood–brain barrier and plays an important part in acetylcholine synthesis [10].

Choline and CDP-choline have shown potential clinical use in various conditions. Abnormal choline metabolism has been observed in various types of cancer, and it has been detected using magnetic resonance spectroscopy (MRS) approaches [11]. CDP-choline has been studied in various neurological disorders, including traumatic brain injury, Alzheimer's

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disease, Parkinson's disease, learning and memory disorders, amblyopia, acute ischemic stroke and glaucoma [12-14]which is identical to the natural intracellular precursor of phospholipid phosphatidylcholine. Following injection or ingestion, citicoline is believed to undergo quick hydrolysis and dephosphorylation to yield cytidine and choline, which then enter the brain separately and are used to resynthesize CDP-choline inside brain cells. Neuroprotective activity of citicoline has been repeatedly shown in preclinical models of brain ischaemia and trauma, but two recent, large, pivotal clinical trials have revealed no benefits in ischaemic stroke and traumatic brain injury. However, the substance seems to be beneficial in some slowly advancing neurodegenerative disorders such as glaucoma and mild vascular cognitive impairment. This paper critically discusses issues related to the clinical pharmacology of citicoline, including its pharmacokinetics/biotransformation and pharmacodynamics/mode of action. It is concluded that at present, there is no adequate description of the mechanism(s. These studies have shown some beneficial effects of CDP-choline, with rare side effects such as stomach pain, diarrhea, and headaches [14].

Besides, its cholinergic interactions via Choline moiety, purinergic receptors has been shown to contribute some of the effects of cytidine moiety of CDP-Choline [15]1.0 and 2.0 µmol which might be responsible for its distinct effects from Cho. However, the differential effects of Choline and CDP-Choline on HRV has not been established yet. The present study investigated the differential effects of CDP-Choline and Choline on parasympathetic nervous system activity via evaluating short-term HRV parameters together with the total choline/ACh levels.

2. MATERIALS and METHODS

Experimental Groups and Heart Rate Variability Analyses

The regional Ethics Committee for Animal Experiments authorized the experimental study (No: 77.637.435/224). Adult male (12-16 weeks old) wistar rats (310 ± 22, 45 g, n=24) were used for the experiments [16]. The animals were kept in the animal care center under ad libitum conditions for at least five days prior to the start of the experiments in 12 hours dark/light cycle, at 20-22 °C. Ketamine/Xylazine (75/15 mg.kg-1, Sigma-Aldrich, PHA568487) was administered intraperitoneally (i.p.) for anesthesia before the operation [17]. Pedal pain reflex and respiration frequency were monitored to ensure the level of anesthesia after the injection.

To assess the impact of Choline and CDP-Choline on the HRV and heart rate complexity, 24 rats were randomized into the three groups (n=8): (i) Control (0.9% sodium chloride (NaCl)), (ii) choline (100 mg/kg) and (iii) CDP-Choline (400 mg/kg) [18], [19]. Choline chloride (C7017, Sigma Aldrich), CDP-choline sodium salt (C0256, Sigma Aldrich) or 0.9% NaCl injections were administered intraperitoneally (i.p.) after the baseline measurements. For ECG recordings, needle electrodes for Lead II were placed on right arm and left leg [20]. ECGs were recorded using Powerlab/SP8 (ADInstruments, Australia). LabChart 7 software (ADInstruments, Australia) was employed for R wave detection.

R waves were detected automatically via the Pan-Tompkins real-time QRS recognition software and a tachogram of RR intervals was created [21]. These RR tachograms were converted to time series using Berger interpolation. For all HRV analyses, Kubios HRV Software (University of Eastern Finland) was used. HRV analysis was conducted in three domains: (i) Time Domain Analyses; root mean square of successive deviations between regular heartbeats (RMSSD), Standard deviation of NN intervals (SDNN), Baseline width of the RR interval histogram (TINN), the stress index (SI), and mean heart rate (HR), (ii) Frequency Domain Analyses; High Frequency (HF), Low Frequency (LF), Very low frequency (VLF), and LF/HF ratio and (iii) Nonlinear Analyses; Detrended Fluctuation Analysis (DFA) [22], Poincaré plot analysis [23] and Entropy Analysis [24] as we described earlier [25].

Choline measurements

A commercially available kit was used to measure the total choline/ACh levels in the serum and cardiac tissues (Sigma-Aldrich, MAK056). Serum samples were obtained with serum-separator collection tubes and centrifuged immediately at the end of the protocol. Homogenates were prepared with the 10 mg of total heart tissues and lysed with choline assay buffer on ice. Total choline/ACh levels were determined by spectrophotometer according to the instructions [18].

Statistical Analysis

The Shapiro-Wilk test was employed to analyze normal data distribution. One-way ANOVA and post hoc Tukey–Kramer tests were used for comparisons between groups (GraphPad Prism 5, La Jolla, CA). Data were presented as mean ± standard error of the mean (S.E.M.) and $P<0.05$ was considered statistically significant.

3. RESULTS

Heart Rate Variability; Frequency Domain, Time Domain and Non-Linear Analyses

Time domain analysis showed mean HR, RMSDD and SI between the time periods (baseline,15th minute, 30th minute and 45th minute) after the drug injection did not significantly change in the control group ($P>0.05$). In choline-treated group mean HR and SI were significantly lower at 15th minute, 30th minute and 45th minute while SDNN, RMSDD, TINN were significantly higher after the injection compared to baseline measurements (*: $p<0.05$; **:$p<0.01$; ***: $p<0.001$). In CDP-choline-treated group mean HR were significantly decreased within 15th minutes while SDNN and RMSDD were significantly elevated at 30th minute after the injection compared to baseline measurements (†: $P<0.05$; ††:$P<0.01$) (Figure 1).

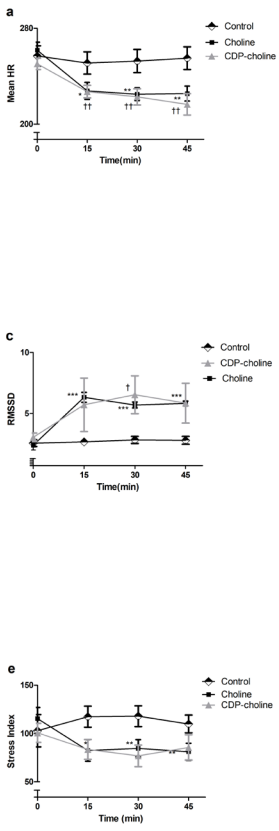

Figure 1. Time domain parameters of experimental groups. *Mean heart rate (HR) (a), Standard deviation of normal intervals (SDNN) (b), Root mean square of successive RR interval differences (RMSSD) (c), Baseline width of the RR interval histogram (TINN) (d) and Stress Index (e). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M. (n=8 per group). (*); P<0.05, (**); P<0.01, (***); P<0.001 vs. baseline measurements of choline group, (†); P<0.05, (††); P<0.01 vs. baseline measurements of CDP-choline group.*

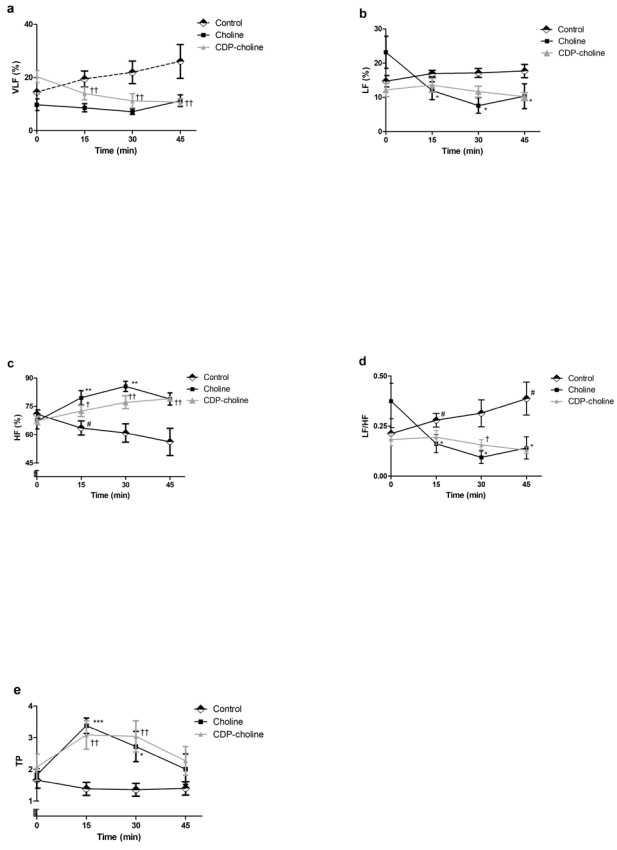

Figure 2. Frequency domain parameters of experimental groups. *Very low frequency (VLF) (a), low frequency (LF) (b), high frequency (HF) (c), LF/HF ratio (d) and total power (TP) (e). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (*); P<0.05, (**); P<0.01, (***); P<0.001 vs. baseline measurements of choline group, (†); P<0.05, (††); P<0.01 vs. baseline measurements of CDP-choline group.*

Regarding the frequency domain analysis; changes in mean relative powers of LF and HF, and TP between the time periods (15th minute, 30th minute and 45th minute) did not significantly change compared to baseline measurements in control group (P>0.05). In Choline-treated group mean LF and LF/HF ratio were significantly lower in 15th minute, 30th minute and 45th minute while HF and TP were significantly higher after the injection compared to baseline measurements (*: P<0.05; **: P<0.01; ***: P<0.001). In CDP-choline-treated group LF and LF/HF ratio were significantly lower in 15th minute, 30th minute and 45th minute while HF and TP were higher after the injection compared to baseline measurements (†: P<0.05; ††: P<0.01) (Figure 2).

Regarding the frequency of nonlinear analysis; changes in mean Sample Entropy (SampEn) between the time periods of baseline measurements, 15th minute, 30th minute and 45th minutes after the operation did not significantly change in control and choline groups. In CDP-choline group mean SampEn were significantly higher in 45th minute compared to baseline measurements (P<0.05; Figure 3e). The changes in mean differences of $DFA\alpha_1$ significantly increased in control group compared to baseline measurements at 30th and 45th minutes in CDP-choline group (P<0.05). In Choline group mean $DFA\alpha_1$ were significantly decreased in 30th minute (P<0.05). $DFA\alpha_2$ did not significantly change in the groups ($DFA\alpha_1$: P<0.05, $DFA\alpha_2$ P<0.01; Figure 3f-g). SD1 and SD2 parameters did not significantly change in the control group compared to baseline measurements. In Choline and CDP-choline-treated groups mean SD1 significantly increased while SD2 and SD2/SD1 ratio significantly decreased

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in 15, 30 and 45 minutes after the injection, compared to baseline measurements (P<0.001). (Figure 3a-c).

