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European Journal of Therapeutics aims to contribute to the international literature by publishing original clinical and experimental research articles, short communication, review articles, technical notes, and letters to the editor in the fields of medical sciences. The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in in all medical disciplines.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489–93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	30	6	7 or total of 15 images
Review Article	5000	250	50	6	10 or total of 20 images
Short Communication	1500	200	20	5	1 or total of 5 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

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While citing publications, preference should be given to the latest, most up-to-date publications. Authors should avoid using references that are older than ten years. The limit for the old reference usage is 15% in the journal. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

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Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

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Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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Contents

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Original Research

Signature of Serum miR-199a/b in Coronary Artery Bypass Graft Surgery

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ABSTRACT

Objective: microRNAs (miRNAs) have important potential as biomarkers in the diagnosis and prognosis of ischemia/reperfusion (I/R) injury in coronary artery bypass grafting surgery (CABG). This study investigated the relationship between preoperative (pre-op) and postoperative (post-op) cardiac parameters and miRNA expressions in CABG.

Methods: We analyzed a total of 94 individuals (CABG, n= 46 and healthy control, n=48). Quantitative real-time polymerase chain reaction (qRT) was performed to determine plasma miRNA expressions (miR-21, miR-181a, miR-199a, miR-199b, and miR-320a-5p) in triplicates: before surgery, 1 hour after surgery, and 24 hours after surgery. The target genes and pathways of miRNA were determined using bioinformatic analysis. The biomarker potentials of miRNAs were evaluated with receiver operating characteristic (ROC) curve analysis.

Results: All miRNAs were significantly downregulated ($p < 0.05$). Troponin I, LVEF, CPK, and CK-MB were found to be statistically significant for operation groups ($p < 0.05$). miRNA expressions and cardiac markers were associated with troponin I and/or CK-MB. In ROC analyses, miR-199a was a good diagnostic marker. CREBRF and ZNF704 genes may be a target for these miRNAs.

Conclusions: Downregulation of miR-199a has a regulatory role in ischemia/reperfusion. They may contribute to CABG pathology through these two genes involved in signaling cascades to turn on protein response and ion binding.

Keywords: coronary artery, microRNA, diagnostic marker, ischemia, reperfusion.

INTRODUCTION

Coronary artery disease (CAD) is a disease characterized by reduced blood flow to the heart muscle due to atherosclerosis, affecting the structure and functions of the heart (1). Types include st/unstable angina, myocardial infarction, and sudden cardiac death (2). There are various risk factors responsible for the root of the disease and the epigenetic basis has a very important place. While the methods used in the diagnosis of the disease detect recent or instantaneous changes, searching for epigenetic markers can make it possible to go to the point and time where these processes started. Most interventions to reduce disease risk are based on cardiac markers and imaging systems. However, there is limited evidence to identify people at low risk or without symptoms. Therefore, from the onset of symptoms, even earlier methods of diagnosis, including genetic markers, may provide significant advances. Ischemia develops due to a lack of oxygen as a result of insufficient perfusion of organs and tissues as a result of decreased arterial or venous blood flow (3). Lack of energy and accumulation of toxic metabolites cause cell death (4). Damage to ischemic tissue due to reperfusion is more serious than the damage caused by ischemia. The cellular structures most susceptible to I/R are proteins, nucleic acids, and membrane lipids (5).

Although significant advances have been made in understanding the mechanisms responsible for myocardial I/R injury, it is difficult to match the findings clinically. miRNAs control a variety of cellular activities by degradation or inhibition of translation of the target mRNA. However, they can be tissue-specific or expressed in more than one tissue. Many studies have shown that miRNAs have an important role in cardiac processes and determine their fate by regulating cell death and regeneration after myocardial infarction (6). There are many known pathways, responsible genes, and epigenetic marker candidates in the miRNA mechanism. Evaluation of the reflections of these markers on clinical parameters and their diagnostic or therapeutic potential

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may enable them to be presented as descriptive and therapeutic in CABG. In this study, we evaluated the regulation of five miRNAs and their relationship with parameters in cardiac processes in the sera of healthy, pre-and post-operative individuals. We demonstrated the contribution of these miRNAs to the pathophysiology of the disease and their diagnostic potential. Finally, we identified the targets and pathways of miRNAs functional in CABG using bioinformatic analyses.

METHODS

Population Data

Ethics approval was obtained from the Medical Ethics Committee (Protocol number: 276). Consent to participate in the study was obtained from the patient and control individuals and the study protocol conforms to the ethical guidelines of the 2013 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The study included 46 CABG patients (female = 26 and male = 20) and 48 healthy volunteers (female = 32 and male = 16) who applied to the University Research and Practice Hospital, Department of Cardiovascular Surgery. The average age of healthy individuals is 62.6±11.2, and the mean age of individuals with CABG was 42.4±11.2. The duration of intensive care and post-op hospital stay, weight, the number of bypasses, cardiopulmonary bypass, and cross-clamp times of the patients were recorded. The criteria for inclusion of volunteers in the patient group; Male and female patients aged 18-65 years, diagnosed with coronary artery disease, at risk of CAD, and undergoing CABG. Inclusion criteria of healthy volunteers in the control group; Healthy males and females between the ages of 18-65, who do not have diseases such as coronary artery disease, hypertension, diabetes, and kidney failure.

Plasma Sampling

5 ml peripheral blood samples were obtained from healthy controls and patients requiring cardiopulmonary bypass operation before the operation (pre-op), 1 hour after the operation (post-op 1), and 24 hours after the operation (post-op 24). Blood samples collected in tubes with 7.5% EDTA and gel tubes were immediately centrifuged at 2000xrpm for 15 minutes for RNA isolation and biochemical analysis (LDH, BUN, glucose, troponin I, CPK, CK-MB). The 2 ml supernatant was taken into Eppendorf tubes (coded for each patient) and stored at –80 °C until RNA was obtained.

miRNA Expression Analysis

MiRNAs that are effective in vivo and in vitro cardiac processes were investigated bioinformatically and selected according to their match scores. Importantly, a new miRNA target and pathway has been proposed that was not previously demonstrated in bioinformatics analyses. Total RNAs were obtained according to the commercial miRNA isolation kit protocol (Qiagen, miRNeasy Mini Kit, 217004). Cell-miR-39 (Qiagen, MS00080247) was used as an internal control (for the isolation stage) to quantify the expression values of miRNAs (by adding the spike-in before). 2-ΔΔCt method was used to calculate the fold change (Fc) of gene expression between the patient and control group (7).

Complementary DNA (cDNA) Reactions for Reverse Transcriptase PCR (RT-PCR)

Reverse transcriptase reactions were performed according to the kit protocol. The mixture prepared in a total volume of 280 µl (for 69 samples + 1 positive sample) contained 112 l 5xmiScript Hispec buffer, 56 µl 10xmiScript Nucleics mix, 56 µl DNase-Rnase free water, and 56 µl transcriptase mix (Qiagen, 205311). The reaction mixture was dispensed into the pico plate as 48 µl and then, 3.5 µl of cDNA followed by 3.5 µl of RNA were added to each well. Finally, thermal cycling conditions were incubated at 37°C for 60 min and 95°C for 5 min, and total cDNA was obtained. Expressions were performed for *hsa-miR-21-5p* (Qiagen, MS00009079), *hsa-miR-181a-5p* (Qiagen, MS00008827), *hsa-miR-199a-5p* (Qiagen, MS00006741), *hsa-miR-199b-5p* (Qiagen, MS00006741), and *hsa-miR-320a-5p* (Qiagen, MS00014707) relative to the endogenous control miRTC (miRNA reverse transcription control, Qiagen miScript II RT Kit, 218161).

Dynamic Array

Dynamic arrays were prepared in 6 steps: 1- PRIME the dynamic array, 2- Assay plate preparation, 3- Preparation of samples, 4- Loading sample into a dynamic array, 5- Array loading to Dynamic Array in IFC, 6- Analysis of Dynamic array in Biomark (BioMark™ 96.96 Dynamic Array). After chip priming, samples premixed with master mix were pipetted into separate sample inlets of the dynamic array, and then assay plates were prepared. The reaction mixture was prepared in a total volume of 25 µl with 2.5 µl of primer (100 µM), 3.8 µl of nuclease-free water, 6.25 µl of microfluid universal primer, 13 µl of 2xAssay Loading Reagent. The samples were prepared for RT-PCR. The reaction mixture was prepared as 255 µl qPCR Master Mix, 25.5 µl 20xDNA Binding Dye, 59.5 µl nuclease-free water in a total volume of 340 l. The reactions were placed in the Dynamic Array IFC controller and analyzed using a gene expression program and SybrGreen probe technology. Thermal cycling conditions were set as follows: 2 min at 50°C, 30 min at 70°C, 10 min at 25°C for the thermal mix step, 10 min at 95 °C for starting temperature, 15 sec at 94 °C for

Table 1. Clinical and demographic data of CABG patients and controls

Clinical characteristics	Reference (adult)	Control (n = 48) (mean±SD)	CABG (n=46) (mean ± SD)	P– value
Age (year)	NA	42.3 ± 11.2	62.6 ± 11.2	0.001*
LDH (U/L)	140–280	192.4 ± 23.8	228.2 ± 66.0	0.001*
BUN (mg/dL)	7–20	13.8 ± 9.9	8.6 ± 8.7	0.001*
Fasting blood glucose (mg/dL)	70–100	94.7 ± 9.9	100.3 ± 8.7	0.002*

* P-values <0.05 are indicated in bold. CABG; coronary artery bypass grafting, LDH; lactate dehydrogenase, BUN; blood urea nitrogen, n; the number of individuals.

Table 2. Expression analysis of five miRNAs in control and pre-op (Control= 48, CABG = 46)

miRNAs	Groups	*Mean ΔCt*	*SD*	*P-value*
miR-21-5p	Control	2.4	1.4	**0.001***
	pre-op	3.7	2.4	
miR-181a-5p	Control	9.1	2.2	**0.001***
	pre-op	11.6	3.5	
miR-199a-5p	Control	7.6	2.5	**0.001***
	pre-op	11.0	3.7	
miR-199b-5p	Control	8.2	1.6	**0.001***
	pre-op	10.6	3.5	
miR-320a-5p	Control	2.0	1.6	**0.001***
	pre-op	3.6	3.1	

* P-values <0.05 are indicated in bold. Pre-op; before from operation, post-op; after from operation. SD; Standart Deviation

denaturation, 30 sec at 55 °C for bonding, 30 sec at 70 °C for elongation, and finally between 60-95 °C for melting temperature.

Receiver Operating Characteristic (ROC) Curve Analysis

The area under the ROC curve (AUC) determines the accuracy of the test in distinguishing between patients and non-patients. The potent miRNA/miRNAs were determined by determining the sensitivity, specificity, and AUC values of five miRNAs before and after the operation.

Bioinformatic Analysis

miRNA::target gene analysis was performed to determine the miRNA target genes and the pathways. The miRDB database (8) and DIANA TOOLS (9) were used for specific targets and matching scores of miRNAs. Genes with a match score of 80% and above were selected for further analysis. Target Scan database was used to detect conserved sequences between miRNA and mRNA (10, 11). Gene ontology (GO) analyses were performed with miR2GO (12). The Reactome database was used for pathways (13, 14). Finally, the proteins with which these genes interact were analyzed for their specific effects (STRING v11.0) (15).

Statistical Analysis

Compliance of numerical data between patient and control with normal distribution was tested with the Shapiro Wilk test. The Mann-Whitney U test was used to compare the variables that were not normally distributed in the two groups. In investigating the regulation of five miRNAs for control, pre-op and post-op patient groups; Repeated measures analysis of variance, LSD multiple comparison tests, Friedman 2-way analysis of variance, and all pairwise multiple comparison tests were used for normally distributed and non-normally distributed variables. The relationship between cardiac processes and miRNA regulation was determined by the Spearman correlation coefficient. In the analysis, using the SPSS package program (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) (16), it was considered significant when $p < 0.05$.

Main Points:

- MicroRNA-199a-5p is strongly associated with ischemia/reperfusion.
- In CABG patients, miR-21-5p levels may vary in correlation with cardiac markers.
- miR-199a-5p can be evaluated as a marker in determining the severity of coronary artery disease.
- miR-320a, miR-199b-5p, and miR-181a-5p levels may have a role in activating inflammation-related pathways involved in the pathogenesis of coronary artery disease.

RESULTS

Analysis of Demographic, Clinical, and Operation Data

LDH was found to be significant in patients as an expected outcome ($p < 0.05$). Serum BUN levels are significant ($p < 0.05$). The plasma glucose levels were also found to be high in our patient group ($p < 0.05$) (Table 1). In patients, intensive care duration (hours); 49.9±8.8, post-op hospital duration (days); 5.5±0.8, weight (kg); 5.5±0.8, cardiopulmonary bypass duration (min); 54.174 ± 9.481, aortic cross-clamp duration (min); 37.7±7.4, number of bypasses; 3.3±0.9 were determined. No relationship was found between miRNA expressions and demographic and clinical data.

Evaluation of Parameters in Cardiac Processes

Troponin I and CK-MB were found to be statistically significant in the analysis of cardiac parameters in the pre-op patient and control groups (Table 2). The mean values for troponin 1 and CK-MB were higher in the patient group than in the controls ($p = 0.001$).

Table 3. Pre-op vs control cardiac parameters

Cardiac parameters	Groups	n	Mean±SD	P– value
Troponin I	Control	48	0.02±0.1	0.001*
	Pre–op	46	0.9±2.8	
CPK	Control	48	79.1±40.3	0.812
	Pre–op	46	88.0±57.1	
CK–MB	Control	48	18.1±4.7	0.001*
	Pre–op	46	27.9±17.6	

* P-values <0.05 are indicated in bold. CPK; creatine phosphokinase, CK-MB; creatine Kinase myocardial band, n; the number of individuals.

Table 4. miRNA expression analysis in between the operation groups (CABG = 46)

miRNAs	Groups	Mean±SD	P-value
miR-21-5p	Pre-op	3.7±2.4	**0.001***
	Post-op 1	3.9±2.6	**0.001***
	Post-op 24	3.9±2.3	**0.001***
miR-181a-5p	Pre-op	11.6±3.5	**0.001***
	Post-op 1	10.4±3.6	**0.001***
	Post-op 24	10.7±3.4	**0.005***
miR-199a-5p	Pre-op	11.0±3.7	**0.001***
	Post-op 1	10.5±3.8	**0.001***
	Post-op 24	11.7±3.4	**0.001***
miR-199b-5p	Pre-op	10.6±3.5	**0.001***
	Post-op 1	10.3±3.3	**0.001***
	Post-op 24	10.8±3.2	**0.001***
miR-320a-5p	Pre-op	3.6±3.1	**0.004***
	Post-op 1	3.9±3.6	**0.002***
	Post-op 24	3.6±3.2	**0.004***

* P-values <0.05 are indicated in bold. Significant according to the Mann-Whitney u test.

CPK, which is defined by the increase in its activity at 4-6 hours after MI, is evaluated together with the increase in CK-MB. It is a maximum of 10-20 hours after chest pain. Although CPK was higher than controls, it was not significant in the analysis.

In univariate and multivariate analysis of pre-op and post-op cardiac parameters, it was observed that troponin I was significantly higher in post-op 1 (14.5±69.03) (p = 0.001) than pre-op (0.9±2.8) and post-op 24 (4.6±7.7). Troponin was detected significant in multiple analyses (pre-op vs post-op 24, p = 0.001; pre-op vs post-op 1, p = 0.001) except for post-op 24 vs post-op (p = 0.348). In the comparison of CPK values, an increase was observed in post-op 1 (429.1±335.2) and post-op 24 (451.1±253.7) compared to pre-op (88.0±57.1) (p = 0.001). In multiple analysis, it was found to be statistically significant according to pre-op/post-op 24 (p = 0.001), pre-op/post-op 1 (p = 0.001), but not significant compared to post-op 24/post-op 1 (p = 0.835). CK-MB values were found to be statistically significant in post-op 1 (63.4±64.5) compared to both pre-op (27.9±17.6) and post-op 24 (55.5±52.0) (p < 0.05). CK-MB values were found to be significant in the comparative analysis of the groups. A distinction can be made between ejection fraction (EF) and systolic-diastolic heart failure, how much the heart contracts with each beat. The normal EF is between 50-70 %. In our patient group, this value is below normal. LVEF analysis showed an increase in post-op 1 (56.39.0) and post-op 24 (56.4±9.0) compared to pre-op (55.6±9.5) and were not significant. LVEF values below the normal level in pre-o started to increase in post-op 1 and showed improvement in post-op 24.

miRNA Expression Analysis

Expression analyzes of five miRNAs in the patient-control group are given as averages using delta Ct values. Five miRNAs were found to be downregulated in pre-op in CBAG patients diagnosed with MI (p < 0.05) (Table 3). In analyzes of each miRNA expression in pre-op and post-op, five miRNAs were significant in post-op 1 and post-op 24 compared to pre-op (p < 0.05) (Table 4)

Correlations Between Cardiac Markers and miRNA Expressions

miR-21-5p

In comparative analysis, there was a correlation between pre-op miR-21-5p expression levels and pre-op troponin values (r=0.34, p=0.01), and a negative correlation between troponin values measured at post-op 24 and post-op 1 (r= -0.38, p=0.00). When evaluated before and after the operation, there is a relationship between increased troponin I levels (95% CI=-10.9-24.2, SD=7.1, SE = 4.1) and upregulation of miR-21 (95% CI=3.6-4.0, SD=0.07, SE = 0.04) (p < 0.001). CPK (95% CI=-183,05-828.5, SD=203.6, SE = 117.6) and miR-21-5p levels increased to post-op 1 and post-op 24 (p < 0.001). LVEF was low in post-op 1 and higher in post-op 24 (95% CI=55.1-57.1, SD=0.4, SE=0.2), while miR-21-5p (95% CI=3.6-4.0, SD=0.07, SE=0.04) was upregulated but not statistically significant (p = 0.06). A statistical significance was determined between the expression of CK-MB and miR-21 (p < 0.001). CK-MB is high in post-op 1, low in post-op 24 (95% CI=2.6-95.2, SD=18.6, SE=10.8), and miR-21-5p is upregulated.

miR-181

In the patient group, there was a negative correlation between miR-181 values measured pre-op and cardiac troponin values measured 1 hour and 24 hours later (r= -0.3, p=0.02; r= -0.3, p=0.01). Troponin I level is high in post-op 1, miR-181a is downregulated (95% CI=-10.9-24.2, SD=7.1, SE=4.1), troponin I is low in post-op 24, and vice versa, miR-181 is upregulated (95% CI=9.3-12.4, SD=0.6, SE=0.4) (p = 0.01). A statistically significant difference was found between miR-181 (p < 0.001) and CPK levels. No correlation was detected between miR-181 and EF (p = 0.596). A correlation was found between post-op 1 of miR-181a and pre-op, post-op 1 of CK-MB (r=0.3, p=0.03; r=0.3, p=0.03) (Table 5). CK-MB is high in post-op 1, low in post-op 24 (95% CI=2.6-95.2, SD=18.6, SE=10.8), and vice versa in miR-181 (p = 0.002).

miR-199a-5p

In the patient group, there was a moderate negative correlation between miR-199a-5p values 24 hours after the operation and

Table 5. Comparative analysis of Troponin I and CK-MB for miR-21-5p (CABG = 46)

			Troponin I			CK–MB		
			Pre–op	Post–op 1	Post–op 24	Pre–op	Post–op 1	Post–op 24
miR–21–5p	Pre–op	r	0.3	0.0	0.0	0.3	0.0	–0.2
		P–value	**0.02***	0.64	0.68	0.09	0.96	0.29
	Post–op 1	r	0.1	–0.2	–0.1	0.2	0.0	–0.3
		P–value	0.33	0.23	0.57	0.12	0.76	0.08
	Post–op 24	r	0.1	–0.4	–0.1	0.1	0.1	0.0
		P–value	0.49	**0.01***	0.59	0.53	0.60	0.83
miR–181a–5p	Pre–op	r	0.2	–0.05	0.1	0.1	0.0	–0.05
		P–value	0.20	0.72	0.68	0.71	0.99	0.72
	Post–op 1	r	0.1	–0.3	0.0	0.3	0.3	0.0
		P–value	0.3	0.03	0.93	**0.04***	**0.03***	0.62
	Post–op 24	r	–0.0	–0,4	–0.1	0.0	0.2	–0.1
		P–value	0.92	**0.01***	0.70	0.94	0.23	0.69
miR–199a–5p	Pre–op	r	0.2	0.0	0.1	–0.1	0.0	–0.1
		P–value	0.15	0.63	0.41	0.74	0.81	0.38
	Post–op 1	r	0.1	0.0	–0.03	–0.0	0.2	–0.03
		P–value	0.44	0.61	0.82	0.87	0.18	0.81
	Post–op 24	r	–0.0	–0.5	–0.2	–0.1	–0.05	–0.2
		P–value	0.14	**0.00***	0.15	0.36	0.74	0.24
miR–199b–5p	Pre–op	r	0.1	0.0	0.0	–0.04	–0.05	–0.2
		P–value	0.37	0.92	0.75	0.77	0.80	0.20
	Post–op 1	r	0.2	0.0	0.1	0.0	0.2	–0.1
		P–value	0.12	0.92	0.75	0.77	0.70	0.20
	Post–op 24	r	0.0	–0.3	–0.1	–0.03	0.1	–0.02
		P–value	0.77	**0.03***	0.66	0.83	0.39	0.86
miR–320a–5p	Pre–op	r	0.3	0.0	0.0	0.2	0.0	–0.2
		P–value	**0.03***	0.99	0.83	0.21	0.97	0.30
	Post–op 1	r	0.2	–0.2	–0.1	0.2	0.2	–0.1
		P–value	0.19	0.10	0.47	0.19	0.16	0.58
	Post–op 24	r	0.0	–0.2	0.0	–0.03	0.2	–0.02
		P–value	0.82	0.15	0.91	0.81	0.13	0.85

* P-values <0.05 are indicated in bold. r; correlation value

cardiac troponin values measured 1 hour after the operation (r=-0.5, p=0.001). While troponin I level increased in post-op 1, miR-199a-5p is downregulated (95% CI=-10.9-24.2, SD=7.1, SE=4.1). miR-199a-5p is upregulated while Trponin I decreases in post-op 24 (95% CI=9.5-12.6, SD=0.6, Std. Er.=0.4) (p = 0.016). Statistically significant difference was found between miR-199a-5p (p < 0.001) and CPK levels. No corelation between EF values and miR-199b-5p expression levels (p = 0.588). There is significant relationship between miR-199a-5p and CK-MB (p = 0.002). CK-MB is high in post-op 1, low in post-op 24, and miR-199a-5p is downregulated in post-op 1 and upregulated in post-op 24.

miR-199b-5p

While miR-199b-5p was associated with post-op 24 troponin levels and there is a negative correlation between groups (r=-0.3, p=0.034). miR-199b is downregulated while troponin I rises in post-op 1 (95% CI=-10.9-24.2, SD=7.1, SE=4.1). miR-199a-5p is upregulated while troponin I decrease in post-op 24 (95% CI=9.9-11.2, SD=0.3, SE=0.1) (p = 0.003). Statistically significant difference was found between miR-199b-5p (p < 0.001) and CPK. No statistical significance was found between miR-199b-5p and LVEF (p = 0.593). CK-MB is high in post-op 1, low in post-op 24, and miR-199b-5p is downregulated in post-op 1 and upregulated in post-op 24 (p < 0.001).

miR-320a

There was a positive correlation between pre-op and pre-op troponin values for miR-320a-5p in the patient group (r=0.3, p=0.031). Troponin I and miR-320a levels increase in post-op 1 (95% CI=-10.9-24.2, SD=7.1, SE=4.1) and decrease in post-op 24 (95% CI=3.2-4.1, SD=0.2, SE=0.1) (p = 0.001). Statistically significant difference was found between miR-320a and CPK levels (p < 0.001). LVEF and miR-320 expressions are not related (p = 0.344). There is no significant relationship between miR-320a-5p expression and CK-MB values (Table 5).