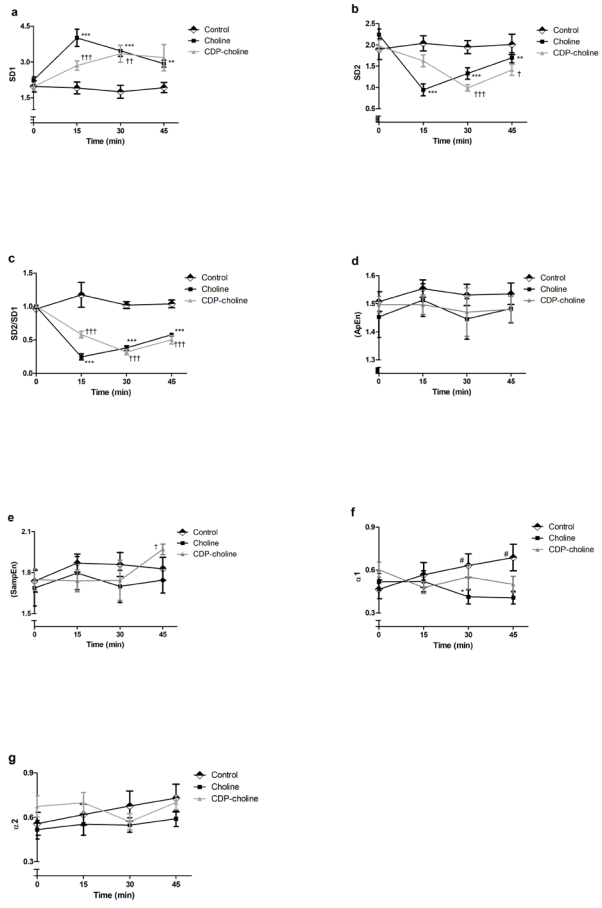

Figure 3. Non-linear parameters of experimental groups. *Standard derivation 1 (SD1) (a), standard derivation 2 (SD2) (b), SD2/ SD1 ratio (c), Approximate Entropy (ApEn) (d), Sample Entropy (SampEn) (e), short-term Detrended Fluctuation Analysis (DFAα1) (f) and long-term Detrended Fluctuation Analysis (DFAα2) (g). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (*); P<0.05, (**); P<0.01, (***); P<0.001 vs. baseline measurements of choline group, (†); P<0.05, (††); P<0.01 vs. baseline measurements of CDP-choline group.*

Total Choline Measurements

Total choline/ACh levels in serum samples increased in Choline (2.5 ± 0.03 nM) and CDP-choline(2.3 ± 0.02 nM) treated groups compared to control group (0.5 ± 0.0005 nM; P<0.001; Figure 4a). The levels in heart homogenates increased in Choline(3.7 ± 0.08 nM) and CDP-choline(3.3 ± 0.04 nM) treated groups compared to control group (1.2 ± 0.01 nM; P<0.001; Figure 4b). Total choline/ ACh was significantly higher in serum and heart tissues of Choline-treated group compared to CDP-Choline groups (P<0.001 and P<0.01 respectively).

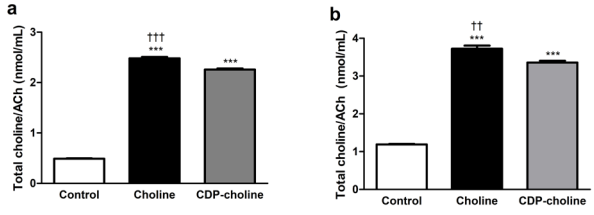

Fig 4. Total choline/acetylcholine levels of experimental groups. *Total choline/ACh levels of serum (A) and heart (B) tissues of experimental groups. One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (***); P<0.001 vs. control group, P<0.01, (†††); P<0.001 vs. CDP-choline group.*

4. DISCUSSION

Heart rate variability is a valuable tool to evaluate the ANS activity, however, effects of parasympathomimetic drugs on HRV analysis have not well established. In present study, our aim was to evaluate effects of parasympathomimetic drugs CDP-choline and choline on HRV parameters of time domain, frequency domain and nonlinear analyses. This study's primary findings can be summarized as follows: (i) Choline and CDP-choline treatment significantly changed HRV parameters that indicated parasympathetic system activation (ii) Effects of choline treatment affected most of the HRV parameters in first 15 minutes, whereas the effects of CDP-choline started within 30 minutes after the injection (iii) total choline/ACh levels in heart and serum tissues were markedly raised by choline and CDP-choline injection, and they were also significantly higher in choline group compared to the CDP-choline group.

Choline treatment has been shown to produce a significant reduction in mean heart rate without any significant change after the CDP-choline treatment in dogs monitored with 2 hours periods. The differences were considered as possibly related with choline's direct agonistic effect on muscarinic receptors along with elevation of the vagal tone by stimulating acetylcholine synthesis within the heart although not directly evaluated in scope of the study [26]. Accordingly, our data showed that choline and CDP-choline significantly changed mean heart rate starting from 15th minutes after the injection compared to baseline values while increasing HF and decreasing LF/HF ratio reflecting elevation in parasympathetic activity. During the monitoring period, we observed that choline and CDP-choline did not exhibit significant differences within the first 45 minutes after the injections. However, it is worth noting that CDP-choline treatment did not lead to any changes in the LF parameters, which reflected sympathetic activation. On the other hand, choline treatment resulted in a significant decrease in LF. This variance in LF levels might account for the slightly different effects we observed in our current findings. We attribute this outcome to the extended monitoring periods and the evident inhibitory influence of choline on sympathetic activity.

Furthermore, we noticed a positive chronotropic effect, which is typically induced by a sudden disruption in the balance between sympathetic and vagal nerves. This results in the predominance of the parasympathetic nervous system in controlling heart functions. As seen in the changes in mean heart rate following vagal activation, our HRV analysis also suggested shifts in the sympathovagal balance towards the parasympathetic nervous system.

CDP-choline is an endogenous intermediate [7] molecule that can be metabolized to choline and cytidine [10]. As a result, choline levels in blood circulation increase and cross the blood brain barrier to contribute acetylcholine synthesis which produce its therapeutic effects in many diseases affecting vascular systems within the body including such as stroke and brain injury [27-30]. Exogenously delivered CDP-choline and choline increase total choline levels in the brain and blood circulation [8, 9, 31]. An earlier study found that intraperitoneal injection of CDP-choline or phosphocholine at equal doses results in significant increases in serum free choline concentrations, which have been shown to cause significant hyperglycemia due to increase in cholinergic neurotransmission [32]. Increase in circulating choline concentrations (ie > 500 μM) has been shown to induce bronchoconstriction by activating PNS. The differences between choline and CDP-choline were possibly related with choline's direct agonistic effect on muscarinic receptors at higher concentrations [26]. Our data indicated that single dose of choline injection elevated plasma total choline/acetylcholine levels significantly compared to CDP-choline which might be responsible for the distinct onset times of effects on HRV parameters.

In present study, HRV parameters indicating parasympathetic activity, RMSSD, HF, and DFAα1, significantly changed after both choline and CDP-choline injection. These parameters, by their nature, reflect relatively fast changes in heart rate time series. Our data indicated that both choline and CDP-choline treatments affect short term HRV parameters and fast changes in heart rate time series within 45-minutes.

HRV parameters of sympathovagal balance including LF/HF ratio and SD2/SD1 ratio decreased after choline and CDP-choline injection indicating parasympathetic system activation and changes in relatively faster oscillations in heart rate time series. However, LF/HF ratio also increased in control group which may be due to the effects of Ketamine/Xlazyne anesthesia [25] although, significant changes between treatment groups and controls indicate prominent parasympathetic system activity with the cholinomimetic drug administrations.

Conclusion

Our results showed, choline and CDP-choline treatments produce a rapid response on short-term HRV parameters related with parasympathetic system activity via increasing total choline/acetylcholine levels in serum and heart tissue while the onset time of effect might differ between them.

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Compliance with the Ethical Standards

Ethics Committee approval: This study was approved by Manisa Celal Bayar University, Institutional Ethics Committee for the Care and Use of Experimental Animals (approval no:77.637.435/224).

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Conflicts of interest/Competing interests: Authors state no conflict of interest.

Authors contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission. HK, SA, HFO and EB: Designed and conducted the experiments, HK and EB: Wrote the draft manuscript. All authors reviewed the final manuscript.

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The role of epidermal growth factor and cholinergic receptor agonists and antagonists in MAPK signal transduction in K562 cells

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ABSTRACT

Objective: Muscarinic receptors (M1-M5) are members of the G protein-coupled receptor superfamily and are effective in physiological functions through G proteins. Recent studies suggested that cholinergic receptors mediate cellular activities in hematopoietic cells. The aim of this study was to investigate the potential role of mitogen-activated protein kinases (MAPK) signaling extracellular signal-regulated kinases 1 and 2 (ERK1/2)/phosphorylated ERK1/ (pERK1/2) pathways in chronic myeloid leukemia (K562) cells.

Materials and Methods: Chronic myeloid leukemia cells were cultured. Cells were incubated in the presence of muscarinic receptor agonist, antagonist and epidermal growth factor (EGF). To detect MAPK activation, ERK/pERK protein expression levels were determined by western blot method techniques.

Results: Our study results showed that cholinergic agents and EGFs affect the MAPK pathway in the human K562 cell line.

Conclusion: Cholinergic and EGF receptors may affect the MAPK pathway in K562 cells.

Conclusion: Cholinergic and EGF receptors may affect the MAPK pathway in K562 cells.

Keywords: G protein coupled receptor, Carbachol, Epidermal growth factor, Mitogen-activated protein kinase, Chronic myeloid leukemia

1. INTRODUCTION

Acetylcholine (ACh) is a classical neurotransmitter. It has been demonstrated in a variety of neuronal and non-neuronal cells. ACh also plays a role as a mediator of cell communication in non-neuronal cells [1]. ACh has widespread physiological effects such as cytoskeletal regeneration, cell proliferation, differentiation, and programmed cell death (apoptosis) [2].