Table 6. miRNA target genes according to database analysis

hsa-miR-21-5p	*YOD1, PRDM11, FASLG, ZNF367, VCL, SKP2, TGFBI, PLAG1, IL12A, **CREBRF***, RAB6D, KRIT1, PELI1, RBPJ, RALGPS2, ADGRG2, GATAD2B, PBRM1, SCML2, RSAD2, PPP1R3B, PLEKHA1, FGF18, SPRY1, FAM13A, GPATCH2L, STAT3, BCL7A, SKI, YAP1, MALT1, ZBTB41, KLF3, MBNL3, CCL1, NKIRAS1, TIAM1, OSR1, PAN3, KDM7A, CASKIN1, PDCD4, GID4, HSD17B4, MAP3K1, PDZD2, UBE2D3, AKAP12, CPEB3, RECK, CCL20, PPP1R3A, NTF3, TIMP3, ANGPTL5, BCL11B, JAG1, FAM3C, MEI4, EPM2A, SLC30A10, BTG2, SYT15, MPRIP, NFIA, KLHL15, CFAP300, GLCCI1, SPRY2, LRRC57, STAG2, KDM1B, GRAMD2B, RMND5A, C7, ALX4, RASA1, SOX5, RNF103, GLIS2, NEGR1, ARHGAP24, NIPAL1, LTV1, ANKS1B, TMEM170A, HIPK3, ELF2, EPHA4, PPP1R3D, **ZNF704***, RAD51AP1, CLDN8, EHD1, ATXN10, MCMDC2, ITCH, MATN2, NPPB, RASA2, CLIC2, SMARCD1, OLFM3, USP15, MAST4, KCNJ10, NIPAL2, PCSK6, TPRG1L, WWP1, ARL1, LPA, GABRB2, CSRNP3, STK40, NFIB, UBR3, CHIC1, CUX1, RASGRP1, LATS1, FDX1, MINDY2, BCL11A, SPEF2, PITX2, SMAD7, PLAA, UBE2D1, THRB, BAHD1, MED21, FRMD3, RP2, TENT5A, KHDC1, CDH7, DLGAP1, RBMS3, KHDC1L, ZDHHC17, BEST3, STK38L, MSH2, KLF6, PTPN20, CNOT6, SOS2, DUSP8, KBTBD6*
hsa-miR-181a-5p	***CREBRF***, C2CD5, ZNF594, ZNF268, ZNF439, DDX3X, ZNF781, PRTG, TRIM2, SESN3, TNPO1, ZFP90, ZNF37A, ZNF780B, KMT2A, FIGN, ZFP36L2, S1PR1, OSBPL3, ZNF780A, PDE5A, DMXL2, GLS, ZIC3, ZNF844, PPIP5K2, PROX1, BEND3, CLIP1, NWD2, LGALSL, ACVR2B, TENT4B, NEXMIF, ZNF559, REPS2, TRIM71, GPD1L, RNF217, SPRY4, TBC1D1, CBX7, KMT2C, ARF6, PLCL2, GABRA1, SPP1, PTBP3, NEK7, ZNF426, AP1G1, KCNQ5, CDYL, IL2, CDON, RLF, SSX2IP, TMEM94, LARP4, TADA2B, BTBD3, SCD, ADAM11, CEP97, GIGYF1, ZNF597, SIPA1L2, POU2F1, **ZNF704***, INO80D, UBE2B, ST8SIA4, TMEFF1, MARK1, ZNF440, ZNF302, ATP2B1, DNAJC13, SPIRE1, NELFA, PITPNB, ZFP36L1, SLC4A10, SLC25A37, DOCK4, TMEM87B, ARSJ, CXCL9, MB21D2, ARHGEF3, MTF2, PCDHA1, PLAG1, PNRC2, ZFP14, AKIRIN1, MFSD6, TRAK1, LMO1, KATNBL1, OSBPL8, GPD2, ZBTB4, ABHD18, TGFBR1, PHF20L1, TOM1L1, LATS1, UBP1, ANKRD44, TCERG1, SIK3, LIN28B, HOXC8, CPNE2, THRB, ADCY9, KIF3A, PPFIA1, KAT2B, PCDHA8, PCDHAC1, HIPK3, ZNF800, HIC2, ATXN3, BIRC6, ZNF584, ZNF468, GSKIP, NOVA1, PCDHAC2, PCDHA13, GATA6, SOWAHA, PCDHA10, PCDHA5, CPD, PCDHA6, CREB1, CTTNBP2NL, PCDHA11, PCDHA3, OOSP2, TCF7L2, RALGAPB, MGAT2, RBBP7, NUS1, TGFBRAP1, PPP3R1, PCDHA4, PRLR, SLAIN2, BRD1, NAALADL2, PCDHA7, PCDHA2, ZNF124, ZNF700, PAX9, PCDHA12, LCLAT1, USP42, PI4K2B, ATM, PEAK1, SALL4, EPHA4, PIAS1, CLASP1, NMBR, TMEM64, KIF1B, KCNH1, E2F5, PSPC1, CHIC1, BHLHE40, FNDC3B, ATXN7, TXNDC12, FUT9, TOGARAM1, WDR82, ADGRB3, KIF3B, KLHL2, G3BP2, OXGR1, PER3, SEC24C, AKT3, NOTCH2, AMER2, GSE1, PRKCD, KLHL29, EPC2, MIER3, IPO8, IL1A, SLITRK1, MAMDC2, ZNF479, ACVR2A, ZNF773, FAM126B, DCBLD2, ELMSAN1, IQCJ-SCHIP1, PAX5, NIPAL4, PHTF2, HMBS, PARP11, ZNF189, SEMA4G, YTHDC2, ZIC2, PCDHA9, C2orf69, KLF6, ZNF266, AFG3L2, EYS, TPRX1, KLF15, BCLAF1, SLC7A11, UBE2D3, BAZ2B, ATP2B3, CNTN4, LYRM1, NCOA2, FAM122B, HSP90B1, RFC1, ONECUT2, SS18L1, PRRC2C, ZNF140, USP33, HRH1, MBOAT2, TMEM144, EXOC5, SYT16, TMEM165, CRIM1, HEY2, ETNK1, ETS1, NR1D2, PAFAH1B2, NR6A1, CLVS1, ZBTB43, PKNOX2, CHMP1B, MAN2A1, LIN28A, ACER3, KPNB1, ADARB1, SAMHD1, SLC35F3, FAM3C, CARM1, GPBP1, KRBOX4, RAI1, CTDSPL, FOXP1, TRDMT1, IGF2BP2, GTSE1, SERTAD2, CBFA2T3, FGD4, RAB3IP, CDKN3, B4GALT1, RNF34, ATP1B1, LRBA, ATP2B2, DNAJC3, SMAP1, CNKSR2, BCL2, SLC2A3, IPMK, RPS6KB1, CCNJ, ZDHHC7, DYNC2H1, CALCR, RPS6KA3, CNKSR3, MYBL1, OTUD4, ENTPD6, LIMCH1, AP1S3, RASSF2, FSBP, SRSF7, LIG4, SPECC1L, ZFP1, CPOX, TTC39B, AGO4, CDC73, EN1, MPP5, QKI, NLN, KIAA1549L, BCL2L11, NCALD, KDM5A, ETV6, ZNF563, RORA, NIPBL, ST6GALNAC5, AHCTF1, PRR27, BLOC1S6, HAO1, GOLGA1, HOXD1, GHITM, TBC1D4, ZNF586, ZNRF2, TNFRSF11B, NSUN7, PDE3A, GOLGA8M, BRAP, PCSK1, LNPK, ZBTB2, KLHL5, NKAIN2, RAB30, DUSP6, HOXA1, UNC80, NR2C2, MUC22, ENAH, YLPM1, RAD54B, PAK5, PDAP1, ARMH4, LOX, SACM1L, FNDC3A, SLC4A8, STARD4, POLQ, DARS, CPEB4, ZFP62, FAM160A1, SIN3B, JARID2, RIMKLB, ADAMTS18, PNISR, GCC2, PAWR, ACSL4, OGFRL1, MS4A1, ZEB2, TMLHE, ARMC8, ZDHHC17, DCN, MEGF9, SLC5A9, LPCAT2, TREML4, VPS41, ESR1, BOLL, RNF169, KCNA1, ZNF527, ABTB2, TNF, ADAMTS6, GRB10, N4BP2, ATP8B2, CNOT2, LRRN1, SLC12A5, KCNJ10, TNFSF4, OTOGL, SCHIP1, PHACTR4, PPP2R3A, AASDHPPT, FBXO34, KANK1, PARM1, PBX3, DCLK1, FSD1L, GPRIN3, TBC1D14, MICU3, SLC25A36, UBL3, PHLDA1, MBNL2, ZDHHC3, FAM135A, TNS1, ZFAND6, APOO, CCNDBP1, GOLGA8J, SYNPR, APBA1, GOLGA8H, FNIP2, NOTCH4, SEC24A, LAMA1, SIX4, TM9SF4, MLXIP, GRM5, KPNA1, ASPH, TAB3, RASSF8, CCNK, GPR137C, ZNF544, FAM19A2, LEMD3, ATP2A2, SLC38A11, TAOK1, OSBPL2, CCL8, RLIM, GOLGA8R, RAB8B, MTPN, MAPK1IP1L, DERL1, PSG11, MTX3, TNRC6B, GOLGA8K, SRGAP2, BAG4, ACAP2, SSB, COX15, TBPL1, CDK17, FKBP1A, DNAJA4, ZFAND4, NR4A3, RAD21, MUC7, PTPDC1, CECR2, ADAMTS5, RAB3GAP1, RASSF1, EYA3, BMP2K, HECA, IKZF5, TESMIN, GOLGA8O, MAP2K1, CALM1, TBCEL, GOLGA8N, ITGA3, ASAH2B, RECK, NAA50, TRDN, ATXN1, NLK, DLG2, MYCBP2, ADO, ATMIN, XPO7, AKIRIN2, PHIP, PAPOLG, RAB3C, CA8, SH2B3, ASTN1, ANKRD13C, PRDM4, UNC5D, GOLGA6L4, ARL5A, E2F7, MCC, IPO7, CDH8, RYR3, AKAP6, GRIK2, LCTL, CCP110, JADE2, AP1AR, BRWD1, SELENOT, C1orf109, LRRC8D, NAB1, STXBP5, CHD1, AK9, ANO1, ESM1, DEPTOR, KLHL42, UBE2D1, DEK, CDC40, TMED4, PLAU, CFAP161, ADCY1, HEATR3, PLEKHJ1, PTPN4, AGFG1, NPEPPS, IGDCC3, CHMP2B, GOT2, QSER1, CPSF6, PTPN22, LPCAT1, PHF3, TAB2, ZNF121, TCFL5, IRS2, YTHDF3, PDGFRA, NAP1L1, SLC35E1, NUCKS1, MTURN, PNMA2, NRXN1, TNFAIP1, HOXA11, C14orf28, MORC3, UBE3C, DDX60L, CDC5L, WNK1, PAM, MYO1E, FBXO33, RNF182, MAGOHB, ADRA1A, ZNF44, TMEM131, MED26, CD69, PIK3R3, TMF1, PSRC1, MBTPS2, SLC10A7, GALNT4, RIOX2, WSB1, MAP3K3, BACH2, MAB21L3, IL25, ZNF823, CAMSAP2, DISC1, ETFBKMT, MSANTD3-TMEFF1, CBLB, HCN2, POC1B-GALNT4*

hsa-miR-199a-5p	*DDR1, SLC25A23, NAA40, ZNF763, MYRF, CLCN3, SLC24A3, CELSR1, LIN7C, HAPLN1, FZD4, MAP3K11, RAD23B, GCNT2, SULF1, AKAP1, KL, ARHGAP12, ZNF189, CDCA7L, FAM222B, GPR63, ZNF773, RASSF2, MICAL3, ZNF426, BCAM, ZNF439, MARCH8, ZBTB20, GNG5, ZNF544, ITGA3, ZNF440, MAB21L1, TSPAN6, ARHGAP21, GPRC5A, **ZNF704***, ZFYVE27, RBM47, ECE1, TUBG1, ZNF516, PAXBP1, SIRT1, ZFAND4, NSG1, CLIP1, ACVR2B, WAPL, VPS26A, M6PR, PDPN, HMCN1, NFIL3, NPAS2, FER, ANK3, SHOC2, CCNL1, KPNA4, TPR, ASRGL1, SOS2, AUTS2, CCDC88C, FZD6, ZNF563, PLXNA2, PPARGC1A, TXNL1, POU3F2, FLRT3, SORCS3, INO80D, BTRC, CSDC2, TMEM245, RASSF3, NINL, LXN, AGAP1, ZNF418, GPR89A, HSPA5, TAB3, ZNF589, PIK3CD, ZNF479, PLEKHF2, ABHD17C, ZNF776, ATG14, P3H2, UBAP1, CYP51A1, GSK3B, ATP13A2, SACS, ZNF584, BICC1, TGFB2, SMARCD1, CECR2, ATXN7, SUN1, FAM19A2, HSPA12A, AP1G1, CCDC120, RALGAPA1, ANKRD23, KLHL29, MINDY3, IPO8, ZNF468, **CREBRF***, SLF2, FAM126B, WDR76, NLK, ZNF329, RFX3, ITGA8, USP31, RGMA, NTNG1, TAF9B, SERPINE1, MARCH7, NCSTN, CELF2, EIF5B, PLXND1, ADD3, ZFP90, ZNF559, PAN3, KDM3B, DENND6A, PPFIBP1, CLEC2D, RNF38, HIF1A, GPR89B, CCNJ, TMEM220, PAX3, ABCA1, BEND3, MGAT3, CRYBG3, KIAA1109, RNF11, ETS1, GIT1, ZNF579, ARF6, ZNF559-ZNF177, FBXO4, IL36B, ARRB2, CCDC43, ZNF195, MGAT4B, CEP350, RBPMS, NUDT5, RAB7A, PDE4D, PODXL, TST, PI4KA, USPL1, CACUL1, COL5A3, AFTPH, RBM24, YIPF6, TMEM135, CACNB2, GAS2L2, NPY2R, MPP5, RCSD1, ZNF547, TVP23C-CDRT4, WDTC*
hsa-miR-199b-5p	*DDR1, SLC25A23, NAA40, MYRF, CLCN3, SLC24A3, CELSR1, ZNF763, LIN7C, HAPLN1, FZD4, MAP3K11, RAD23B, GCNT2, SULF1, AKAP1, ZNF773, ARHGAP12, ZNF189, CDCA7L, GPR63, RASSF2, KL, MICAL3, ZNF426, BCAM, ZNF439, MARCH8, ZBTB20, GNG5, ZNF544, ITGA3, FAM222B, ZNF440, MAB21L1, TSPAN6, ARHGAP21, GPRC5A, **ZNF704***, ZFYVE27, RBM47, ECE1, TUBG1, ZNF516, PAXBP1, SIRT1, ZFAND4, NSG1, CLIP1, ACVR2B, WAPL, VPS26A, M6PR, PDPN, HMCN1, NFIL3, NPAS2, FER, ANK3, SHOC2, CCNL1, KPNA4, UBAP1, TPR, ASRGL1, SOS2, AUTS2, CCDC88C, FZD6, ZNF563, PLXNA2, PPARGC1A, TXNL1, POU3F2, FLRT3, SORCS3, INO80D, BTRC, CSDC2, TMEM245, RASSF3, NINL, LXN, AGAP1, ZNF418, GPR89A, HSPA5, TAB3, ZNF589, PIK3CD, ZNF479, PLEKHF2, ABHD17C, ZNF776, ATG14, P3H2, CYP51A1, GSK3B, ATP13A2, SACS, ZNF584, BICC1, TGFB2, SMARCD1, CECR2, ATXN7, SUN1, FAM19A2, HSPA12A, AP1G1, CCDC120, RALGAPA1, ANKRD23, KLHL29, MINDY3, IPO8, ZNF468, **CREBRF***, SLF2, FAM126B, WDR76, NLK, ZNF329, RFX3, ITGA8, USP31, RGMA, NTNG1, TAF9B, SERPINE1, MARCH7, NCSTN, CELF2, EIF5B, PLXND1, ADD3, ZFP90, ZNF559, PAN3, KDM3B, DENND6A, PPFIBP1, CLEC2D, RNF38, HIF1A, GPR89B, CCNJ, TMEM220, PAX3, ABCA1, BEND3, MGAT3, CRYBG3, KIAA1109, RNF11, ETS1, GIT1, ZNF579, ARF6, ZNF559-ZNF177, FBXO4, IL36B, ARRB2, CCDC43, ZNF195, MGAT4B, CEP350, RBPMS, NUDT5, RAB7A, PDE4D, TST, PI4KA, USPL1, CACUL1, COL5A3, AFTPH, RBM24, YIPF6, TMEM135, CACNB2, GAS2L2, NPY2R, MPP5, RCSD1, TVP23C-CDRT4*
hsa-miR-320	*ZC3H12B, RIMKLB, CLCN3, DUSP18, SOX4, KCNB1, TMEM178B, GFRA1, TNRC6B, LMLN, MAP3K9, HOXA10, RAB29, CHRM2, CCR5, ANKRD13C, PTGER4, SSTR3, RASSF5, ZSWIM6, TTC7B, MON2, MANEAL, UBR3, ANKRD37, SMARCD1, CNDP2, POU2F1, FBXW11, **ZNF704***, ZSLC16A4, CD300E, NR6A1, SDK1, XG, TIA1, MMP2, RAD54L2, NAV1, RORA, NIPBL, KIAA1211L, NFASC, PARM1, ERICH5, CENPA, CACNA1E, UGGT2, LOC403312, ERBB4, FXYD3, XKR7, TNS1, MPDZ, TNFRSF19, TBK1, TRAF3, SV2B, ARMC2, NMT1, VAPB, AGAP1, GAREM1, PRICKLE2, LARP1, TMEM267, ABCC4, SRP19, FGF13, SLC9A7, RPL36A-HNRNPH2, PAX2, AFTPH, XYLT1, SH3TC2, SNED1, RFX7, RAPGEF4, STAT5B, HTR5A, ATL3, NMD3, EEF1A1, TAOK1, COG3, ZNF366, HAO1, FABP7, SORCS1, CLMP, ZNF287, PPBP, NEUROG2, PRDM16, AAK1, KDM3B, GRAMD1B, INSR, CREB5, TRIM9, NFATC2, FAM84B, CENPBD1, PKNOX2, MCM6, C6orf136, **CREBRF***, SMCR8, C1orf115, CA8, ST8SIA5, WARS2, ETS1, RIDA, ZSWIM1, NUDT15, ST3GAL3, STK35, TMPRSS15, INTS14*

* The *ZNF704* and *CREBRF* genes are common targets for the five miRNAs.

ROC Curve Analysis of Five miRNAs

miR-21-5p (AUC=0.77, CI=0.68-0.86), miR-181a-5p (AUC=0.78, CI=0.69-0.86) and miR-320a-5p (AUC=0.78, CI=0.69-0.86) were determined as weak, miR-199a-5p (AUC=0.81, CI=0.71-0.88) and miR-199b-5p (AUC=0.80, CI=0.72-0.88) were determined as good (control vs pre-op).

In Silico Analysis for miRNA Targets and Pathways

Target genes with a matching score between 80-100% were selected. We analysed 469 genes for miR-21-5p, 1408 genes for miR-181a-5p, 562 genes for miR-199a-5p, 556 genes for miR-199b-5p and 607 genes were analysed for miR-320a-5p (Table 6). In comparisons for miRNA target genes, CREB3 Regulatory Factor (CREBRF) and Zinc Finger Protein 704 (ZNF704) genes targeted by all five miRNAs were detected (Table 7). The association of target genes with miRNAs was analyzed by gene ontology (GO). Parameters used in this association with miRpath 3.0; micro T-CDS (v5.0) was chosen as p-value threshold = 0.05, MicroT threshold = 0.8. In the analysis of miRNA pathways, we determined that CREBRF is responsible for the metabolism of proteins, as a CREB3 factor activating gene in the formation of unfolded protein response, and it binds to CREB. ZNF704 shows DNA binding transcription factor activity. The proteins they work with were identified using the protein interaction database (STRING V.11.00). According to these results; CREBRF is associated with CREB3 and ZNF704 with TRIM28 for its potential effects on CABG.

DISCUSSION

In this study, cardiac parameters and miRNA expressions (miR-21-5p, miR- 199a-5p, miR-199b-5p, miR-181a-5p ve miR-320a-5p) were investigated in the I/R status of patients who underwent CABG. Surgery was compared in plasma samples with healthy controls, as well as between preoperative, postoperative, and intraoperative hours. The target genes of miRNAs and the pathways

Table 7. miRNA::target mRNA matching specificity data

miRNAs	Target mRNA	Target end	Site type	Position (nucleotide)
miR-21-5p	CREBF	3'-UTR	7mer	1221-1227
	ZNF704	3'-UTR	7mer	354-360
miR-181a-5p	CREBF	3'-UTR	8mer	736-743
	ZNF704	3'-UTR	8mer	4357-4364
			8mer	11162-11169
			7mer	12207-12213
miR-199a-5p	CREBF	3'-UTR	7mer	**3259-3266***
	ZNF704	3'-UTR	7mer	**10021-10027***
			7mer	**12361-12367***
			7mer	**12572-12578***
miR-199b-5p	CREBF	3'-UTR	8mer	**3259-3266***
	ZNF704	3'-UTR	7mer	**10021-10027***
			7mer	**12361-12367***
			7mer	**12572-12578***
miR-320a-5p	CREBF	3'-UTR	8mer	2831-2838
	ZNF704	3'-UTR	7mer	9279-9286

*The same target position of miR-199a-5p and miR-199b-5p (they are miRNAs belonging to the miR-199a/b family strengthens the target similarity) for *CREBRF* and *ZNF704* genes.

in which these genes are functional in CABG were determined by bioinformatic analyses. These analyses aimed to investigate the relationship between clock-related blood values of cardiac parameters and miRNA expressions, determine their behavior in CABG, their contribution to I/R, and cardiac remodeling, and reveal the potential for early diagnosis in these patients with MI.

miRNAs overexpressed in the cardiovascular system, such as miR-16 and miR-499, have been suggested as markers in CAD (17). Targeting the 3'-UTR of PTEN, miR-21 is functional in I/R. While these in vitro studies showed negative regulation of miR-21 in the I/R, in in vivo studies miR-21 is down-regulated in the ischemic region (18). miR-21 was also downregulated in our group. Differently, in the analyzes in which we determined the operation status, it is upregulated in the first 1 hour and the next 24 hours after the operation. Post-operative analyzes in plasma show that miR-21 increases with time and can be detected 24 hours after the operation, therefore, it may be related to cardiac markers in this process both time and expression level. We suggest that the expression can be used for prognosis and perioperative biomarker even after the operation.

The significant increase in plasma miR-181a levels at 6 and 12 hours after the onset of MI and the correlation with CK-MB, cardiac troponin I, and ROC analyses indicate that this miRNA has diagnostic value in MI (19). In our study, miR-181a levels are downregulated. Downregulation of miR-181a in patients diagnosed with MI is likely to be associated with injury, overlapping with preoperative elevated and abnormal clinical presentation. Therefore, the stability and continuity of miR-181a in plasma and its association with troponin, CK-MB, CPK, and EF are very important for monitoring the process.

MiR-199a, which is among the cardiomyocyte-specific miRNAs, stands out with the regulation of the SIRT1 gene in heart tissue. The downregulation of this miRNA is associated with high levels of biochemical markers associated with inflammation, angiogenesis, and endothelial dysfunction that play a role in the development and progression of atherosclerosis (20, 21). Here we propose for the first time that CREBRF and ZNF704 and their downregulation of cardiac parameters may be effective in CABG. Post-op upregulation of miR-199b suggests that this miRNA may be entitled to cardiac remodeling. Bioinformatics analyzes also show that this task is done through CREBRF and ZNF704. In addition, its correlation with troponin I indicate that it can be an effective miRNA in post-op. Based on ROC analysis results, two miRNAs are potential candidates for determining postoperative prognosis.

miR-320 plays a role in the correction of I/R-induced cardiac injury (22). The closest findings to our study are the investigation of miR-34a, miR-15a, and miR-320a gene expressions before and 24 hours after surgery in plasma samples of patients undergoing cardiopulmonary bypass. In this study, it was determined that the miR-320a level increased during surgery (23). In contrast to this study, we examined I/R in CABG patients with MI. Similarly, we found high levels of miR-320 in post-op. The expression of miR-320 after I/R injury and its upregulation after bypass graft surgery show that it can be a marker that can be monitored in this process, and most importantly, it can be a treatment target in I/R injury.

As a result of our bioinformatic analyzes, we have achieved two common targets that may be effective in CABG: CREBRF and ZNF704 for miR-199a/b, and their co-operating proteins, CREB3 and TRIM28 are involved in cardiac processes in CBAG. In addition, it is functional in diagnosis, treatment, and prognosis. These

miRNAs are important tools for the management of I/R injury and their diagnostic potential in MI-diagnosed CABG.

LIMITATIONS

This is a case-control study with relatively small sample size. The molecular processes, oscillations, transports, targets of miRNAs, and the mechanisms mediating these processes have not been fully elucidated. Interventional studies are needed to detect circulating miRNAs and make them available in routine laboratories. In addition, profiling of circular and tissue-specific miRNAs will strengthen the association studies of CABG and miRNAs.

CONCLUSION

In our study, the expression levels of miR-21-5b, miR-199a-5p, miR-199b, miR-181a, and miR-320a were lower in patients with coronary artery disease compared to the control group. Moreover, miR-199a-5p was higher in patients with coronary artery disease compared to the first hour and 24th hour after the operation. Accordingly, it can be said that the decrease in the levels of these miRNAs may pave the way for the pathogenesis of coronary artery disease. miR-199a can be used to predict future adverse events, optimize patient care, and improve the patient clinic in coronary artery bypass graft surgery patients. Clinicians need to know the morbidity and mortality of patients, and miR-199a-5p has the potential to address this need as an epigenetic marker.

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Original Research

Systemic Immune Inflammation-Index and CANLPH Score in Patients with Mitral Stenosis Undergoing Balloon Valvuloplasty

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ABSTRACT

Objective: to evaluate CANLPH score and systemic immune inflammation index (SII) in patients with symptomatic rheumatic mitral stenosis (MS) undergoing percutaneous mitral balloon valvuloplasty (PMBV).

Methods: 62 patients who underwent PMBV in our clinic between 2018 and 2021 were included retrospectively. The patients were divided into 2 groups according to echo score. The CANLPH score was calculated from the cut-off values of C-reactive protein to albumin ratio (CAR), neutrophil to lymphocyte ratio (NLR) and platelet to hemoglobin ratio (PHR), determined with the Youden index and SII by the formula platelet x neutrophil/lymphocyte. P <0.05 was considered statistically significant.

Results: The mean age of the patients was 44.5±10.4 years (40 female, 64.5%). The mean values of SII and CANLPH scores were higher in the Echo score >8 group (p <0.001, both). The mean mitral gradient before and after PMBV was 12.6±5.7mm Hg and 5.0±2.4 mm Hg, and the mean valve area was 1.12±0.27 cm2 and 1.85±0.29 cm2. A statistically significant and negative correlation was observed between the gradient decrease after the procedure and the CANLPH score and SII (r=-0.426, p=0.001 and r=-0.418, p=0.001, respectively). In receiver operating characteristic (ROC) curve analysis, it was concluded that the ability of CANPLH score to predict the higher Echo scores was noninferior to SII with an area under curve (AUC:0.820 (0.701-0.906) and AUC:0.786 (0.664-0.880), z statistics 0.576 and p=0.564.

Conclusion: SII and CANLPH scores are correlated with Echo score. A significant negative relationship was found with both biomarkers and gradient decrease after the PMBV.

Keywords: CANLPH score, mitral stenosis, percutaneous balloon valvuloplasty, systemic immune-inflammation index

INTRODUCTION

The most important cause of mitral stenosis is rheumatic carditis, and isolated mitral stenosis is seen in 40% of patients with carditis.[1] The time until symptoms develop is long, and symptoms increase gradually in 50% of patients.[2] Procedure is necessary in symptomatic patients and if the valve anatomy is suitable, percutaneous balloon valvuloplasty (PMBV) is the first choice.[3]

The plasma levels of stokines and chemokines such as interleukin-1 (IL-1), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), interleukin-6 (IL-6) and interleukin-12 (IL-12) to decrease after PMBV indicates the link between inflammation and severity of mitral stenosis.[4] There are also studies supporting that high neutrophil to lymphocyte ratio (NLR) values are associated with severity of mitral stenosis.[5]

The systemic immune-inflammation index (SII) and CANLPH score can be used to evaluate the host's current degree of inflammation.[6,7] Both have prognostic importance for many clinical situations.[8,9] The SII can predict prognosis and survival after coronary artery disease and mortality in patients with infective endocarditis.[10] Tosu et al. determined that SII could predict short-term mortality in patients with aortic stenosis who underwent transcatheter aortic valve implantation.[11] CANLPH score is a prognostic biomarker especially used in malignancy.[12] In a recently published study, it has been mentioned that the CANLPH score can be used to determine mortality after coronary artery bypass,grefting.[13] This result was based on the thesis that the inflammatory process is involved in most of the mortality and complications in coronary artery disease. The aim of this study is to evaluate CANLPH score and SII in patients who underwent PMBV

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and the relationship between echo score and gradient decrease after balloon valvuloplasty and inflammatory markers.

METHODS

Study Population and Laboratory Measurements

Sixty-five patients with mitral stenosis that had been treated with PMBV in our clinic between January 2018 and December 2020 were included in this retrospective study. Three patients, two had atrial fibrillation and one patient who was taken to emergency surgery after the development of pericardial tamponade during the procedure, were excluded. Patients with a history of transischemic attack or cerebrovascular disease, with known inflammatory disease, autoimmune disease, or chronic liver disease, malignity, valvular disease requiring procedures other than mitral stenosis, presence of thrombus in the left atrium or atrial appendage, active infection were also excluded. The demographic and medical characteristics of the patients were obtained from their files and in the hospital digital recording system. Routine laboratory parameters of the patients were examined before PMBV procedure and were recorded in the hospital digital system. C-reactive protein to albumin ratio (CAR), neutrophil to lymphocyte ratio (NLR) and platelet to hemoglobin ratio (PHR) are all associated with the systemic inflammatory status of the host and the CANLPH score, a combination of these three markers, was calculated from the cut-off values of CAR, NLR and PHR determined with the Youden index. SII was calculated by the formula platelet X neutrophil/ lymphocyte.[12]

Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or current use of antihypertensive medication. Diabetes mellitus was defined as fasting serum glucose ≥ 126 mg/dL, hemoglobin A1c ≥ 6.5%, or the use of blood glucose lowering agents

A successful procedure was defined as an increase in the valve area above 1.5 cm^2 and the degree of mitral regurgitation less than severe.

The study was approved by the local Clinical Research Ethics Committee of our hospital (2.6.2021, 1432). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Main Points:

- Mitral stenosis is an inflammatory process and it has been shown that inflammation regresses after treatment.
- CRP, SII and CANLPH are biomarkers of host's inflammatory status and all of them were higher in the patients with mitral stenosis with higher Echo scores.
- The decrease in mean gradient after balloon valvuloplasty was associated and moderately correlated with both SII and CANLPH.

Transthoracic and Transesophageal Echocardiography

Transthoracic and transesophageal echocardiography (TTE-TOE) were performed using commercially available ultrasonographic equipment according to recommendations of the American Society of Echocardiography before the procedure and TTE after the procedure.14 TTE examinations included M-mode, two dimensional and Doppler flow imaging measurements. Left ventricular ejection fraction was measured and recorded using the modified biplane Simpson method. The transmitral mean gradient assessed by means of tracing mitral inflow was calculated and mitral valve area with the direct planimetry of the orifice in the parasternal short-axis view was performed. After calculating the mean gradients before and after the procedure, the difference was expressed as percentage. Systolic pulmonary artery pressure was determined according to formula (4 x [peak TR velocity]2) +RA pressure where TR is tricuspid regurtitation and RA is the right atrium. Echo score of Wilkins was used to classify the patients into two groups according to echo score of 8 or less and higher than 8. TOE was performed to rule out left atrial and appendage thrombi.

Statistical Analysis

All statistical analysis were performed with SPSS 17 (SPSS, Inc. Chicago, Illinois, USA) and MedCalc (v19.6.1) for Windows. Continuous variables were expressed as mean ± standard deviation (mean ± SD) or median (interquartile range) and categorical variables as numbers and percentages. Comparisons of the continuous variables between groups were performed using the independent samples t-test and Mann-Whitney U test, as appropriate and categorical variables using the $\chi 2$ test or Fisher's Exact test. Whether continuous variables have normal distribution was analyzed with Kolmogorow-Smirnov test. Univariate and multivariable logistic regression analyses were performed to assess the relationship between the CANLPH score and SII and mitral stenosis. Variables with a p value ≤ 0.05 in univariate analysis were included in the multivariate analysis. Reciever operating characteristic (ROC) curve analysis was performed to demonstrate the cut-off values and sensitivity and specificity of SII and CANLPH score in showing the MS severity. The results are expressed as relative risk and 95% confidence interval (CI). A p value less than 0.05 was considered as statistically significant.