Acetylcholine is involved in the regulation of many physiological processes, involving muscarinic acetylcholine receptors (mAChR) (M_1-M_5) from the G protein-coupled receptor (GPCR) superfamily [3]. Muscarinic acetylcholine receptors expressed in different tissues and cells mediate intracellular responses by interacting with G protein subtypes [4]. Recent studies have shown that mAChRs functionally mediate cellular activities in non-neuronal and hematopoietic cells. It has also been reported that they are expressed in various cancer cell types, including the brain, mammary, colon, skin, lung, and prostate cancer cells. It is also stated that there are interactions between mAChRs and epidermal growth factor receptors (EGFRs) in different cancer cell types [5, 6]. Different mAChR subtypes are expressed in B and T lymphocytes [2]. Activation of M_1, M_2, and/or M_3 receptors in these cancer cell types causes an increase in cell proliferation. However, it has also been reported that mAChR receptor activation can cause cell cycle arrest and reduce cell proliferation [6]. Muscarinic receptors activate different signal transduction pathways via G proteins. M_1, M_3, and M_5 receptor subtypes interact with αq/11 subunits of G proteins, while M_2 and M_4 receptor subtypes interact with αi/o subunits of G proteins [7,8]. Different researchers showed that many G protein-coupled receptors also activate MAPK [9]. It has been determined that MAPKs are activated by cholinergic receptors. Activation of MAPK via the acetylcholine signal transduction pathway in different cells causes phosphorylation of ERK1/2 , DNA synthesis, and cell proliferation [4]. It has previously

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been shown that mAChRs increase SNU-407 colon cancer cell proliferation via the ERK1/2 pathway. The researchers showed that EGFR, protein kinase C, ERK1/2, and ribosomal S6 kinases (RSK) and mAChR-mediated activation affect proliferation of SUNU 407 colon cancer cells. EGFR activates MAPK, ERK1 and ERK2 (p44 and p42 MAPK), which are activated by mitogens, and their central role in cell proliferation has been established. These receptors activate the transcription of genes necessary for cell survival and cell proliferation [10]. The M_3 mAChR is determined to be expressed and functional in colon cancer cells [11]. The role of these receptors in cancer is largely unknown, they are known to mediate cell growth through different signal transduction pathways [11]. The Ras, small GTP-binding protein, /Raf, a serine/threonine protein kinase, /MEK tyrosine and serine/threonine dual specificity protein kinases,/ERK cascade is an important signaling pathway in MAPKs. Over activation of the Ras /Raf/MEK, /ERK pathway has been reported in 30% of human tumors [12]. In cancer, hormones, growth factors, differentiation factors, and tumor-promoting agents use the Ras/Raf/MEK/ERK signal transduction pathway. This signal transduction pathway starts with Ras activation, and followed by activation of Raf, MEK, and ERK [12]. mAChRs have been shown in animal and human cell lines to stimulate mitogen-activated protein (MAP) kinases, which act by activating extracellular signal-regulated kinases 1 and 2 (ERK1/2) [13]. Our previous studies demonstrated the semi-quantitative RT-PCR method by which K562 cells express M_2, M_3, and M_4 muscarinic receptor subtypes [14]. We also demonstrated the expression of muscarinic receptors at protein level in K562 cells using specific antibodies specific for M_2, M_3, and M_4 subtypes by western blot method [15]. In our previous studies, cholinergic system was found to be functional in K562 cells by muscarinic receptor-mediated protein kinase C , nitric oxide , cylic adenosine mono phosphate, c-fos, an intermediate early gene, and Ca^{+2} measurement assays [14,15]. We also showed that alpha 7 nicotinic receptor expression was determined in K562 cells. The effects of cholinergic agonist ACh and/or atropine (ATR), nicotinic antagonist, methyllycaconitine on cell proliferation and intracellular Ca^{2+} levels in K562 cells were determined. [16]. The epidermal growth factor receptor is one of the key players in the MAP kinase signaling cascade that is activated through the cholinergic system. Different investigators have shown that MAPK/ERK activation by GPCR occurs via Ca^{2+} dependent or independent mechanisms, depending on receptor type and cellular background [17,18]. EGFR is a member of the tyrosine kinase family. Various signaling pathways activate these receptors. Several studies have found that stimulation of various GPCRs activates the epidermal growth factor receptor [19, 20]. The signaling pathways involving mAChRs are not fully clear. It appears to depend on the context of cellular growth [21].

This study aimed to determine the changes in ERK, pERK protein expression levels in the presence of carbachol (CCh) and/or EGF to determine the role of EGFRs in muscarinic receptor-mediated mitogen-activated protein kinase activation in human erythroleukemia cells.

2. MATERIALS and METHODS

Carbachol, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP), atropine (ATR) , Roswell Park Memorial Institute 1640 (RPMI-1640) medium, and epidermal growth factor (EGF) were purchased from Sigma Chemical Co, St. Louis, MO, USA. Fetal bovine serum (FBS) was obtained from Biol. Ind. (Beit Haemek, Israel). The Lowry kit (SMART TM BCA Protein Assay Kit iNtRON Biotechnology (Korean), The Nitra Blue Tetrazolium/5-Bromo-4-Chloro-3-indolyl phosphate (NBT/BCIP) was provided by Promega (Madison, WI, USA). ERK, phosphorylated ERK (pERK) (137F5 – p44/42 MAPK (Erk1/2) Rabbit mAb #4695), Phospho-p44/42 MAPK (ERK 1/2) (Thr202/Tyr204) (197G2) Rabbit mAb #4377 and β-actin antibodies (Sc190657) were supplied by Cell Signaling Technology (CST-USA) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Sigma (St Louis, MO, USA) provided the secondary antibodies.

Cell culture

The American Type Culture Collection (ATCC), Manassas, VA, USA, provided the K562 cells. K562 cells were incubated in RPMI-1640 culture medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 200 mM L-Glutamine, at 37°C, in a humidified atmosphere of 5% CO_2 and 95% air, with one half of the medium being replaced every 3-4 days.

Cell proliferation and viability

Cells were stained with 0.4 % trypan blue and counted using a hemocytometer. The trypan blue exclusion test calculated cell viability and proliferation.

K562 cells were seeded into culture flasks containing RPMI-1640 medium under serum-free conditions. After 24 h, these "starved cells" were placed into a medium containing 1% FBS. K562 cells were exposed to CCh in the presence or absence of the M_3 muscarinic selective antagonists 4-DAMP. 4-DAMP was administered for 30 min before CCh/EGF was added to the culture medium. Cells were harvested and washed in phosphate buffered saline for 15 minutes at 400 g before being frozen at –80 °C.

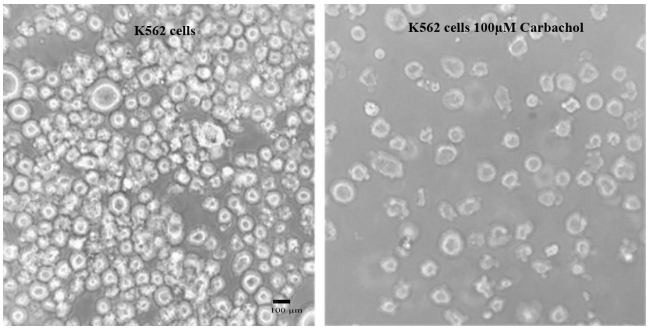
Preparation of homogenates and western blot analysis

Semi-quantitative western blotting assessed the levels of ERK and pERK protein. Cells treated with drugs and cell lysates were prepared in lysis buffer. Lysates were lysed with Dounce homogenizer in a lysis buffer that contained 20 mM HepesKOH, pH 8.0, 0.1 mM, ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonylfluoride, 10 µg/mL leupeptin and 2 µg/mL aprotinin. The Lowry kit (SMART TM BCA Protein Assay Kit, iNtRON Biotechnology Korean), was used to detected protein levels in cell homogenates. Western blot analyses were as described in our previous studies with minor modification [14,15]. 150 µg of protein was loaded onto sodium dodecyl sulfate-polyacrylamide gels and electrophoretically transferred onto nitrocellulose membranes (Schleicher and Schuell,

0.45 μM, Germany). The membranes were blocked at room temperature for 60 min. Later, the membranes were incubated overnight at 4°C with antibodies against ERK and pERK (1/100) (Cell Signaling Technology,USA). The blots were washed with Tris-buffered saline (TBS) containing 0.05% Tween-20 (TBS-T) and were later incubated with alkaline phosphatase-conjugated secondary antibodies for 1 h at room temperature (20°C). β-actin antibody was used for loading control in each blot (Santa Cruz, CA, USA). Secondary antibody and dilutions were used: goat-antirabbit 1:10,000 (Sigma, St Louis, MO, USA). Nitra blue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) was used to detect the antibody-antigen complex. The free edition of ImageJ software quantified band intensities by optical density. The apparent molecular weights of ERK, pERK, and β-actin are 42 kDa, 44 kDa, and 47 kDa, respectively.

3. RESULTS

K562 cells were incubated in RPMI-1640 culture medium containing 10% fetal calf serum, L-Glutamine, at 37°C, in a humidified atmosphere of 5% CO_2 and 95% air. K562 cells were observed by Phase contrast light microscopy (Figure 1).



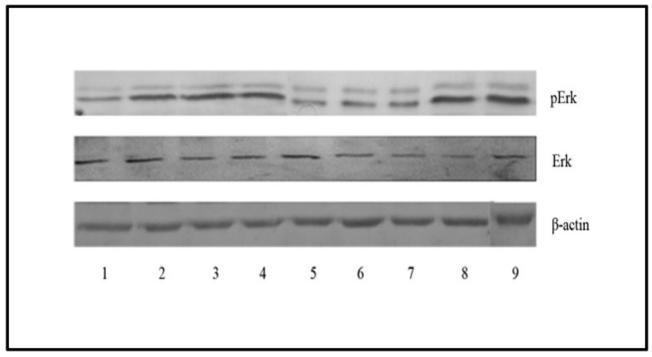
a b

***Figure 1**. Phase contrast light microscopy of K562 cells. K562 cells were seeded at a density of 1X10^5 cells/ml in RPMI 1640 medium containing L-glutamine and 1% fetal calf serum. a) K562 cells b) Carbachol treated K562 cells*

Effect of CCh and EGF on pERK/ERK expression in K562 cells

K562 cells were seeded into culture flasks containing RPMI-1640 medium under serum-free conditions. After 24 h, these "starved cells" were placed into a medium containing 1% FBS.

K562 cells were exposed to 100μM CCh or 16nM EGF in the presence or absence of the M_3 muscarinic selective antagonists 4-DAMP at 0, 30, and 120 min. Expression of pERK/ERK was detected by western blot analysis of whole lysates of K562 cells. CCh increased the time-dependent expression of pERK/ERK, while EGF caused a decrease at 30 min. The epidermal growth factor was determined to decrease pERK/ERK expression in 30 minutes (Figure 2).



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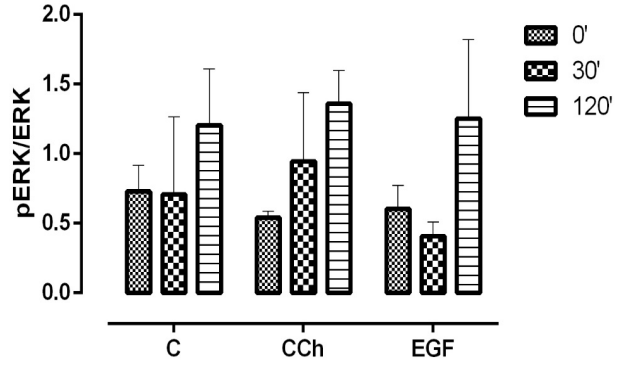

b

***Figure 2.** Effects of CCh /EGF on ERK expression. K562 cells were treated with 100 μM CCh /or 16nM EGF for 0, 30 min, and 120 min. 1) Control 0 min 2)CCh 0 min 3)EGF 0 min 4) Control 30 min 5) CCh 30 min 6) EGF 30 min 7) Control 120 min 8) CCh 120 min 9) EGF 120 min. Western blotting for β-actin was used as the control for the assay. a) Typical western blot figure is representative of three separate experiments. b) The results were shown as ± SEM by taking the average of 3 independent experiments. Statistical analysis was performed with Two-way ANOVA; P>0.05 (ns).*

In order to determine the role of EGF in muscarinic receptor-mediated mitogen-activated protein kinase activation, we investigated the effect of cholinergic agonist/antagonists and EGF on pERK/ERK. Results are shown in Figure 3. pERK/ERK expression decreased compared to the control group (P<0.05) (Figure 3). Exposure of K562 cells to the M_3 muscarinic receptors the antagonist 4-DAMP significantly inhibited pERK and ERK responses to CCh (P<0.05) (Figure 3). Our results show that pretreatment of K562 cells with the M_3 selective muscarinic receptor antagonist 4-DAMP (10^{-6} M, 30 min) reduced pERK/ERK expression compared to control (P<0.05).

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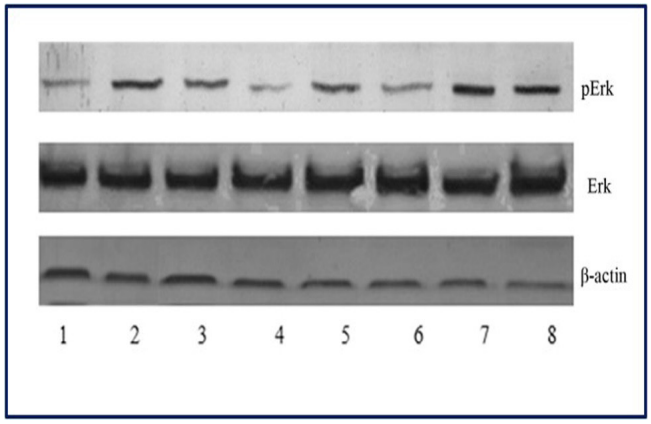

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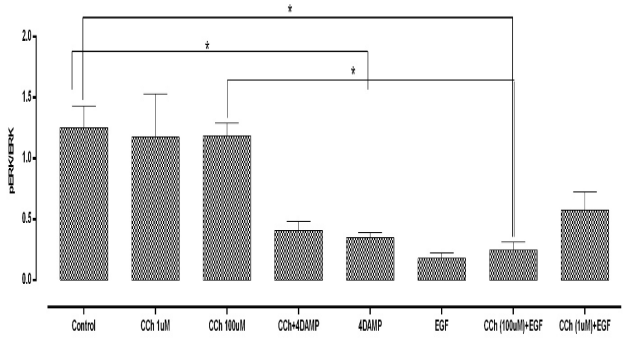

Figure 3. *Effects of CCh, 4-DAMP, and EGF on pERK/ERK expression, K562 cells were treated with 100 µM CCh for 30 min. The 10 µM 4-DAMP antagonist was added 30 min prior to CCh. 1) Control, 2) 1µM CCh 3) 100 µM CCh 4) CCh+4-DAMP 5)4-DAMP 6) 16nM EGF 7) 100 µM CCh +EGF 8) 1 µM CCh +EGF. a) Representative western pERK1/2 and ERK1/2 phosphorylation blots are shown, western blotting for β-actin was used as the control for the assay. b) The results were shown as the mean of the 3 independent experiments as ± standard error (SEM) (P<0.05). Statistical analysis was performed with Two-way ANOVA (P<0.05).*

Strong inhibition of pERK/ERK by both 4-DAMP and EGF was observed. pERK/ERK ratio was significantly inhibited in the presence of CCh+EGF compared to CCh.

4. DISCUSSION

The present study showed that mAChR agonists, antagonists, and/or EGF caused a change in MAPK in K562 cells. Expression of pERK/ERK in K562 cells was decreased in the EGF, 4-DAMP, and EGF+CCh groups compared to the control. We have previously shown that cell proliferation is inhibited when K562 cells are exposed to CCh in a cell growth medium supplemented with 1% or 10% serum [15]. However, it has been shown that CCh stimulates K562 cell proliferation (increased DNA synthesis) in a serum-free medium. We also showed that growth inhibition elicited by CCh in K562 cells is reversed by atropine and 4-DAMP. CCh also caused a decrease in the expression of M_2 and M_3 proteins in K562 cells. [15]. This study showed that blocking M_3 mAChR signaling using the selective antagonist 4-DAMP inhibited MAPK pathway protein expression. 4-DAMP appeared to suppress M_3R mAChR-mediated phosphorylation of ERK. Because EGF also inhibited ERK expression levels after incubation with CCh in K562 cells, the change in ERK expression was independent of EGFR activation. Kuol et al., showed the effect of atropine and 4-DAMP on the phosphorylation of protein kinase B (AKT) and ERK. They also demonstrated EGFR activation in CT-26 cells. They also showed that blocking all muscarinic receptors with atropine and M_3R with 4-DAMP significantly suppressed CT-26 cell proliferation in a dose-dependent manner and induced apoptosis through inhibition of the EGFR/AKT/ERK signaling pathways. They suggested that atropine exerts its effect by inhibiting the EGFR/AKT/ERK pathway, and 4-DAMP by suppressing the AKT/ERK signaling pathway [22]. Different researchers have noted that mAChR activation can stimulate or inhibit cellular growth depending on previous levels of cellular activity [21, 23]. According to Metzger et al., CCh inhibited EGF-induced HaCaT cell migration [24]. Prenzel et al., stated that M_3R activation promotes CRC progression by both EGFR-dependent and independent mechanisms [25]. Yu et al., found a close link between M_3R expression and AKT and ERK phosphorylation and EGFR activation in gastric cancer cells [26]. Different researchers have reported that M_3 mAChR mediated EGFR activation, signaling pathway is activated in different types of cancer, thereby phosphorylating ERK1/2 and AKT [27, 28]. In different studies, the ability of muscarinic agonists to stimulate growth and M_3 receptor antagonists to inhibit tumor growth has been demonstrated for breast, melanoma, lung, stomach, colon, pancreatic, ovarian, prostate, and brain cancer. While the cholinergic system is found in a wide variety of cancers, mAChRs have been shown to be organ-specific [29]. Kanlı et al., showed that pilocarpine (M_3 AChR agonist) exhibits antiproliferative effects in the presence of 1% and 0% FBS [30]. Antiproliferative effects of pilocarpine were not reversed by 4-DAMP (M_3 AChR antagonist) in K562 cells. Pilocarpine did not change M_3 mAChR expression. But 4-DAMP+pilocarpine group caused significant increase in M_3 mAChR expression compared to the control group [30, 31]. Treatment with CCh for 48h decreased the K562 cell number, indicating that CCh had a very fast and irreversible effect to promote cells to necrotic cell death [32]. The results of present study show that besides the inhibitory effect of cholinergic receptor antagonists, M_3R blocking may exhibit antitumor effects through a variety of mechanisms, including antitumor response through suppression of the pErk/ERK signaling pathway. Because these receptors can activate multiple signaling pathways, the growth-promoting and inhibiting effects of muscarinic agonists/antagonists in cancer cells are still unclear. This study showed that cholinergic and EGFRs may affect the MAPK pathway in human chronic myeloid erythroleukemia cells.

Compliance with the Ethical Standards

Ethics Committee approval: This research using cell lines does not require ethical approval.

Financial support: The authors have no relevant financial information to disclose.

Conflict of interest: The authors have no potential conflicts of interest to disclose.

Authors contributions: SGA and BA: Carrying out the experiments, H C, B A, and S GA: Analyzing the results and conducting the project, H C: Writing – Original draft preparation, writing – reviewing and editing. All authors read and approved the final version of the article.

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The evaluation of the processes of problem based learning tutorials: Online or face-to-face?

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ABSTRACT

Objective: The purpose of this research is to evaluate e-PBL tutorials and compare these sessions with face-to-face PBL sessions.

Materials and Methods: This research is a program evaluation study in which quantitative methods were used. In the research, four 90-minute e-PBL sessions held between April and June 2020 were evaluated. Sessions conducted online were realised using the seven-step approach in groups of 13-14 students and a tutor. Video recordings were analysed with the thin slicing method. In addition, various quantitative data on evaluation were analysed using multiple tools and materials, including the end-of-program evaluation form. Friedman test and Mann-Whitney U test were used in the analyses of quantitative data.

Results: Upon evaluating the analyses of the feedback received from the students about the structure, content and process of the program regarding the e-PBL tutorials, the students gave a positive opinion of 80% or more. In regards with the cases, the students had positive opinions of over 80% in terms of "motivation for learning and researching", "daily life and its relation to their individual development", "suitability to their levels of knowledge and skills", "reinforcement of topics". Support, guidance and feedback received from the tutor as a group and individually during online tutorials were statistically significantly higher than the face-to-face PBL tutorials ($P<0.05$).

Conclusion: Research on the effectiveness of e-PBL tutorials, including ours, point out that e-PBL practices may constitute a viable alternative besides face-to face ones. However, for a sounder framing and better results, the subject should be studied in different aspects and more evidences be gathered in this area. These studies will provide evidence to educational institutions and practitioners on how to adapt and modify educational practices, including PBL.

Keywords: Evaluation, Problem-based learning, Tutorial, Online, Face-to-face

I. INTRODUCTION

Problem-based learning (PBL) has been widely used in medical schools for over 50 years. In recent years, various e-learning applications, including e-PBL, have been on the rise [1,2]. E-learning can be defined as formal learning system with the help of electronic resource or learning conducted via electronic media. E-learning, e-assessment applications have taken their places among the sine qua non of the new normal after the corona virus 19 (COVID-19) pandemic, and educational practices, especially lecturer courses and PBL, have mostly been transformed into e-learning applications [3,4]. Accordingly, PBL sessions held face-to-face since 2000-2001 during the preclinical education period of Marmara University, School of Medicine were made online starting 2019-2020 academic year and including 2020-2021 academic year.

With the learner-centered PBL carried out with small groups of students, the objectives are the acquisition of high level cognitive and metacognitive learning such as in-depth learning, problem solving and reflective thinking. PBL session processes have a complex nature in terms of their emotional, motivational and group interaction aspects. Several parameters like the characteristics of the cases used in PBL sessions, group dynamics, tutor's orientational skills, students' and tutor's motivation may affect the learning environment/climate and tutorial processes and learners' acquisitions [2,5-10]. Although, relevant research exists in literature, similar research need to be made on e-PBL practices.

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Learners may have various communication and interaction ways with the group and the tutor in e-learning platforms via chat rooms, forums, e-mail or interactive white boards [11]. Synchronous and asynchronous tools may be used in sharing materials and their sources related to the problem. Also learning administration systems incorporating all of these together may be used in e-PBL sessions [12,13].

The aim of this study is to evaluate e-PBL tutorials and compare these sessions with face-to-face PBL sessions. As per this aim the research problems have been defined as follows:

1. What are the characteristics, in terms of learning content and process, of the e-PBL of four sessions carried out with the seven-step approach?
2. What kind of a learning environment and climate have been formed in the e-PBL practice?
3. Are PBL sessions carried out online as effective as the ones carried out face-to-face?

2. MATERIALS and METHODS

The study was approved by the Trakya University Faculty of Medicine Scientific Research Ethics Committee (approval no: 09/21, date:12.04.2021).