RESULTS

The mean age of the patients was 44.5±0.4 (40 female, 64.5%). Of the 27 patients in the group with an echo score of 8 and below, 16 were female; while 24 of the 35 patients in the group with an echo score above 8, were female. The average of the echo scores was 4.5±1.1 and 9.1±0.9, respectively. When the demographic characteristics of the patients were compared, it was found that the groups were similar in terms of diabetes mellitus, hypertension, gender, age and ejection fraction (p=0.626, p=0.715, p=0.447, p=0.089 and p=0.803, respectively). Baseline demographic characteristics, laboratory results and echocardiorapic findings of groups are summarized in Table 1. The only laboratory parameter that the difference was significant between the groups was C-reactive protein (CRP) (p=0.042).

Table 1- Clinical, echocardiographic and laboratory data of the study population

Variables	Echo score ≤8, n=27	Echo score >8, n=35	p
Age, years	42±9	46±11	0.089
Female, n(%)	16 (59.2)	24(68.5)	0.447
HT, n(%)	6(22.2)	9(25.7)	0.715
DM, n(%)	4(14.8)	6(17.1)	0.626
EF,%	59±4	60±3	0.803
Echo score	4.5±1.1	9.1±0.9	<0.001
Pre-PMBV mean gradient, mm Hg	12.5±5.8	12.7±5.7	0.895
Pre-PMBV MVA, cm^2	1.0±0.2	1.1±0.3	0.376
Post-PMBV mean gradient, mm Hg	4.6±1.6	5.3±3.0	0.285
Post-PMBV MVA, cm^2	1.8±0.2	1.8±0.3	0.689
SPAP, mm Hg	44.6±13.4	48.6±15.4	0.512
Δ mean gradient post-PMBV, %	61.4±14.3	52.3±16.4	0.035
CRP, mg/L	3.0±2.6	4.6±3.3	0.042
Albumin, g/dL	4.1±0.3	4.0±0.4	0.604
Neutrophil, 10^3/µl	4.6±1.8	5.4±1.4	0.055
Lymphocyte, 10^3/µl	2.1±0.7	1.9±0.5	0.112
PLT, 10^3/µl	229.6±68.3	236.2±50.3	0.662
HG, g/dL	12.9±1.7	13.0±1.6	0.752
WBC, 10^3/µl	7.5±2.0	8.2±1.7	0.136
MPV, fL	9.8±1.2	9.7±1.2	0.962
RDW, %	14.7±2.1	14.7±1.7	0.707
Glucose, mg/dL	92.0±18.0	110.2±31.8	0.059
Creatinine, mg/dL	0.6±0.1	0.7±0.1	0.166
Uric acid, mg/dL	4.9±1.1	5.6±1.7	0.250
Triglyceride, mg/dL	140.2±101.5	151.2±87.1	0.538
LDL, mg/dL	126.7±28.9	136.5±34.8	0.284
CAR	0.7±0.6	1.0±0.6	0.036
NLR	2.2±1.1	3.0±1.3	0.014
PHR	18.0±5.9	18.2±4.5	0.872
SII	463.2±159.5	689.6±238.4	<0.001
CANLPH	0.9±0.6	2.0±0.9	<0.001

HT: hypertension, DM: diabetes mellitus, EF: ejection fraction, PMBV: percutaneous mitral baloon valvuloplasty, MVA: mitral valve area, SPAP: systolic pulmonary artery pressure, CRP: C-reactive protein, PLT: platelets, HG: hemoglobin, WBC: white blood cell count, MPV: mean platelet volume, RDW: red cell distribution wide: LDL: low density lipoprotin cholesterol, CAR: CRP/ albumin ratio, NLR: neutrophil/lymphocyte ratio, PHR: platelet/hemoglobin ratio, SII: systemic immune inflammation index, CANLPH: CRP/albumin, neutrophil/lymphocyte, platelet/hemoglobin

When the echocardiographic records of the groups were compared, no significant difference was found between the mean gradient, mitral valve area and pulmonary artery systolic pressure before the procedure (p=895, p=0.376 and p=0.512, respectively). Although the mean gradient, and mitral valve area were similar between the groups after percutaneous balloon valvuloplasty, the percentage decrease in the mean gradient was statistically significantly lower in the group with an echo score above 8 compared to the other group (p=0.285, p=0.689 and p=0.035, respectively).

The mean CAR, NLR, SII and CANLPH score were higher in the group with echo score >8 (p=0.036, p=0.014, p<0.001 and p<0.001 respectively) although mean PHR was similar (p=0.872). When the cut-off values of PHR, CAR and NLR were calculated using the Youden index; the cut-off value for PHR was 16.18 (sensitivity 71.4%, specificity 48.1%); 0.84 for CAR (sensitivity 62.9%, specificity 76.9%) and 2.37 for NLR (sensitivity 80%, specificity 70.4%). The cut-off value of SII for the higher echo score group was >667.79 with a sensitivity of 57.1% and specificity of 96.3% while >1 with a sensitivity of 77.1% and specificity of 81.4 for CANLPH score. Although female sex was dominant in both group; SII and CANLPH score and the decrease in percentage in mean gradient after the procedure were similar between females and males (p=0.324, p=0.414 and p=0.314, respectively).

When the relationship of CRP, CAR, NLR, SII and CANLPH scores with the echo score was evaluated, it was seen that all biomarkers were positively correlated with the echo score and the correlation coefficient determining the relationship with the CANLPH score was higher than the others. The results of the correlations between inflammatory markers and echo score and gradient decrease after the procedure are shown in Table 2. The best correlation with the gradient decrease (in percentage) after the procedure was again with the CANLPH score and was in the opposite direction (Figure 1-4).

Table 2- Correlations between Echo score, Δ mean gradient post-PMBV % and SII, CAR, NLR and CANLPH

Variables	r	p
CRP- Echo score	0.251	0.049
CAR-Echo score	0.256	0.047
NLR-Echo score	0.390	0.002
SII- Echo score	0.562	<0.001
CANLPH- Echo score	0.602	<0.001
Δ mean gradient postPMBV,% - CRP	-0.084	0.544
Δ mean gradient postPMBV,% -CAR	-0.304	0.025
Δ mean gradient postPMBV,% -NLR	-0.279	0.039
Δ mean gradient postPMBV,% - SII	-0.418	0.001
Δ mean gradient postPMBV,% - CANLPH	-0.426	0.001

CRP: C-reactive protein, CAR: CRP/albumin ratio, NLR: neutrophil/lymphocyte ratio, SII: systemic immune inflammation index, CANLPH: CRP/albumin, neutrophil/lymphocyte, platelet/hemoglobin, PMBV: percutaneous mitral baloon valvuloplasty

Figure 1- Correlation of Echo score and CANLPH score

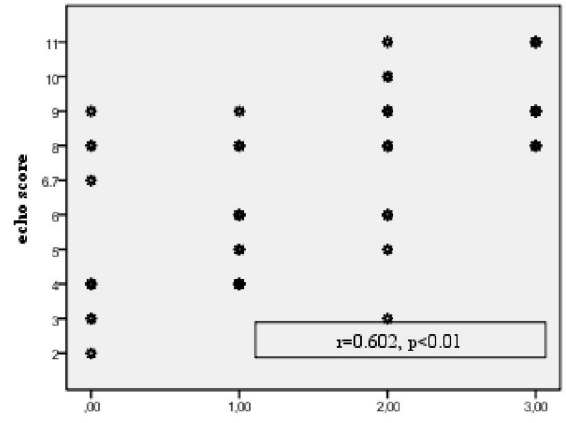


Figure 2- Correlation of Echo score and SII

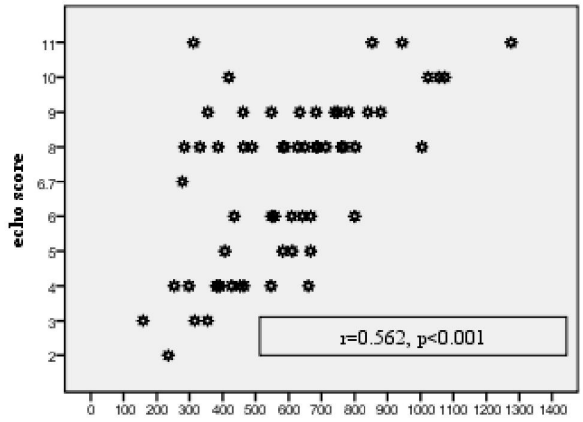


Figure 3- Correlations of Amean gradient (%) and CANLPH score

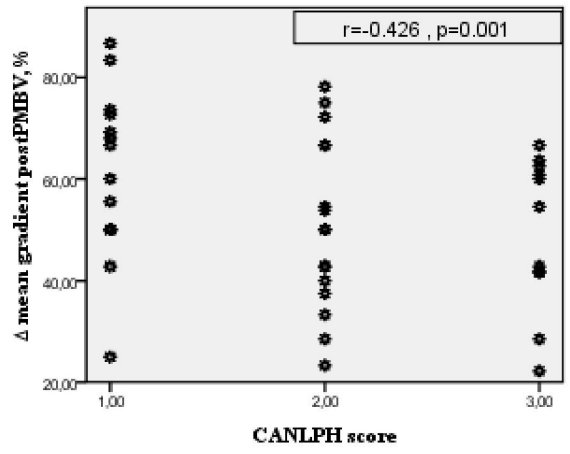


Figure 4- Correlations of Amean gradient (%) and SII

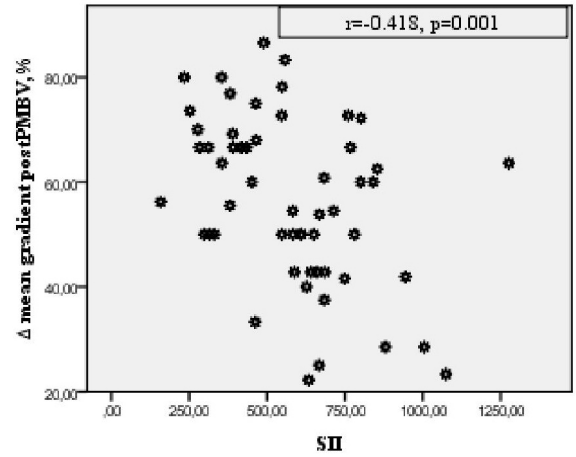
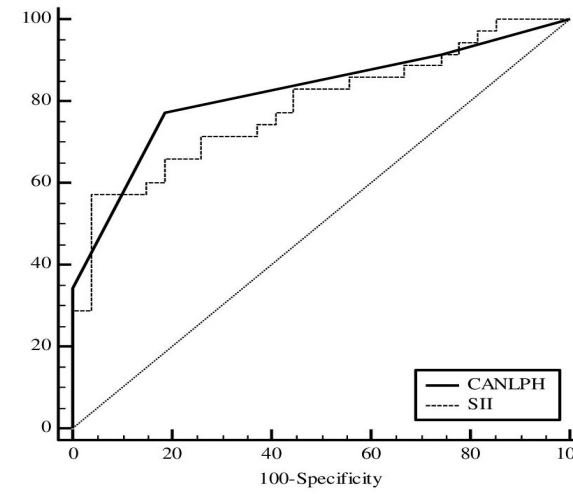


Figure 5- ROC curve analysis of SII and CANLPH score



Pairwise comparison of ROC curve analysis for determining the predictive value of higher Echo scores revealed that the CANPLH score was noninferior to SII with an AUC: 0.820 (0.701-0.906) and AUC: 0.786 (0.664-0.880), difference between AUC:0.034, z statistics 0.576 and p=0.564 (Figure 5).

DISCUSSION

The main results of this study were: 1) the increase in Echo score was correlated with the increase in inflammatory biomarkers such as CRP, NLR, and CAR, 2) new markers as SII and CANLPH score were also associated with the Echo score, 3) the relationship between the CANLPH score and the MS echo score was further, and this result is also provided by the relation of mitral mean gradient decrease after PMBV, 4) Our study was the first to consider the SII and CANLPH scores in patients with symptomatic MS with PMBV indication, and showed that they are related with the Echo score and the success of the procedure.

There are many factors that affect the success of PMBV. These parameters include age, sex, echo score, presence of atrial fibrillation, right heart function and degree of pulmonary artery pressure, and biomarkers that determine the host's inflammation status, such as the NLR in recent studies.15,16 Krishnamoorthy and Dash suggested that advancing age reduces the immediate and late recovery of atrial contribution after MBV and younger patients, especially below 18, achieve better atrial filling.17 Most of the patients in our study were in their 40s. Therefore, it is possible to state that the probability of a complete recovery in atrial function in long-term follow-up is low compared to these data. It is controversial whether gender has a positive or negative effect on the success of the procedure.18,19 Although female gender was dominant in the patients in our study group; no gender-related differences were observed in echo score, SII and CANLPH score, or post-procedure gradient decrease. The echo score is considered to be the most important factor in the success of the procedure. In the chronic inflammatory process, proliferation and fibrosis formation are observed due to the increase of lymphocytes and fibroblasts in the valvular tissue. This factor, which affects the success of the percutaneous balloon procedure, is also directly related to the level of inflammation. There are many studies stating that success is low in patients with the score over 8, considering the components of the echo MV score. In the study of Ekinci et al., it was shown that the success rate was high in patients with echo scores between 9 and 11. 20 Mahfouz et al. also emphasized that the global echo-Doppler score correlated better with the success of the procedure than the classical echo score.21 In our study, successful application was also performed in patients with echo scores between 9 and 11. The presence of atrial fibrillation shows that the severity of MS is increased and the inflammation process is accelerated, and it has a negative effect on the success of the valvuloplasty. Since inflammatory markers were considered in this study, patients with atrial fibrillation were excluded in terms of possible bias. The increased pulmonary artery pressure before valvuloplasty is important both because it is an indicator of increased right ventricular afterload and has effect on the success of the procedure and the outcomes after the procedure. Studies have shown that performing valvuloplasty before pulmonary hypertension develops is more ap-

propriate for preserving right ventricular functions.22,23 Mean systolic arterial pressure in our patient group was over 40. Since we do not have long-term results, it is not possible to interpret how the increased pulmonary arterial pressure plays a role in the post-procedure clinic. We can only say that there was no negative effect on the success of the procedure, at least in the acute period.