This research is a program evaluation study in which quantitative methods were used. In the research an e-PBL program of four sessions of 90 minutes each held with year 1 medical students (age range 18-22 and gender distribution close to each other) between April and June 2020 was evaluated. Sessions carried out online were realised using Maastricht's seven-step approach in groups of 13-14 students and one tutor for each group [14]. Video recordings of the sessions were taken with permission of the groups. Sessions were held with 12 groups. Due to the limited number of groups consenting to be recorded and technical reasons such as unable to take records for various reasons or sound quality, connection problems and interruptions in the process in recorded videos, video recordings of only four groups out of 12 groups could be used. Quantitative data for evaluation were gathered using more than one tool and material. Methods and tools used are as follows:

Thin slicing method

Video recordings of the sessions were evaluated on macro and micro levels using two tools for observation and evaluation prepared by the researchers. The evaluation of the sessions was made globally from the beginning to the end (macro evaluation) by three researchers (OE, AG, EA), and the chosen slices were evaluated (micro evaluation) by two researchers (OE, AG) separately. Thin slicing method was used for micro evaluation. In thin slicing method, an evaluation of slices of 30-40 seconds taken from different steps of each session instead of the whole of video recordings is made [15-17]. In this research, slices of 40 seconds from each pre-discussion and discussion step of sessions were taken. In order to evaluate each session, over all 30 slices were taken and 20 minutes out of 90 minutes of a session recording was analysed.

A two-axis tool of 5-degree scale titled "Session Environment/ Climate Observation and Evaluation Tool" and "Content and Process Observation and Evaluation Tool" formed by the researchers was used for the evaluation. Three researchers separately evaluated the recordings of overall four e-PBL cases as a whole over the "problem identification and pre-discussion" and "discussion" parts using the "Session Environment/Climate Observation and Evaluation Tool". After the first evaluations, two researchers evaluated one by one the thin slices taken out of these two parts. A similar evaluation was made using the "Content and Process Observation and Evaluation Tool".

The consistency in the evaluation among the three researchers was analysed using the "intraclass correlation coefficient (ICC)", and after a consensus was reached among the researchers over the observation and evaluation scales on the items whose ICC values were not on requested levels, the parts in question were re-evaluated by the researchers. And the researchers reached a consensus on their average rates. The ICC was computed using the two-way random effect models and "average" unit to assess the agreement among the three raters in rating each aspect (interaction, togetherness, participation, confirmation, openness/flexibility, liveliness, conflict, ease, support/guidance, mood) of the first tool. There was a good consistency among the three raters where ICC ranged from 0.71 to 1. Regarding the agreement among the three raters in rating each aspect (content sharing, content presentation, content arrangement, arrangement of the session process, relating with the case, asking questions, tutorial support/guidance, emotional environment and support) of the second tool, there was also a good consistency among the three raters where ICC ranged from 0.62 to 0.97.

End of program evaluation

At the end of the program, students' opinions on e-PBL sessions were gathered via a questionnaire of 26 questions. The evaluation questionnaire prepared by the researchers consisted of three parts that are "general functioning" (12 items), "cases" (6 items), "face-to-face compared to online" (8 items), and the evaluations were requested to be made over scales of 5 and 10.

Statistical Analyses

R 0.4 program was used to analyse quantitative data statistically. Friedman test was employed to compare cases based on relation to the subjects handled in the related course. Scores for Pre-discussion and Discussion parts evaluated in thin slicing were compared using Mann-Whitney *U* test. Lastly, one sample median test was employed to compare face-to-face and online PBL. $P< 0.05$ was accepted for statistical meaningfulness.

3. RESULTS

Observation and Evaluation of e-PBL Tutorial Processes

Two different tools were used to evaluate the video recordings of e-PBL tutorial processes. With the first tool, the 10 aspects (interaction, association, participation, confirmation, openness/flexibility, liveliness, conflict/disagreement, ease,

support/guidance, mood) of the e-PBL session environment/climate were graded over a scale of two axes (negative axis of 5-point Likert scale and the positive axis of 5-point Likert scale). As seen on Table I, the average grades were calculated on the positive axis, in each aspect, except for the conflict/disagreement grade, between 3.0-4.0; and in the conflict/disagreement aspect as 2.0. Although, the level of constructive conflict appears to be low, these scores point out a medium-high level learning environment and climate. Furthermore, the fact that a meaningful difference was not identified between the Pre-discussion and Discussion parts of e-PBL processes shows that a confirmatory learning environment was attained for all the tutorial processes of seven steps.

With the second tool, the eight aspects related to handling the content and coordinating the session process (content sharing, content presentation, content arrangement, arrangement of the session process, relating with the case, asking questions, tutorial support/guidance, emotional environment and support) were graded over a scale of two axes (negative axis of 5-point Likert scale and the positive axis of 5 – point Likert scale). As seen on Table II, the grade averages for the aspects of asking questions, tutorial support, emotional environment were calculated between 2.8-4.0 on the positive axis. The average scores in the remaining 5 aspects were between 0.3-3.0. These grade averages point out a positiveness of medium-high level. No statistically significant difference between average scores of Pre-discussion and Discussion parts was identified in any of 8 aspects.

End of Program Student Evaluations

In this part, first of all, results of the evaluations received from students at the end of the program by way of three different forms were included. With the first form, the structure, content and process of the program, and with the second form results related with the cases used were obtained. By way of data obtained with the third form student opinions on face-to-face and online tutorial processes were compared.

Upon evaluating data regarding the structure, content and process of the program, it was observed that students expressed positive opinions above 90% in 9 items. As for the remaining three items (sufficiency of personal performance during tutorials, usability/functionality of the learning media and sufficiency of the recommended sources) the percentage of students who expressed positive opinions was just 80%, and 12-19% of opinions were identified to be not positive (Figure 1).

When student opinions on the quality of the cases used were examined, all three cases were evaluated above 80% positively in terms of "motivation for learning and researching", "daily life and its relation to their individual development", "suitability to their levels of knowledge and skills" and "reinforcement of topics". Percentage of students with positive opinions on the cases regarding "relationship with their professional life" and the "integration with the other topics in the program" varied between 50-79. Especially student opinions on "integration with the other topics in the program" were observed to shift toward medium or low level of 17-50% (Figure 2).

Table 1. *Scores on learning environment/climate in e-PBL tutorial processes*

Aspects related to Learning Environment/Climate	e-PBL Tutorial Process			
	Pre-discussion *Median (Q1, Q3) Min-max*	Discussion *Median (Q1, Q3) Min-max*	Whole Process *Median (Q1, Q3) Min-max*	p
Interaction (interactive, multidirectional, complex)	4.0 (3.0, 4.0) 3.0 – 4.0	3.8 (3.2, 4.0) 3.0 – 4.0	4.0 (3.0, 4.0) 3.0 – 4.0	0.893
Togetherness (collaborative)	4.0 (3.0, 4.0) 3.0 – 4.0	3.8 (3.2, 4.0) 3.0 – 4.0	4.0 (3.0, 4.0) 3.0 – 4.0	0.893
Participation (engaged)	4.0 (3.0, 4.0) 3.0 – 4.0	4.0 (4.0, 4.0) 3.0 – 4.0	4.0 (3.0, 4.0) 3.0 – 4.0	0.456
Confirmation (confirmatory, respectful, non-judgmental attention/care)	4.0 (4.0, 4.0) 4.0 – 5.0	4.0 (4.0, 4.0) 4.0 – 4.0	4.0 (4.0, 4.0) 3.0 – 5.0	0.359
Openness, flexibility (open and flexible attitude and behaviour)	4.0 (4.0, 4.0) 3.0 – 5.0	4.0 (4.0, 4.0) 3.0 – 4.7	4.0 (4.0, 4.0) 3.0 – 5.0	0.659
Liveliness (energetic, uplifted)	3.0 (3.0, 4.0) 2.0 – 4.0	3.3 (3.0, 3.9) 3.0 – 4.0	3.0 (3.0, 4.0) 2.0 – 4.0	0.699
Conflict (constructive conflict, seeking consensus, seeking for the better)	2.0 (2.0, 2.5) 2.0 – 3.0	2.0 (2.0, 2.0) 2.0 – 3.0	2.0 (2.0, 2.3) 2.0 – 3.0	0.401
Ease (safe, challenging)	4.0 (3.0, 4.0) 3.0 – 4.0	4.0 (3.2, 4.0) 2.7 – 4.0	4.0 (3.0, 4.0) 2.7 – 4.0	0.814
Tutorial support/ guidance (supportive, guiding)	4.0 (4.0, 4.0) 2.0 – 5.0	4.0 (4.0, 4.0) 4.0 – 4.0	4.0 (4.0, 4.0) 2.0 – 5.0	1.000
Mood (interested, willing, enthusiastic)	3.7 (3.0, 4.0) 3.0 – 4.0	3.0 (3.0, 3.0) 3.0 – 4.0	3.0 (3.0, 4.0) 3.0 – 4.0	0.153

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Table II. *Scores on content and practice process of e-PBL tutorials*

Aspects related to content and practice process	e-PBL Tutorial Process			
	Pre-discussion	Discussion	Whole Process	p
	Median (Q1, Q3) Min – max	*Median (Q1, Q3) Min – max*	*Median (Q1, Q3) Min – max*	
Content sharing (unidirectional, linear, passive, relational, in-depth)	2.7 (1.8, 3.7) -1.3 – 4.0	2.0 (1.7, 3.1) -1.7 – 4.7	2.7 (1.7, 3.7) -1.7 – 4.7	0.432
Content presentation (information transmitting - - concretization, relating, narrating)	2.0 (1.3, 3.0) -1.3 – 3.0	1.8 (-0.8, 2.0) -3.3 – 3.3	2.0 (1.3, 3.0) -3.3 – 3.0	0.528
Content arrangement (piece by piece, superficial – - – complete, detailed)	2.7 (2.0, 3.7) 1.7 – 4.0	2.5 (-0.4, 3.4) -2.3 – 4.7	2.7 (2.0, 3.7) -2.3 – 4.7	0.753
Arrangement of the session process (step by step, superficial – - – complex, in depth)	2.0 (1.7, 3.2) -2.0 – 4.0	0.3 (-1.6, 3.0) -1.7 – 4.0	2.0 (-1.3, 3.3) -2.0 – 4.0	0.503
Relating with the case (limited reference – - – relating the whole process with the case)	3.0 (2.3, 3.8) -1.7, 4.3	2.2 (-0.3, 2.9) -4,3, 4.3	2.7 (2.0, 2.3) -4,3, 4.3	0.240
Asking questions (constructive, opening and deepening learning)	3.0 (2.8, 3.7) 2.0 – 4.7	2.8 (2.7, 3.5) 2.3 – 4.0	3.0 (2.7, 3.7) 2.0 – 4.7	0.581
Tutorial support/guidance (sufficient support, guidance)	4.0 (3.5, 4.0) 2.0 – 4.7	3.8 (3.7, 4.2) 3.7 – 4.7	4.0 (3.7, 4.0) 2.0 – 4.7	0.660
Emotional environment and support (emotional awareness, arrangement)	3.0 (2.7, 3.2) 1.3 – 4.3	3.0 (3.0, 3.0) 2.7 – 3.7	3.0 (2.7, 3.0) 1.3 – 4.3	0.654

The three cases were compared with each other using the Friedman test, and results are presented in Table III.

A statistically significant difference was observed in all items over the Freidman test (P <0.001). The values for each item were calculated as follows: for "integration with other subjects in the program"; X^2 (2) = 118; P <0.001; Kendall's W effect size = 0.438 (large); for "reinforcement of topics"; X^2 (2) = 102.83; P<0.001; Kendall's W effect size = 0.381 (moderate); for "suitability to their levels of knowledge and skills"; X^2 (2) = 106.58; P<0.001; Kendall's W effect size=0.381 (moderate); for "motivation for learning and researching"; X^2 (2) = 118; P<0.001; Kendall's W effect size=0.437 (moderate); for "daily life and its relationto their individual development"; X^2 (2) = 140.3; P<0.001; Kendall's W effect size=0.520 (large).

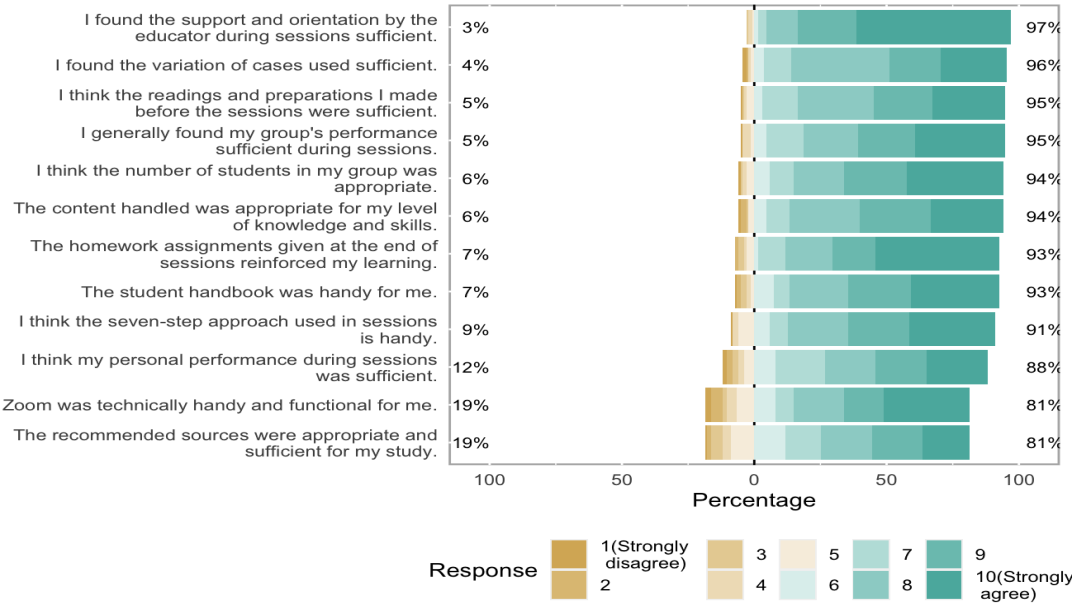

Figure 1. *Student opinions on the structure, content and process of the program*

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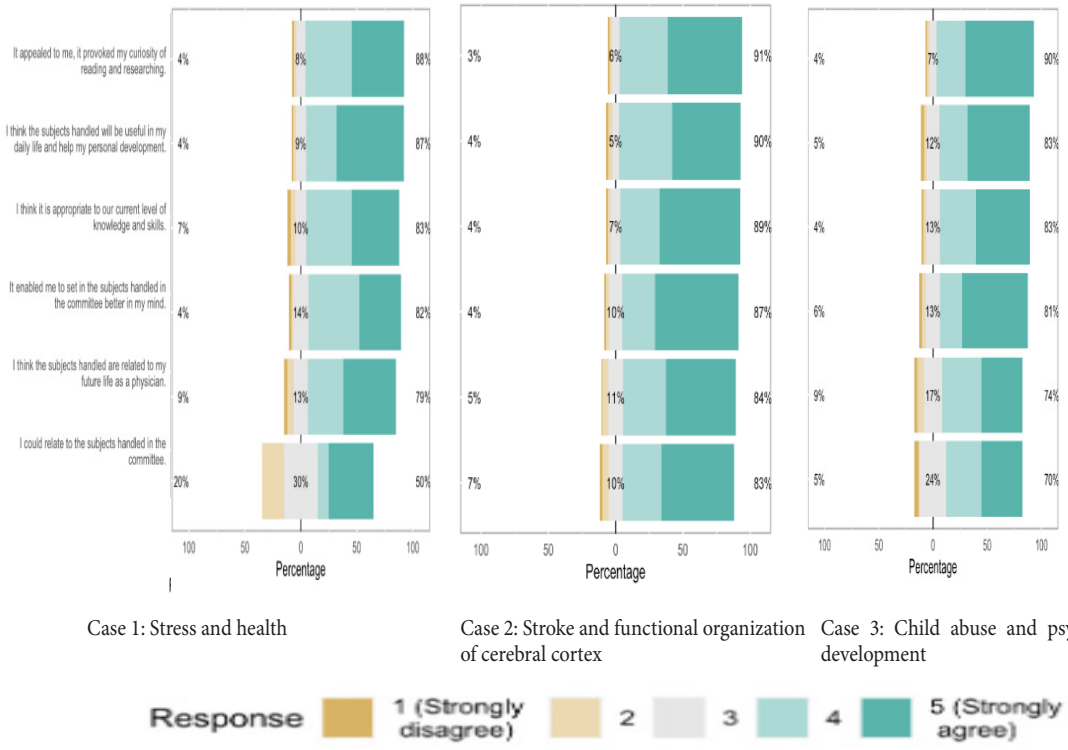

Case 1: Stress and health

Case 2: Stroke and functional organization of cerebral cortex

Case 3: Child abuse and psychosocial development

Figure 2. *Student opinions on the quality of the cases used*

Table III. *Comparison of student feedbacks on cases*

	Case 1 (n=135) Md (IQR) Min; Max	Case 2 (n=135) Md (IQR) Min; Max	Case 3 (n=135) Md (IQR) Min; Max	p	Effect size
I could relate to subjects handled in the courses	5 (1) 1; 5	4 (2) 1; 5	3 (2) 1; 5	<0.001	0.438
It helped me better comprehend the subjects handled in the courses	5 (1) 1; 5	4 (2) 1; 5	3 (2) 1; 5	<0.001	0.381
I think it was in line with our actual level of knowledge and skill	5 (1) 1; 5	5 (1) 1; 5	3 (1) 1; 5	<0.001	0.395
It attracted my attention; it aroused curiosity of reading and researching	5 (1) 1; 5	5 (1) 1; 5	3 (2) 1; 5	<0.001	0.437
I think the subjects handled are related to my future profession as a medical doctor	5 (1) 1; 5	5 (1) 1; 5	3 (0) 1; 5	<0.001	0.520
I think the subjects handled will help me in daily life and will improve me	4 (1) 1; 5	5 (1) 1; 5	3 (1) 1; 5	<0.001	0.444

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The significant differences were followed by pairwise Wilcoxon post-hoc test with Bonferroni adjustment where "integration with the other topics in the program" for case 1 is significantly higher than case 2 and case 3 (P<0.001 and P<0.001 respectively) and case 2 is significantly higher than case 3 (P<0.001); where "reinforcement of topics" for case 1 is significantly higher than case 2 and case 3 (P<0.001 and P<0.001 respectively) and case 2 is significantly higher than case 3 (P<0.001); where "suitability to their levels of knowledge and skills" for case 1 and case 2 are significantly higher than case 3 (P<0.001 and P<0.001 respectively); where "motivation for learning and researching" for case 1 and case 2 are significantly higher than case 3 (P<0.001 and P<0.001 respectively); where "daily life and its relation to their individual development" for case 1 is significantly higher than case 2 and case 3 (P<0.001 and P<0.001 respectively) and case 2 is significantly higher than case 3 (P<0.001). These results show that in general the "Stress and Health" case is relatively the most qualified one, "Stroke and Functional Organisation of Cerebral Cortex" case is a fairly qualified one, and "Child Abuse and Psychosocial Development" case is relatively less qualified.

Comparisons of student opinions on face-to-face and online PBL tutorials are provided in Figure 3. It was observed that online tutorials were evaluated more positively by 93% of the students in terms of "discussion level", 65% in terms of "tutor support/direction" and 50% in terms of "group environment/climate" compared to face-to-face tutorials. 31-62% of the students are of the opinion that there is no difference between online and face-to-face tutorials in all other items, except for the "discussion level". 19% of the students evaluted the online tutorial weaker than the face-to-face tutorial in terms of "tutor support/direction", 26% of them evaluated it weaker in terms of "group environment/climate", 33% of them in terms of "practicality of seven-step", 26% of them in terms of "individual participation level" and 13% of them in terms of "effective use of time".

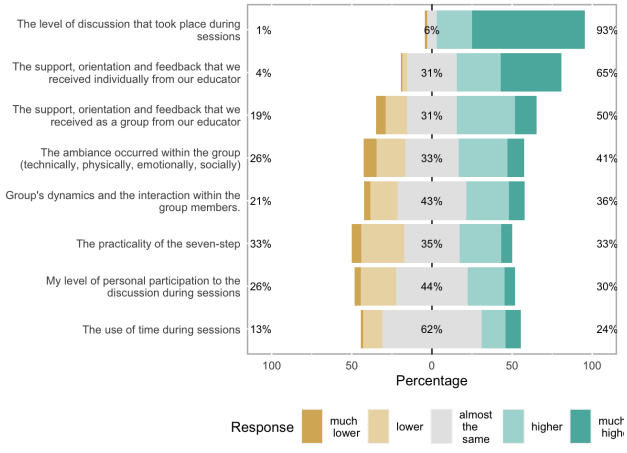

Figure 3. *Comparison of face-to-face and online PBL tutorials*

In this comparison between online and face-to-face tutorials, it was tested whether the median of each item was 3, assuming the average degree of positivity was 3. As a result of the analyses made with the one sample median test, the level of the discussion during the online sessions was found to be much higher (P<0.001, high impact) than the level of the face-to-face discussion. The support, guidance and feedback received from the trainer as a group (P<0.001, medium impact) and individually (P<0.001, high impact) during online tutorials were statistically significantly higher than face-to-face PBL tutorials.

4. DISCUSSION

The quality of the cases used, the arrangement of the content handled, the coordination of the learning process, the learning environment formed during the tutorials and the group dynamics related to it are among the factors that determine the quality of the learning processes in PBL. Studies have pointed to problems such as superficial processing of knowledge, inadequate integration, insufficient individual preparation, unsatisfactory student participation, poor group dynamics and lack of motivation in face-to-face PBL tutorials. According to these results, various improvements are made in this direction in PBL practices [18, 19]. With the pandemic, e-PBL practices have become widespread, and this has led to the need for similar studies to be conducted in online PBL tutorials. The results obtained in this study indicate that a medium-high level of positive tutorial environment/climate has been formed in all aspects except "constructive conflict". The results regarding the handling of the content and the coordination of the e-tutorial process indicated a medium-high level of positive e-PBL practice in terms of asking questions, tutorial support and the emotional environment created. These results point to the effectiveness of online PBL tutorials. However, the e-PBL practice was evaluated positively at a low-medium level in the aspects of the coordination of the content and process as well as in associating the content with the case. More data on the effectiveness of e-PBL practices are needed with new studies to be carried out in this direction.