Valvular diseases, especially rheumatic mitral stenosis and calcific aortic stenosis, are among the cardiac pathologies in which inflammation is detected most intensely. Erdogan and colleagues showed that SII is related to valve area and transaortic mean gradient. in aortic stenosis. In both rheumatic mitral stenosis and calcific aortic stenosis, a decrease in the inflammation level of the host was observed after treatment. Furthermore, it is known that plasma levels of many inflammatory cytokines decrease after PMBV. These data support that the severity of MS is related to the inflammatory state. The NLR is one of these parameters, and studies show that it correlates well with the severity of the MS.24 SII and CANLPH score are biomarkers that reflect systemic inflammation and in this paper, we revealed that both are correlated well with the Echo score and decrease in mean gradient after PMBV. CANLPH is a new biomarker and can be used as an SII equivalent in determining the host's inflammation status and can determine the success of the procedure used in MS.

Ethics Committee Approval: Ethics committee approval was obtained from Adana City Training and Research Hospital Clinical Research Ethics Committee (02.06.2021, approval number 1432).

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Original Research

Efficacy of Colistin Therapy in Patients with Hematological Malignancies: What if There is Colistin Resistance?

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ABSTRACT

Objective: The objective of this study was to evaluate the clinical efficacy and appropriateness of colistin therapy in patients with hematological malignancies.

Methods: Age, gender, type of hematologic malignancy, and potential carbapenem-resistant microorganism risk factors were all noted in this retrospective study. In empirical and agent-specific treatment groups, differences in demographic features, risk factors, treatment responses, and side effects were compared.

Results: Sixty-three patients were included, 54% were male, and the median age was 49. In the last three months, the hospitalization rate history was 68%, and four patients had a hospitalization history in the ICU. Carbapenem-resistant K. pneumoniae colonization was present in 22 patients (35%). Gram-negative microorganisms were isolated in 34 patients (54%). The carbapenem, quinolone, and colistin resistance rates were 82%, 76%, and 4% respectively. Clinical and microbiological response rates were 60% and 69%. 7 and 28-day mortality rates were 17% and 35%. There was no significant difference in demographic data and comorbidities in empirical (n=48) and agent-specific (n=15) treatment groups. The rate of carbapenem and glycopeptide treatments before colistin was higher in the empirical treatment group ($p = 0.004$; $p = 0.001$). The rate of starting combined antibiotics was higher in the empirical treatment group ($p = 0.016$). Two of the patients developed renal failure in the first week after treatment.

Conclusion: The use of empirical colistin may be unavoidable given the risk considerations. Shortly, colistin-resistant strains may also be a factor affecting treatment success negatively.

Keywords: Colistin, hematological malignancy, empirical treatment, carbapenem resistance, colistin resistance

INTRODUCTION

Patients with hematological malignancies are more susceptible to infections due to immunosuppression, neutropenia, long-term hospitalization, invasive procedures, bone marrow depression, and mucosal barrier impairment (1). Infections are a leading cause of morbidity and mortality in individuals with hematological malignancies who have received intensive treatment (2,3). Antibiotics may be overused or misused, resulting in the development of antibiotic-resistant bacteria (4,5). Pathogens include multidrug-resistant *Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia* posed a substantial risk in patients with hematological malignancies and who received hematopoietic stem cell transplantation (4,6).

Polymyxin B and Colistin are primarily used as last-resort antibiotics against Gram-negative bacteria that are resistant to other antibiotics. Colistin treatment is as effective and safe as beta-lactam antibiotics or fluoroquinolones in treating infections caused by MDR P. *aeruginosa*, according to recent trials in cancer patients (1). Colistin is typically administered to patients who have a proven or suspected MDR pathogen infection, as evidenced by signs and symptoms of sepsis during broad-spectrum antibiotic therapy and positive culture findings. The possibility of nephrotoxicity is concerning, especially when combined with other nephrotoxic medications. Kidney toxicity is reported to occur in a range of 0 to 50% of people. However, there is a limitation of data on colistin therapy and stem cell transplantation in patients with hematological malignancies (1,3). Although the use of colistin in empirical treatment reduces mortality in eligible patients, the drug's usage is limited because to potential adverse effects, pharmacological interactions, and the rapid development of resistance in gram-negative bacteria. In our hospital's intensive care units, where colistin is often administered, the frequency

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of colistin-resistant *A. baumannii* and *K. pneumonia* isolates has been progressively increasing over the years (7,8). As a consequence, in every department and for each antibiotic, the principles of rational antibiotic usage should be considered (9).

The objective of this study was to determine the clinical efficacy of colistin and whether it should be used as an empirical therapy in patients with hematological malignancies.

METHODS

Patients diagnosed with hematological malignancy and treated with colistin therapy in the Erciyes University Faculty of Medicine Hematology Clinic between January 1, 2019 and January 1, 2020 were included in this retrospective study. The patients' medical records were obtained from hospital information records and medical files.

Age, gender, type of hematologic malignancy, presence of neutropenia even before to colistin therapy, presence of rectal colonization due to Vancomycin-Resistant Enterococci (VRE) and Carbapenem-Resistant K. pneumonia, recent hospitalization in clinics and intensive care units, antibiotic use prior to colistin, and duration of antibacterial therapy were all recorded.

The results of blood cultures taken three days prior to the initiation of colistin therapy were analyzed, and the treatment was classed as either empirical or based on the isolated microorganism. The use of antibiotics in combination with colistin therapy was documented. The antibiotic susceptibility of microorganisms isolated following colistin therapy was documented. It was determined whether empirical colistin therapy was appropriate. The length of the treatment and the use of nephrotoxic medications in combination with the colistin treatment were recorded. C-reactive protein (CRP) levels were measured at the start, third, seventh, and end of treatment.

At the start of treatment, the seventh day of treatment, and the seventh day after treatment, creatinine, glomerular filtration rate (GFR), and potassium values were observed.

Antibiotic initiation criteria: An antipseudomonal antibiotic was started in patients who met the criteria for febrile neutropenia. Antibiotic treatments given to patients who did not receive a clinical response on the third day of treatment or to whom antibiotic changes were made according to the culture results were recorded.

Main Points:

- Infections are important in patients with hematological malignancies
- Antibiotic resistance is gradually increasing in causative microorganisms.
- Colistin can be used in empirical treatment in patients with risk factors
- The nephrotoxic effect of colistin treatment is reversible.
- Colistin resistant strains should also be kept in mind.

The patients who were started on colistin therapy were separated into two groups: those who received empirical treatment and those who received agent-specific treatment. The agent-specific therapy group included patients who had carbapenem-resistant gram-negative bacteria isolated in their blood culture before and on the day of treatment and were started on colistin treatment. In terms of demographic characteristics, risk factors, treatment responses, and side effects, statistical differences between the groups were compared. There were two outcomes in the study:

Clinical response: A clinical response was defined as a 25% decrease in CRP on the third day compared to the initial CRP value, or a decrease of more than 75% on the seventh day, fever response, or hemodynamic stability on the third day.

Nephrotoxicity: The occurrence of nephrotoxicity was classified using RIFFLE criteria based on GFR and creatinine levels.

The patients' fatalities were recorded on the seventh and 28th days.

Confirmation of carbapenem resistance: The BD Phoenix (Beckton Dickinson, USA) automated system was first used to identify isolates and determine antibiotic susceptibility. Kirby Bauer disc diffusion method was used to confirm carbapenem resistance, and liquid microdilution method, The SensiTitreTM system (Thermo Fisher Scientific) (Sensititre GNX3F plates (TREK Diagnostic Systems, Oakwood Village, Ohio)) was used to confirm colistin resistance.

Statistical Analysis

The data of our study was transferred to the SPSS 25.0 package program. Data analysis was done in the same program. Whether the distribution was normal or not would be evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. The parameters were evaluated with mean ± standard deviation or percentages according to their status. Student's t-test or Mann-Whitney U-test was used to analyze continuous parameters, and chi-square or Fisher's exact test was used for the analysis of categorical variables. Analyzes with a p-value of <0.05 were considered significant.

RESULTS

The study included 63 patients who underwent colistin therapy and were hospitalized in a hematology clinic due to hematological malignancy. The median age of the patients was 49 years, and 54 percent of them were men. Acute myeloid leukemia was the most frequent type of hematological malignancy (40%). The hospitalization rate in the last three months was 68 %, including four patients (6 percent) in the intensive care unit having previously been hospitalized. In periodic rectal screenings before treatment, Carbapenem-resistant K. pneumoniae colonization was found in 22 patients (35%), while VRE colonization was found in 13 patients (21%). In 76 percent of the patients, carbapenem was started before colistin, and in 73 %, glycopeptide therapy was started before colistin. A double antibiotic combination was used to treat 82 percent of the patients. Prior to beginning colistin therapy, the average duration of antibiotic treatment was 13 days (Table 1).

Table 1. Comparison of demographic characteristics, hematological malignancy, and risk factors for resistant bacteria, antibiotics combined with Colistin therapy, nephrotoxic agents, clinical response, microbiological response and mortality

Variables	All patients n=63	Empirical treatment n=48	Specific treatment n=15	p
Age (years)	49 (19–76)	48 (19–76)	52 (25–72)	0.955
Male (gender)	34 (54.0)	26 (54.2)	8 (53.3)	0.999
Hematological malignancy				
AML	25 (39.7)	19 (39.6)	6 (40.0)	0.999
Lymphoma	19 (30.2)	15 (31.3)	4 (26.7)	
MM	7 (11.1)	5 (10.4)	2 (13.3)	
ALL	4 (6.3)	3 (6.3)	1 (6.7)	
Other	8 (12.7)	6 (12.5)	2 (13.3)	
Presence of neutropenia	36 (57.1)	28 (58.3)	8 (53.3)	0.772
Colonization of carbapenem resistant Klebsiella pneumonia	22 (34.9)	18 (37.5)	4 (26.7)	0.544
Hospitalization for the last 3 months	43 (68.3)	34 (70.8)	9 (60.0)	0.528
ICU hospitalization for the last 3 months	4 (6.3)	2 (4.2)	2 (13.3)	0.238
Before Colistin				
Carbepenem treatment	48 (76.2)	41 (85.4)	7 (46.7)	**0.004**
Glycopeptide treatment	46 (73.0)	41 (85.4)	5 (33.3)	**0.001**
Combined antibiotic treatment	52 (82.5)	43 (89.6)	9 (60.0)	**0.016**
Duration of antimicrobial treatment (day)	13 (1–56)	14 (1–56)	7 (3–23)	0.071
Antibiotics combined with Colistin				
Carbapenem	49 (77.8)	38 (79.2)	11 (73.3)	0.725
Piperacillin tazobactam	5 (7.9)	2 (4.2)	3 (20.0)	0.083
Glycopeptide	43 (68.3)	37 (77.1)	6 (40.0)	**0.011**
Tigecycline	5 (7.9)	5 (10.4)	0 (0.0)	0.326
Quinolone	5 (7.9)	5 (10.4)	0 (0.0)	0.326
Triple combination therapy	50 (79.4)	42 (87.5)	8 (53.3)	**0.009**
Duration of colistin treatment	9 (2–39)	8 (3–30)	10 (2–39)	0.377
Gram negative bacteria isolation rate	38 (65.0)	23 (47.9)	15 (100)	0.137
Gram positive bacteria isolation rate	8 (12.7)	5 (10.4)	3 (20.0)	0.382
Concurrent cyclosporine therapy	3 (4.8)	2 (4.2)	1 (6.7)	0.564
Concurrent vancomycin therapy	28 (44.4)	25 (52.1)	3 (20.0)	**0.039**
Concurrent nephrotoxic therapy	28 (44.4)	25 (52.1)	3 (20.0)	**0.039**
Clinical response	38/58 (65)	29/43 (67.4)	9/15 (60.0)	0.999
Microbiological response	29/43 (69.0)	19/28 (67.9)	10/15 (75)	0.999
7-day mortality	11 (17.5)	9 (18.8)	2 (13.3)	0.999
28-day mortality	22 (34.9)	19 (39.6)	3 (20.0)	0.222

Values are expressed as n (%), mean ±SD or median (1st-3rd quartiles). AML, Acute myeloid leukemia; MM, multiple myeloma; ALL, Acute lymphoblastic leukemia; ICU, intensive care unit

Because the causative microorganism was isolated before colistin therapy, 15 individuals were treated with colistin based on their antibiotic susceptibility. In the other 48 patients, empirical treatment was started. Carbapenem (78%) and glycopeptide group antibiotics were the most commonly combined antibiotics with colistin (68 %). 79 percent of the patients underwent triple antibiotic combination therapy. In the culture results of 34 patients, Gram-negative bacteria were found (54 %). Carbapenem resistance was found in 82 percent of these isolates, fluoroquinolone resistance was identified in 76 %, piperacillin-tazobactam resistance was found in 68 %, and colistin resistance was found in 4%. (Figure 1). In 13% of the patients, Gram-positive bacteria were found. 44 percent of the patients had previously used nephrotoxic medications while on colistin therapy.

Vancomycin was the most commonly used nephrotoxic agent (44%), and three patients were on cyclosporine therapy. The Gram-positive bacteria isolated in the five patients in the empirical treatment group was used to evaluate clinical response in

the 58 patients. In 65 percent of the patients, a clinical response was obtained, and in 69 percent, a microbiological response was established. The mortality rates at 7 and 28 days were calculated to be 17% and 35%, respectively (Table 1). Figure 2 shows the change in CRP levels of the patients during colistin therapy. It was observed that the decrease in CRP level was slighter in the group in which colistin therapy was started empirically.

Table 2 shows the rate and severity of renal failure after colistin treatment. Nephrotoxicity was observed in 32 patients during the first week of treatment, 23 patients after treatment, and 20 patients in the first week after treatment. Renal failure occurred in two of the individuals.

Between patients who received empirical and agent-specific treatment, there was no significant difference in demographic data or co-morbid diseases. As compared to the other group, the rate of usage of carbapenem and glycopeptide before colistin was higher in the patient group who started empirical treatment ($p = 0.004$; $p = 0.001$). The group receiving empirical treatment had a higher rate of starting combination antibiotics (89% vs. 60%, $p = 0.016$). In the empirical treatment group, the use of glycopeptide antibiotics in combination with colistin was also found to be higher. The empirical treatment group also used more triple-combination antibiotics with colistin. There was no significant difference in clinical response, microbiological response, or mortality in the patients who received empirical and agent-specific treatment.