In our study the scores on interaction, togetherness, participation, confirmation, openness, flexibility, liveliness, constructive conflict, ease, support and guidance as well as mood in e-PBL sessions were determined to be high. The positive learning environment created in e-PBL sessions, group collaboration and interaction make learning more interesting and attractive, as well as supporting the learners' exchange of information with their peers [20]. It is claimed that the self-efficacy perception and transfer skills of the learners increase with the PBL conducted in the online medium [21]. The e-PBL practice is seen as an important alternative for solving the problem of organizing the physical space and the tutor in terms of taking learning outside the classroom walls [13].

When observed in terms of learning outcomes, e-PBL practices were seen to support learners' knowledge acquisition, critical thinking and clinical reasoning skills [22-24]. In the research by Gavgani et al., it was found that there was no difference between digital and paper-pencil based scenario presentation in terms of its effectiveness and contribution to clinical reasoning skills [25].

In our study, it was determined that 80% or more of the students reported positive opinions about the PBL program carried out online. Regarding the quality of the cases used, it was evaluated that the over 80% of students had positive opinions in terms of "motivation for learning and researching", "daily life and its relation to their individual development", "suitability to their levels of knowledge and skills" and "reinforcement of topics". The rate of positive opinions about the integration of cases with professional life and with other subjects in the program is between 50-79%.

Several studies conducted on e-PBL indicate parallel findings with ours. For instance, according to a 2013 study a vast majority of students were satisfied with the overall learning process in e-PBL and perceived it positively in fostering knowledge acquisition and clinical reasoning [22]. Students felt that e-PBL increased their flexibility for learning, enhanced their ability to deeply process content, and provided access to valuable learning resources [26]. The general attitude of another group of students was found to be high in a positive way towards the web-based problem-based learning process [27]. A recent study from 2021 identified that e-PBL sessions were acknowledged positively by students, and "contribution of the quality of group discussion to students" in PBL sessions received high score [28]. A study conducted with a computer-mediated problem-based learning group reveals that the group spent significantly more time on learning than the traditional problem-based learning group in face-to-face [29]. A 2022 study puts forward that the majority of students found e-PBL to be efficient and effective, despite having deficiencies compared to face-to-face application [30]. The results of a different one showed that e-PBL sessions are as good as face to-face sessions [31].

In our study students evaluated online PBL sessions more positively compared to face-to-face PBL sessions held in previous years in terms of discussion level by 93%, tutor support and guidance by 65% and group environment/climate by 50%. In other aspects, one third to two thirds of the students think that there is no difference between online and face-to-face sessions. It was found that students evaluated the face-to-face PBL sessions more positively than online sessions in terms of the usefulness of the seven-step approach by 33%, in terms of the group environment and individual participation by 26%, in terms of tutor support by 19% and in terms of effective use of time by 13%. It was determined that the discussion level in online PBL was significantly higher than the face-to-face, and the support, orientation and feedback received as a group and as individuals from the tutor in online PBL tutorials were statistically significantly higher compared to face-to-face PBL tutorials ($P<0.001$, high impact).

In conclusion, studies on the effectiveness of online PBL tutorial processes including ours point to the fact that e-PBL practices may be considered an important alternative besides face-to-face practices. However, for a sounder framing and conclusion, it is important that the subject be researched with different aspects and more evidence be obtained. Further research will provide evidence to educational institutions and practitioners in the process of reconsidering all educational practices, including PBL, according to the "new normal" that started with the pandemic process.

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Compliance with the Ethical Standards

Ethics Committee approval: Trakya University Faculty of Medicine Scientific Research Ethics Committee (approval no: 09/21, date:12.04.2021).

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Authors contributions: AG has provided substantial contributions to the conception, design of the work, literature review, interpretation of data and writing the manuscript. OE has provided substantial contributions to the literature review, interpretation of data, writing the manuscript. EA has provided substantial contributions to the analysis and interpretation of data and writing the manuscript. MAG has provided substantial contributions to the design of the work, interpretation of data, revising and final approval of the manuscript. All authors reviewed the manuscript.

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Case Report

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Successful diagnosis of a ruptured ectopic pregnancy: A woman without abdominal pain and vaginal bleeding

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ABSTRACT
Ectopic pregnancy is the implantation of the developing embryo outside the uterine cavity. It usually occurs in the fallopian tubes. One of the critical complications of ectopic pregnancy is rupture. The most common symptoms of ectopic pregnancy rupture are vaginal bleeding and abdominal pain. In atypical presentations, the diagnosis is based on suspicion. Herein, we presented a case of ruptured ectopic pregnancy with an atypical presentation. The diagnosis of ruptured ectopic pregnancy should be considered when women with childbearing potential apply to the emergency department.
Keywords: Diagnose challenge, Ectopic pregnancy, Hemoperitoneum

1. INTRODUCTION

Ectopic pregnancy is when embryo implantation occurs outside the uterine cavity. In more than 96% of cases, implantation occurs in the fallopian tubes [1]. It occurs in approximately 0.7-2.0% of all pregnancies [2,3]. The most common clinical sign of ectopic pregnancy is vaginal bleeding or abdominal pain in the first trimester. Clinicians should be aware of the diagnosis of ectopic pregnancy in any patient of reproductive age who presents with vaginal bleeding or abdominal pain. Ectopic pregnancy patients may also be asymptomatic. In this case, the diagnosis becomes even more challenging. Bleeding due to ectopic pregnancy is one of the leading causes of pregnancy-related maternal deaths, so early diagnosis is essential. Herein, we presented a case of a woman with an atypical presentation of ruptured ectopic pregnancy. Hence, we aimed to raise awareness of ectopic pregnancy rupture diagnosis and prevent possible mortalities in patients presenting with atypical symptoms.

2. CASE REPORT

A 42-year-old female presented to the emergency department (ED) complaining of nausea, vomiting, and fatigue that began two days ago after eating fast food. She was vomiting about four to five times a day, and the contents of her vomiting were usual. As a result, she said that her nutrition was seriously reduced, and she had difficulty even drinking water. Fatigue made this situation even more complicated, and she did not want to get out of bed. She had no abdominal pain, diarrhea, constipation, dysuria, vaginal bleeding, discharge, or any other symptoms.

The patient was Uzbek. She barely could speak the local language (Turkish), so she had some limitations in communication. She had no significant medical history. She had no alcohol or drug history and smoked one pack of cigarettes daily for about ten years. She declared that she had not had sexual intercourse recently and that there was no possibility of pregnancy. She had a single healthy pregnancy history (14 years ago). She had presented to another center with similar complaints the day before. During this presentation, the patient was diagnosed with food poisoning and anemia and was recommended to apply to hematology for the latter. She did not have any of those results or medical documentation.

In admission, her blood pressure was 82/53 mmHg, pulse was 83 beats/minute and regular, respiratory rate was 18 breaths/min, temperature was 36.9°C (98.5°F), and Glasgow coma scale was 15. Finger stick glucose level was 87 mg/dL. Her pulmonary and cardiac exams were unremarkable. On abdominal exam, bowel sounds were normal, and the patient had no tenderness, rebound,

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or rigidity. The patient's electrocardiogram (ECG) showed 87 beats per minute with sinus rhythm without signs of ischemia or dysrhythmia. Other examinations were unremarkable.

Gastroenteritis with severe fluid loss was considered a preliminary diagnosis in the patient. Then, symptomatic treatment was started. For this purpose, hydration was performed, and 8 mg of ondansetron was given. Venous blood gas was requested regarding possible electrolyte disturbance due to decreased oral intake of the patient and excessive fluid loss. A complete blood count test was requested, considering that the patient's anemia might have deepened because she had severe fatigue. Venous blood gas results were within normal limits. However, the hemoglobin level was 3.7 g/dL, red blood cell count was 1.2 x 10^6/mcL (Normal range: 3.5-5.7 x 10^6/mcL), mean corpuscular volume was 87.3 fL (in normal range), red cell distribution width was 14.8 (in normal range). In line with these results, acute blood loss was considered in the patient. Erythrocyte replacement was planned, and the blood bank was contacted quickly. Meantime, the relatives of the patients brought the previous results, and it was seen that the hemoglobin was 6.4 g/dL two days ago.

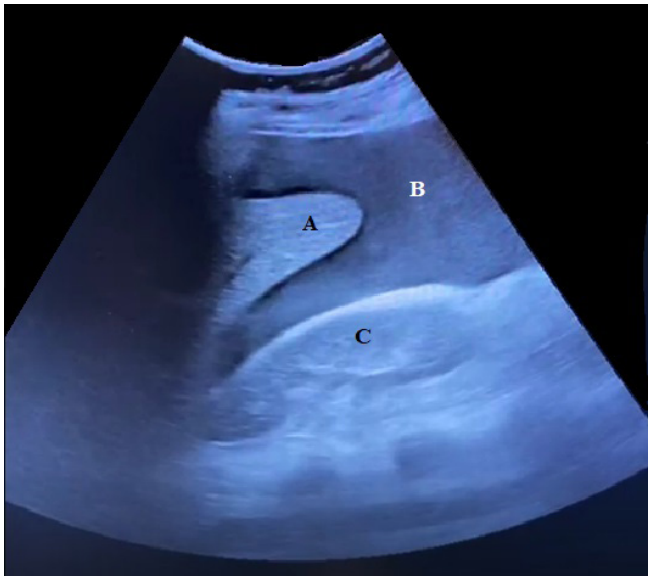

Figure 1. *A: Liver B: Morison's Pouch filled with fluid C: Right kidney*

These results strengthened the pre-diagnosis of acute bleeding. When the patient was questioned about any trauma or bleeding history again, she denied it. There was no evidence of bleeding on the rectal examination. No blood was observed in the vomit content of the patient who vomited in the hospital. When her menstrual cycle was questioned, the patient did not remember her last menstrual period, and she described that there was no change in her cycle and her bleeding amount was average (3 pad changes per day, for approximately 4-6 days). Then, bedside ultrasonography was performed, and it showed extensive hemoperitoneum in the abdomen (Figure 1). There was no evidence of liver, spleen, or kidney injury. The internal genitals could not be visualized adequately because the patient's bladder was empty. However, it was seen that the uterus was prominent, but no pregnancy material was observed in the uterus. Beta-human chorionic gonadotropin (B-hCG) test was taken while two units of erythrocyte suspension were replaced. The patient was consulted to Departments of Gynecology and General Surgery. With the result of B-hCG as 34,773 U/L, the patient was taken to surgery with the diagnosis of ectopic pregnancy rupture. Laparoscopy revealed a hemoperitoneum with 3000 mL of blood with a ruptured right fallopian tube ectopic pregnancy. Evacuation of the clot and a right salpingectomy was successfully performed. The patient was discharged without complications on the sixth postoperative day.

3. DISCUSSION

Ruptured ectopic pregnancy is a life-threatening condition, and the prevalence among pregnant patients presenting with abdominal pain and first-trimester bleeding is 16% [4]. An ectopic pregnancy's clinical signs (mainly abdominal pain and bleeding) typically appear six to eight weeks after the last menstrual period. It may sometimes be accompanied by standard pregnancy disorders (e.g., breast tenderness, frequent urination, nausea). Since, progesterone, estradiol, and hCG levels are lower than in healthy pregnancies, early pregnancy symptoms are less or milder in patients with ectopic pregnancy [5]. Because pregnancy symptoms are mild, patients may not realize they are pregnant during this period, as in our case.

The most important finding that leads the clinician to the diagnosis is abdominal pain or vaginal bleeding in pregnant women. In a study conducted on 2026 pregnant patients admitted to the emergency department, 376 patients were diagnosed with ectopic pregnancy, and vaginal bleeding was found in 76% of them and abdominal pain in 66% [6]. Therefore, it is even more challenging to reach this diagnosis in a patient who does not know that she is pregnant and does not have abdominal pain and vaginal bleeding. Moreover, in our case, attributing the patient's chief complaints (nausea and fatigue) to food poisoning was another factor that concealed the primary diagnosis.

The presence of hypotension (lower than baseline) at the patient's admission indicated something was wrong, but the absence of a tachycardia response excluded the preliminary shock diagnosis. Although, the patient did not use a drug that may hide this response (beta blocker or calcium channel blocker), the absence of a tachycardia response suggested that chronic anemia may be accompanying acute anemia. In such cases, it is crucial to learn the patients' baseline values and make patient-based management. The severe decrease in the hemoglobin level (2.7 g/dL) within two days in the case of an indistinct clinical condition was one of the most critical factors leading us to the diagnosis. Gastrointestinal bleeding or trauma is usually the most common cause of acute blood loss. In our case, we deepened the story and advanced our examinations and evaluations to exclude them. The fact that we detected widespread fluid in the abdomen in the ultrasonography allowed us to diagnose ruptured ectopic pregnancy. Bedside ultrasonography has been used successfully in emergency

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departments for years. It provides much information that guides the diagnosis as well as shortens the diagnosis time [7].

Our country has been receiving many immigrants from East and South Asia lately. Another reason that makes diagnosis difficult is that there are many gaps in the anamnesis due to the language barrier when these people apply to the hospital. In summary, the patient's denial of the possibility of pregnancy, vague symptoms, poor communication due to language barrier, no abdominal pain or vaginal bleeding, and previous diagnosis of food poisoning are the reasons that made the diagnosis of ectopic pregnancy rupture difficult in our case. Ectopic pregnancy rupture is a condition with high mortality and morbidity; it is essential to be among the differential diagnoses in women of fertile age to avoid possible mortality. If there is even a slight suspicion of pregnancy, a pregnancy test should be performed on every female patient of childbearing age.

Compliance with Ethical Standards

This work was conducted ethically by following per under Helsinki World Medical Association Declaration.

Patient Consent: The patient gave her consent for images and other clinical information relating to her case to be reported in a medical publication.

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End-point nystagmus and EMDR

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ABSTRACT

Eye movement desensitization and reprocessing (EMDR) is a psychotherapy that helps people to heal from trauma or other disturbing life experiences. In this article, the appearance of nystagmus in the eye during the application of the EMDR method was discussed while treating a 16-year-old adolescent girl who presented with a complaint of not eating meat following a traumatic event. Although, eye movements are used in EMDR, nystagmus can impede the patient's ability to maintain eye movements. After excluding additional pathologies related to the eye, alternative bilateral stimulations can be used in EMDR.

Keywords: Adolescent, Chidhood trauma, EMDR, Nystagmus

1. INTRODUCTION

Eye movement desensitization and reprocessing (EMDR) is an empirically supported, 8-stage psychotherapeutic intervention that was developed based on the adaptive ınformation processing (AIP) model. The primary aim of EMDR is to ameliorate the distress caused by traumatic memories [1]. In EMDR, bilateral stimulation (BLS) is utilized through various methods. Most therapists use finger movements, but some therapists use alternatives to finger movements such as butterfly hug, vibration, and two-directional touches to body parts like legs or shoulders as part of EMDR therapy. As it can be challenging to follow eye movements depending on the age group of children, BLS can be applied through alternative methods mentioned above, while EMDR can be easily applied through eye movements in adolescents. In this report, we aim to share a situation related to the eye movements of a 16-year-old adolescent girl that emerged during the use of EMDR.

2. CASE REPORT

A 16-year-old female patient, presented to the Child and Adolescent Psychiatry Clinic with complaints of fatigue, hair loss, and anxiety during exams. She was attending 10th grade at a boarding high school. In her history, it was noted that she had been following a "vegetarian" diet for the past 7-8 months. She expressed discomfort with eating meat after holding the head of a sacrificed animal at the age of 12 and reduced her meat consumption. Although, she felt the need for a diet containing meat, it was revealed that the memory of the event she experienced 8 monts ago, came to her mind frequently, preventing her from eating meat.

In the patient's medical and family history, the older of the two healthy children of a 45-year-old nurse mother and a 50-year-old veterinarian father, it was learned that during the mother's pregnancy, the glucose loading test was elevated, but no medication was used, and she was born healthy through a normal pregnancy by vaginal delivery, weighing 3500 grams. The developmental milestones were reached on time. At the age of 1.5, the child was thought to be not gaining weight because she was breastfed, so efforts to wean her were made by attaching something to the nipples. Walking and speaking milestones were met without any issues, and there were no problems during toilet training.

Until the age of 1, the child was cared by her mother, and from then on, the grandmother provided care until the age of 3.5 when she started daycare school due to the mother's work. She had a history of appendectomy at the age of 8. The child learned

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to read and write in the 1st grade of elementary school, and her academic performance was consistently good. There have been no issues in terms of relationships with friends and teachers.

During the mental status examination, her external appearance was age-appropriate, cooperative, her speech was clear and purposeful, psychomotor activity was slightly increased, and mood was mildly anxious. Her affect was appropriate, consciousness was clear, and her orientation was intact. Thought associations were regular, and the content of her thoughts revolved around exams and meals. She experienced flashbacks, had no perceptual disturbances, and clinically, her intelligence was normal. She also had intrusive, involuntary memories and imaginations related to the traumatic event, as well as feelings of nausea and disgust triggered by the smell or sight of meat. Additionally, she displayed avoidance behaviors, such as avoiding the kitchen, cafeteria, or dining areas to distance herself from these distressing situations. Post-event negative cognitions and emotions were mentioned during the EMDR session below. She was diagnosed with post-traumatic stress disorder according to the The Diagnostic and Statistical Manual of Mental Disorders (DSM) – 5 diagnostic criteria [2].

She expressed that she has been feeling tired and fatigued lately, especially during study periods for exams, which has hindered her ability to focus on her studies and has caused anxiety. She mentioned that she does not consume meals from the cafeteria but eats non-meat-containing foods. There is no significant weight loss, and laboratory investigations ruled out iron deficiency anemia.

It was decided to apply the EMDR therapy to work on the specific incident, as the individual had developed meat aversion due to a traumatic experience, and her current issue revolved around this matter.

During the preparation phase, she was introduced to EMDR therapy and techniques such as bilateral stimulation (BLS) through eye movements and tapping were demonstrated. The application distance, tracking distance, and speed of eye movements were adjusted and demonstrated to the patient. Based on the patient's formulation, a treatment plan was created, and the standard protocol was planned to be implemented [1]. A safe place was planned to be established during the preparation phase. During the safe place exercise, it was observed that the patient's lateral gaze crossed the saccadic midline during slow BLS through eye movements. This occurrence made it difficult for the patient to follow finger movements in almost every slow set. Consequently, the patient was switched to tapping on the leg after discussing the issue. The patient did not have any medical conditions, and the Ear-Nose-Throat (ENT) examination was unremarkable. The patient's current nystagmus was determined not to be related to any peripheral vestibular pathology during the ENT examination. After a consultation by an ophthalmologist, she was diagnosed as having bilateral myopia according to the examination findings. Jerk nystagmus (end-point nystagmus) was observed in both lateral gaze positions. The patient was treated with glasses for myopia.

The preparation phase was completed with breathing exercises, mindful exercises, establishing a safe place, and the use of the container exercise.

During the assessment phase, the target memory was identified, along with the image that was most disturbing to the individual, the negative cognition related to the event ("I am a terrible person"), and the positive cognition ("I am a good person"). The Validity of Cognition (VoC) Scale score was 3. The VoC is a 7-point Likert-type scale where a score of 1 indicates an adaptive cognition that is entirely implausible, while a score of 7 indicates a cognition that is entirely plausible. The emotions experienced included anger, hatred, and sadness. The Subjective Unit of Distress (SUD) scale, which ranges from 0 (no disturbance) to 10 (the highest level of disturbance), was rated as 7. The location of the body sensation was identified as the shoulders. In the desensitization phase, BLS was continued by tapping on the legs. The session was completed with a VoC score of 7 and a SUD score of 0, with the body feeling cleansed at the end of the session. It was learned that the individual ate sausages before the next session.

During the follow-up appointments, reassessment was conducted, and triggers were systematically addressed in accordance with the standard protocol. On the 5th session, it was observed that the patient had consumed baked fish and chicken, was not bothered by meat odors, and did not experience discomfort when utensils came into contact with meat. During the sessions, preventive interventions were discussed with her and her family to prevent retraumatization as the Sacrifice Feast approached. Academic performance and family and peer relationships were functioning well.

3. DISCUSSION

Nystagmus is the rhythmic, involuntary, rapid, jerky, and uncontrollable movement of the eyes. These involuntary eye movements are classified as horizontal (side-to-side), vertical (up-and-down), and rotatory (torsional) nystagmus according to their movement axis, and as jerk, pendular, or combined nystagmus according to their amplitude and speed [3]. Manifest nystagmus is continuous, while latent nystagmus occurs when binocular vision is prevented. Pathological nystagmus may be associated with ocular, vestibular, or central nervous system pathologies, while physiological nystagmus such as end-point, optokinetic, and vestibular nystagmus can be observed in healthy individuals [4]. End-point nystagmus is a physiological condition that occurs at extreme lateral gaze positions. Studies have shown that it can occur in varying proportions in the general population, depending on the gaze angle, ranging from 0-71% at up to 30 degrees lateral gaze positions and 0-100% at up to 45 degrees lateral gaze positions [5-8]. The characteristics of end-point nystagmus are that it occurs during slow eye movements and particularly after lateral gazes of 30 degrees or more, it causes a jerk movement towards the midline and then the eye turns towards the direction that the patient is looking at. Since, this condition is physiological, no treatment is required. However, as in the case presented, it caused difficulty during

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EMDR's eye movements with BLS. When such a situation is noticed, it would be appropriate to discuss with the patient and switch to other types of BLS. The patient continued with slow and fast BLS using tapping movements.

If eye movements other than fixed tracking are observed during finger movements, consulting a physician for medical evaluation may be important for differential diagnosis. However, not every eye movement that occurs is pathological, and therapy can be continued with a change in the type of BLS used during the application of the treatment plan.

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Patient consent: The patient and her guardian gave their written consent for clinical information related to her to be reported in a medical publication.

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