DISCUSSION

In this study, the rate of starting empirical colistin in the hematology clinics seems to be high. However, patients undergoing empirical treatment appear to have more than one risk factor for carbapenem-resistant Gram-negative microorganisms. In a meta-analysis involving 3627 patients from 16 clinical trials, risk factors for carbapenem-resistant K. pneumoniae were, corticosteroid use OR = 1.43, central catheter use (OR 2.3), previous antibiotic use (OR = 3.31), and exposure to carbapenems (OR = 4.01), aminoglycosides (OR = 2.05), glycopeptides (OR = 2.40), fluoroquinolones (OR = 2.28), and anti-pseudomonal penicillins (OR = 2.67) (10). In an observational retrospective study that analyzed risk factors for carbapenem-resistant K. pneumoniae bacteremia, the use of antibiotics and carbapenem in the previous

Figure 1. Antibiotic resistance rates in isolated Gram negative bacteria

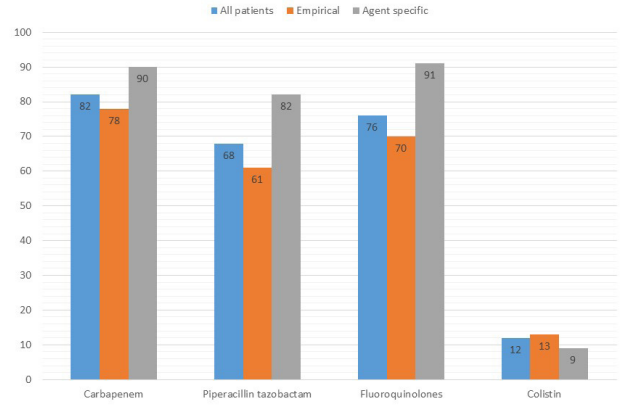

Figure 2. Change in CRP values before treatment, third, seventh and end of treatment

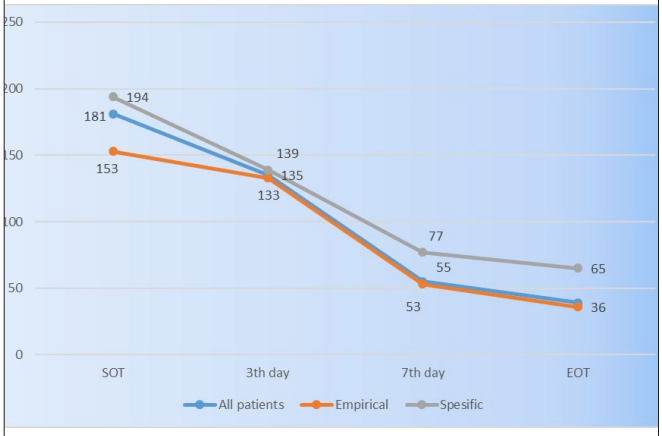

Table 2. Changes in renal function tests according to RIFLE criteria during and after Colistin therapy

	First week of treatment n=32	End of treatment n=23	First week after treatment n=20
Risk (R)	18 (56.3)	4 (17.4)	14 (70)
Injury (I)	5 (15.6)	8 (34.8)	4 (20)
Failure (F)	8 (25.0)	10 (43.5)	2 (10)
Loss (L)	1 (3.1)	1 (4.3)	0 (0.0)
ESKD (E)	0 (0.0)	0 (0.0)	0 (0.0)

ESKD, end stage kidney disease

30 days was reported as a risk factor for carbapenem-resistant K. pneumoniae bacteremia (11). When the risk factors for mortality were evaluated in the same study, inappropriate initiation of empirical antibiotic treatment was found to be a risk factor (11). Hospitalization in the previous three months (68 %), neutropenia (58 %), and carbapenem use (76%) were all risk factors for hospitalization in the patients in this study. In the group that received empirical Colistin, the rate of initiating carbapenem and glycopeptide before Colistin was higher. Because the infectious agent could be MDR or XDR gram-negative bacteria, colistin therapy was selected.

Due to factors such as the patients' malignancies and their severe immunosuppressive treatment, hematology units are departments where empirical antibiotic treatment should be chosen carefully. The colistin-resistant gram-negative bacteria was isolated in four patients in this study. The causative microorganism was isolated at the beginning of treatment in one of these patients. The other three, on the other hand, had ineffective empirical treatment. Patients with colistin-resistant gram-negative isolates had a higher mortality rate (75%) due to a lack of empirical treatment. When the entire patient group in the trial was reviewed, 28-day mortality was found to be 35%, while 28-day mortality was determined to be 40% in patients who were given Colistin as an empirical treatment. The 28-day mortality rate in a study involving carbapenem-resistant A. baumannii as a nosocomial infection agent was calculated to be 52 percent (12), whereas it is 45 percent (13) in P. aeruginosa infections and up to 75 % in K. pneumoniae infections (14). Mortality rates may vary according to the patients' co-morbid diseases and the causative microorganisms (15). In the Extensively-drug resistant A. baumannii bacteremia epidemic reported in our hematology unit in 2012, the mortality rate in 28 patients was reported as 82% (16). Since the carbapenem resistance in Gram-negative bacteria isolated in the patient group included in the study was at a high level of 82%, it seems appropriate to prefer colistin in empirical treatment.

A nephrotoxic agent was co-administered with colistin therapy in % of the patients. Vancomycin was the most common of these medications. In vitro studies have shown that combining colistin and vancomycin had a synergistic impact in colistin-resistant A. baumannii isolates (17,18). Other than colistin and vancomycin, however, nephrotoxic treatment, such as chemotherapy protocols, antibacterial, antiviral, and antifungal prophylaxis, is frequent in this patient population. Nephrotoxicity in this patient group should be closely monitored. In a study comparing nephrotoxicity in 26 patients with hematological malignancies who got colistin therapy to a control group who did not receive colistin therapy, it was found that there was no significant difference in terms of side effects (19). During the treatment, different levels of renal failure were detected in 40% of the participants in this study. However, one week after the treatment was stopped, all of the patients' renal functions improved.

CONCLUSION

Infections in patients with hematological malignancies must be treated with suitable empirical medication immediately away. When beginning empirical treatment, risk factors for probable factors should be considered. In our hospital's hematology unit, empirical initiation of colistin therapy in suitable patients seems to be effective and safe. Colistin-resistant strains, on the other side, may have an immediate negative impact on treatment success.

Ethics Committee Approval: Ethics committee approval was received for this study from the Noninvasive Clinical Research Ethics Committee of Erciyes University (Approval date: 25.12.2019; approval number: 2019/883).

Peer-review: Externally peer-reviewed.

Competing interest for all authors: No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article. The authors declare that they have no relevant conflict of interest.

Author's contributions: ZT, GKU, HNK; performed the analysis and collect the data, ZT, HNK; collect the patient data, ZT; wrote the paper, ZT, AUK,MK critised and edited the paper.

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Original Research

Utilization of Injectable Drugs for Communicable and Non-communicable Diseases in Primary Healthcare: A Retrospective Study in Turkey

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ABSTRACT

Objective: Primary care, which is often the first level of contact for patients with various communicable diseases (CDs) and non-communicable diseases (NCDs), might exhibit patterns of injectable drug utilization different from hospitals. We aimed to examine injection prescribing to adults with CD or NCD in primary care.

Methods: In this retrospective study, single-diagnosis injectable drug-containing prescription data from Family Medicine Information System comprising 32 provinces of Turkey were analysed. The prescriptions were grouped by diagnosis as "CD" (n=3848) and "NCD" (n=9338). Injectable drug utilization patterns in these groups were analysed by demographics, diagnoses, and drug subgroups.

Results: Out of 13186 prescriptions, 70.8% were issued for NCDs. NCD prescriptions were mostly generated for women and elderly ($p<0.05$ for both). About 63.3% (n=2948) of injectable drugs in CD prescriptions were antibiotics and 12.6% were analgesics. Cefazolin (15.2%) was the most commonly prescribed antibiotic for acute pharyngitis and acute sinusitis, and benzathine benzylpenicillin (12.8%) was the top-choice for acute tonsillitis and rheumatic fever. In NCD prescriptions, 34.0% (n=4214) of injectable agents were analgesics and 16.9% were muscle relaxants. The most frequently encountered drug in NCD prescriptions was thiocolchicoside (16.3%), which was the top-choice in all seven common musculoskeletal diagnoses.

Conclusion: Muscle relaxants and analgesics were the most commonly prescribed injectable drugs for NCDs, musculoskeletal diseases in particular. Antibiotics were frequently encountered in CD prescriptions, mostly as broad-spectrum for lower respiratory tract infections (RTIs) and narrow-spectrum for upper RTIs. These findings may elucidate the issues to especially focus on regarding excessive use of injections.

Keywords: injectables, antibiotics, analgesics, primary care, thiocolchicoside.

INTRODUCTION

Parenteral route of drug administration provides precise dosing and produces better bioavailability and faster effect. Besides, it also has remarkable disadvantages such as higher cost, requirement of experienced staff and essential equipment, and increased risk of adverse outcomes such as injection site reactions including infections.[1] Thus, parenteral drugs should be preferred in cases where the need and the superiority of the parenteral route over the enteral is clearly demonstrated.[2] According to the World Health Organization (WHO), overuse of injections when oral formulations would be more appropriate is a common example of irrational drug use. In this respect, WHO included the percentage of prescriptions with an injectable drug as one of the prescribing indicators to measure the degree of irrational drug use.[3] A previous study in Turkey reported that approximately 10% of the prescriptions collected from different levels of healthcare institutions contained an injectable preparation, which was similar to the median of Middle East and North African countries, and lower than that of European and Central Asian countries.[4,5] Injectable drugs can be preferred for the treatment of both communicable diseases (CDs) and noncommunicable diseases (NCDs), which can affect a large number of people in the world. The estimated worldwide prevalence and incidence of CDs, ma-

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Table 1. Comparison of prescriptions containing communicable and noncommunicable disease diagnosis by patient gender, age, season and SEDI category.

		Total (n=13186) (n) (%)	CD (n=3848) (n) (%)	NCD (n=9338) (n) (%)	p-value
Gender	Male	5205 (39.5)	1591 (41.3)	3614 (38.7)	0.005
	Female	7981(60.5)	2257 (58.7)	5724 (61.3)	
Age	18-44*	4862 (36.9)	1841 (47.8)	3021 (32.4)	<0.001*
	45-64	4849 (36.8)	1147 (29.9)	3702 (39.6)	
	≥65	3475 (26.3)	860 (22.3)	2615 (28.0)	
Season	Winter	3314 (25.1)	1049 (27.3)	2265 (24.3)	<0.001
	Spring	3241 (24.6)	897 (23.3)	2344 (25.1)	
	Summer	3482 (26.4)	773 (20.1)	2709 (29.0)	
	Autumn	3149 (23.9)	1129 (29.3)	2020 (21.6)	
SEDI	Above average	5363 (40.7)	1650 (42.9)	3713 (39.8)	0.001
	Below average	7823 (59.3)	2198 (57.1)	5625 (60.2)	

** The difference between the groups was due to 18-44 age group.*

CD: Communicable diseases, NCD: Noncommunicable diseases, SEDI: Socio-economic development index.

ternal, neonatal and nutritional diseases in 2017 were reported as 4.8 billion and 27.2 billion, respectively, and these were 7 billion and 10.8 billion for NCDs, respectively.[6] NCDs and their effects are more prominent in developed and developing countries.[6,7] Primary health care centres are often the first level of contact for patients with various CDs and NCDs, and they provide the care needed for the diagnosis and treatment of these diseases.[8] The prescribing practices for injectable drugs can also be different in primary health care centres than those in hospitals due to various factors including the inadequate physical infrastructure of primary health care centres (i.e. the absence of emergency and inpatient services) and patients' reasons of visits (i.e. mostly uncomplicated cases).[9] Hence, studies investigating the prescribing practices of injectable medicines for CDs and NCDs in primary care might identify problems for the treatment of these diseases and guide for implementation of appropriate interventions. Besides, the results of these studies might provide important clues about the frequency, severity, and treatment burden of these diseases. In this study, we aimed to examine injectable drug prescribing to adults with CD or NCD in primary care.

METHODS

In this retrospective cross-sectional study, data of injectable drug-containing prescriptions issued throughout the year of 2010 from Family Medicine Information System (FMIS) were used. Per national legislation of Turkey, ethical approval was not required for this retrospective study using anonymized data.

FMIS was an electronic prescription database designed for monitoring and evaluation of prescriptions issued by the family physicians and providing feedback to the prescribers.[10] Out of 33 provinces of Turkey selected as pilot regions to collect data for FMIS, 32 provinces with available primary care prescription data for the whole year of 2010 were included in this study. The dataset included 38400 prescriptions, for which 100 injectable drug-containing prescriptions issued in each month of the year were selected by simple random sampling for each province. Among the prescriptions generated for ≥18 years (n=32953), those with single diagnosis (n=15574) were selected. These prescriptions were classified by diagnosis based on the Global Burden of Disease Study 2017, which assessed the disease and injury-related burden.[6] In that study, the conditions related to disease burden were classified as "communicable, maternal, neonatal and nutritional diseases", "NCD" and "injuries" according to International Classification of Diagnosis-10 (ICD-10) codes.[6] After that, we excluded prescriptions with a diagnosis of any maternal, neonatal or nutritional disease, or injury. Accordingly, the remaining 13186 prescriptions were included in the study, 3848 of which were in "CD group" and 9338 were in "NCD group".

The prescriptions in both groups were compared by age and gender of the patients, injectable drugs included, month of issue, and the socio-economic development index (SEDI-2011) category of the provinces they issued in. SEDI-2011 is an index in which the socioeconomic development status of 81 provinces in Turkey are evaluated using 61 different indicators under eight domains as demography, employment, education, health, competitive and innovative capacity, finance, accessibility, and quality of life.[11] Before evaluation, we categorized the provinces as "above average" and "below average" according to the SEDI values. Moreover, the most common ten diagnoses in CD and NCD groups, and the top five injectable drugs prescribed for each of those were examined. The drugs were analysed according to the fifth level of Anatomical Therapeutic Chemical (ATC) classification.[12]

Table 2. Distribution of the most frequently encountered ten diagnoses in prescriptions issued for communicable and noncommunicable diseases.

CD prescriptions		NCD prescriptions	
Diagnosis (ICD-10)	n (%)	Diagnosis (ICD-10)	n (%)
Acute tonsillitis (J03)	1133 (29.4)	Diabetes mellitus (E10-E14)	1355 (14.5)
Acute bronchitis (J20)	823 (21.4)	Other arthrosis (M19)	1271 (13.6)
Acute pharyngitis (J02)	463 (12.0)	Dorsalgia (M54)	676 (7.3)
Viral immunization (Z25)	445 (11.6)	Other arthritis (M13)	577 (6.2)
Acute sinusitis (J01)	188 (4.9)	Pain, not elsewhere classified (R52)	517 (5.5)
Unspecified upper RTI (J06)	178 (4.7)	Gonarthrosis (M17)	246 (2.6)
Unspecified lower RTI (J22)	109 (2.8)	Schizophrenia (F20)	192 (2.1)
Infectious diarrhea and gastroenteritis (A09)	103 (2.7)	Biomechanical lesions, not elsewhere classified (M99)	180 (1.9)
Acute bronchiolitis (J21)	67 (1.7)	Cystitis (N30)	177 (1.9)
Rheumatic fever without mention of heart involvement (I00)	64 (1.7)	Other soft tissue disorders, not elsewhere classified (M79)	176 (1.9)
Others	275 (7.1)	Others	3971 (42.5)
Total	3848 (100.0)	Total	9338 (100.0)

CD: Communicable diseases, NCD: Noncommunicable diseases, ICD-10: International Classification of Diseases-10, RTI: Respiratory tract infection.

Statistical Analysis

Data were analysed using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA) software. Results were expressed as numbers, percentages, or mean ± standard deviation values. Frequency analysis was used for statistical evaluation. Categorical variables were compared by chi-square and Student t-test were used for comparison of continuous variables. P-value under 0.05 was inferred as statistically significant.

Main Points:

- Seven out of 10 prescriptions were issued for NCDs, which were mostly generated for women and older patients.
- Near two-thirds (63.3%) of injectable drugs in CD prescriptions were antibiotics and 34.0% of injectable agents were analgesics in NCD prescriptions.
- Antibiotics were frequently encountered in CD prescriptions, being mostly as broad-spectrum for lower respiratory tract infections (RTIs) and narrow-spectrum for upper RTIs.
- The most frequently prescribed injectable drug in NCD prescriptions was thiocolchicoside (16.3%), which was the top-choice in all seven common musculoskeletal diagnoses.
- This study may provide guidance in determining the roadmap of any intervention to limit unnecessary use of injectable agents.

RESULTS

A total of 13186 single-diagnosis prescriptions included in the study comprised 34.3% of all injectable drug prescriptions in the FMIS-derived database. Among these, 29.2% were issued for CDs, while 70.8% were for NCDs. Women made up 60.5% for whom the prescriptions were generated, and this was significantly higher in NCD group (61.3% vs. 58.7% in CD, $p<0.05$). The mean age of recipients was lower in CD prescriptions (47.2±18.3) compared to that in NCD prescriptions (52.9±16.9), ($p<0.05$). Patients aged 18-44 years comprised a higher proportion in CD group (47.8%) when compared to NCD group (32.4%), ($p<0.001$). Prescriptions were mostly written in provinces that were "below average" (59.3%) in terms of SEDI category. NCD prescriptions were more common in those provinces (60.2%) compared to CD prescriptions (57.1%), ($p=0.001$), (Table 1). The highest number of prescriptions were issued in August (8.9%), whereas the months with the fewest prescriptions issued were May, October, and November (7.9%). Prescribing was most common in October (11.1%) and January (9.5%) in CD group and in August (9.9%) and June (9.6%) in NCD group (Figure 1).

The most common diagnoses in prescriptions were acute tonsillitis (29.4%), acute bronchitis (21.4%), and acute pharyngitis (12.0%) in CD group, while these were diabetes (14.5%), other arthroses (13.6%), and dorsalgia (7.3%) in NCD group (Table 2).

In CD group, 63.3% (n=2948) of injectable drugs were antibiotics and 12.6% were analgesics. Nine of the 20 most commonly encountered injectable drugs in CD prescriptions

Table 3. Distribution of the most frequently prescribed drugs for the top ten diagnoses in prescriptions with a diagnosis of communicable disease.

Rank	Diagnosis (total number of drugs on prescriptions)	1st drug (n) (%)	2nd drug (n) (%)	3rd drug (n) (%)	4th drug (n) (%)	5th drug (n) (%)
1	**Acute tonsillitis (n=1387)**	Benzathine penicillin (422) (30.4)	Cefazolin (229) (16.5)	Procaine penicillin (204) (14.7)	Metamizole (136) (9.8)	Ceftriaxone (136) (9.8)
2	**Acute bronchitis (n=1008)**	Ceftriaxone (458) (45.4)	Cefuroxime (154) (15.2)	Metamizole (91) (9.0)	Methylprednisolone (65) (6.4)	Ampicillin and beta lactamase inhibitor (54) (5.4)
3	**Acute pharyngitis (n=575)**	Cefazolin (126) (21.9)	Metamizole (86) (14.9)	Benzathine penicillin (85) (14.8)	Cefuroxime (53) (9.2)	Ceftriaxone (38) (6.6)
4	**Viral immunization (n=455)**	Influenza vaccine* (442) (97.1)	Pneumococcal vaccine$^{\delta}$ (12) (2.6)	Diclofenac (1) (0.2)	-	-
5	**Acute sinusitis (n=231)**	Cefazolin (43) (18.6)	Metamizole (36) (15.5)	Cefuroxime (32) (13.8)	Ceftriaxone (28) (12.1)	Lincomycin (26) (11.2)
6	**Unspecified upper RTI (n=227)**	Metamizole (39) (17.1)	Cefazolin (36) (15.8)	Benzathine penicillin (20) (8.8)	Ceftriaxone (20) (8.8)	Lincomycin (15) (6.6)
7	**Unspecified lower RTI (n=121)**	Ceftriaxone (33) (27.2)	Cefuroxime (28) (23.1)	Cefazolin (24) (19.8)	Methylprednisolone (6) (5.0)	Metamizole (6) (5.0)
8	**Infectious diarrhea and gastroenteritis (n=172)**	Sodium chloride (31) (18.0)	Metoclopramide (16) (9.3)	Electrolyte combinations (15) (8.7)	Metronidazole (13) (7.7)	Vitamin B complex (11) (6.4)
9	**Acute bronchiolitis (n=73)**	Ceftriaxone (21) (28.8)	Cefuroxime (14) (19.2)	Cefazolin (12) (16.4)	Methylprednisolone (9) (12.3)	Betamethasone (2) (2.7)
10	**Rheumatic fever without mention of heart involvement (n=70)**	Benzathine penicillin (48) (68.6)	Betamethasone (7) (10.0)	Meloxicam (3) (4.3)	Dexamethasone (2) (2.9)	Tenoxicam (2) (2.9)

** Inactivated purified antigen, $^{\delta}$ Purified polysaccharide antigens.*
RTI: Respiratory tract infection.
Color coding is used for visualization of the table. Green: Corticosteroids; Grey: Vaccines; Light Yellow: Electrolyte solutions and vitamins; Navy Blue: Analgesics; Orange: Antiinfectives; Red: Antivomiting agent; White: Blank.

were antibiotics, followed by analgesics, vaccines (flu and pneumococcal vaccines), and corticosteroids (Figure 2a). Thirty-four percent (n=4214) of the injectable drugs were analgesics and 16.9% were muscle relaxants in NCD group. Thirteen percent of NCD prescriptions included both analgesics and muscle relaxants, while only 1.8% included muscle relaxant as thiocolchicoside, without any concomitant analgesic. Of the 20 most commonly prescribed injectable drugs in NCD group, six were analgesics, five were insulins, and three were corticosteroids. Thiocolchicoside (16.3%) was the most frequently encountered injectable drug in NCD prescriptions (Figure 2b).

Antibiotics were the most commonly encountered injectable drugs for eight of the top ten diagnoses in the CD group. Ceftriaxone was the most frequently prescribed injectable drug for the diagnosis of acute bronchitis, acute bronchiolitis, and unspecified acute lower respiratory tract infections (RTIs). Benzathine benzylpenicillin was the top choice for acute tonsillitis and rheumatic fever without cardiac involvement, and cefazolin for acute pharyngitis and acute sinusitis (Table 3).

Thiocolchicoside was the most commonly prescribed injectable drug for seven of the ten most common diagnoses in NCD group. These prescriptions mainly included analgesics, with diclofenac being the most commonly preferred one, second to thiocolchicoside. All top five injections prescribed for diabetes treatment were insulin preparations (Table 4).

DISCUSSION

At least sixteen billion injections are administered worldwide every year. Ninety percent of those are given for therapeutic purposes, and in many cases, injections are reported to be overused despite available oral alternatives.[2] In this study, we examined the utilization of injectable drugs included in prescriptions with

Table 4. Distribution of the most frequently prescribed drugs for the top ten diagnoses in prescriptions with a diagnosis of noncommunicable disease.

Rank	Diagnosis (total number of drugs on prescriptions)	1st drug (n) (%)	2nd drug (n) (%)	3rd drug (n) (%)	4th drug (n) (%)	5th drug (n) (%)
1	**Diabetes mellitus (n=1540)**	Insulin aspart + protamine aspart (437) (28.4)	Insulin glargine (311) (20.2)	Insulin lispro (183) (11.9)	Insulin detemir (155) (10.1)	Insulin aspart (140) (9.1)
2	**Other arthrosis (n=1768)**	Thiocolchicoside (412) (23.3)	Diclofenac (341) (19.3)	Betamethasone (295) (16.7)	Tenoxicam (161) (9.1)	Etofenamate (139) (7.9)
3	**Dorsalgia (n=1048)**	Thiocolchicoside (329) (31.4)	Diclofenac (259) (24.7)	Etofenamate (89) (8.5)	Dexketoprofen (87) (8.3)	Betamethasone (76) (7.3)
4	**Other arthritis (n=793)**	Thiocolchicoside (179) (22.6)	Diclofenac (148) (18.7)	Betamethasone (125) (15.8)	Tenoxicam (62) (7.8)	Etofenamate (61) (7.7)
5	**Pain, not elsewhere classified (n=733)**	Thiocolchicoside (191) (26.1)	Diclofenac (180) (24.6)	Dexketoprofen (70) (9.5)	Etofenamate (61) (8.3)	Betamethasone (52) (7.1)
6	**Gonarthrosis (n=353)**	Thiocolchicoside (75) (21.2)	Betamethasone (67) (19.0)	Diclofenac (50) (14.2)	Tenoxicam (32) (9.1)	Etofenamate (26) (7.4)
7	**Schizophrenia (n=199)**	Zuclopenthixol (76) (38.2)	Risperidone (69) (34.7)	Fluphenazine (19) (9.5)	Biperiden (17) (8.5)	Flupenthixol (13) (6.5)
8	**Biomechanical lesions, not elsewhere classified (n=292)**	Thiocolchicoside (111) (38.0)	Diclofenac (101) (34.6)	Dexketoprofen (22) (7.5)	Etofenamate (15) (5.1)	Tenoxicam (13) (4.5)
9	**Cystitis (n=209)**	Gentamicin (70) (33.5)	Ceftriaxone (31) (14.8)	Diclofenac (20) (9.6)	Cefazolin (13) (6.2)	Metamizole (10) (4.8)
10	**Other soft tissue disorders, not elsewhere classified (n=246)**	Thiocolchicoside (72) (29.3)	Diclofenac (57) (23.2)	Dexketoprofen (29) (11.8)	Etofenamate (17) (6.9)	Tenoxicam (16) (6.5)

Color coding is used for visualization of the table. Blue: Muscle relaxant; Green: Corticosteroids; Navy Blue: Analgesics;

Orange: Antiinfectives; Pink: Antidiabetics; Yellow: Antipsychotics.

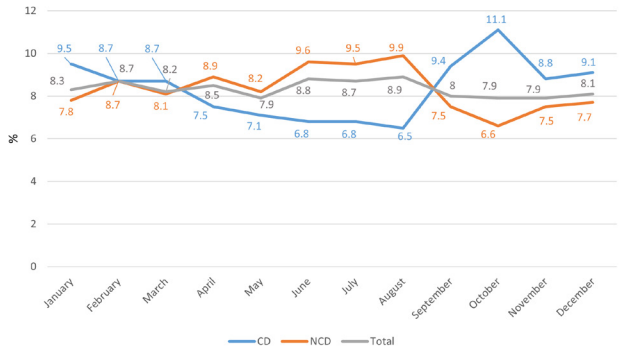
a CD or NCD diagnosis in primary care. Muscle relaxants and analgesics were common in NCD prescriptions due to high number of musculoskeletal diagnoses. Antibiotics were prescribed frequently for CDs, broad-spectrum ones mostly to treat lower RTIs and narrow-spectrum for upper RTIs.

Single-diagnosis prescriptions (n=15574) constituted 40.6% of all prescriptions with an injectable drug (n=38400). This number was lower than previously reported in a study (54.5%) examining prescriptions written in primary care in Turkey between 2013 and 2016, regardless of inclusion of any injectable drug.[13] This might be partly related to high number of concomitant diseases contributing to injectable drug preference. We also found that approximately three-fifths of the prescriptions (60.5%) were written to women, and this gender difference was slightly more pronounced (61.3%) in NCD group ($p<0.05$). That might be partly explained by gender differences in the utilization of healthcare services, as women are reported to be more likely to visit primary care in comparison to men.[14]

The Global Burden of Disease study (1990-2017) indicated that the burden of CD (along with maternal, neonatal, and nutritional diseases) has declined over the years, with being 46.4% of the disease burden in 1990, 34.0% in 2010 and 28.0% in 2017. On the other hand, the burden of NCD increased from 43.2% in 1990 to 55.4% in 2010 and 62.0% in 2017.[15,16] This shift was attributed to significant reduction in communicable and preventable diseases due to increased income and improved health and living standards.[16] The same study reported the burden of NCDs in Turkey as 80.9% and CDs along with other preventable diseases as 12.0% in 2010.[15,16] In our study, 70.8% of injectable drug-containing prescriptions had a diagnosis of NCD and the remainder (29.2%) included CD, which points out proportionally more common utilization of injectable drugs for CDs in primary care. "15-49 years old" reported to have the highest share of NCDs (33.3%) in Turkey in 2010, followed by "50-69 years old" (30.8%).[15,16] However, NCDs were most common in the "45-64 years old" age group (39.6%) in our study, which might be potentially related to the increased need for injectable drug use in relatively older NCD patients.

Approximately three-fifths of the prescriptions evaluated were issued in the provinces "below average" by SEDI category. The high rate of injectable drug prescribing in these provinces with low socioeconomic development indicates that regional differ-

Figure 1. Distribution of prescriptions with a diagnosis of communicable or non-communicable diseases by months. CD: Communicable diseases, NCD: Noncommunicable diseases.



ences should be taken into account when planning of regulatory interventions on promoting rational use of medicines. Several factors that can potentially show local variations such as level of healthcare institution, availability of resources, physician knowledge and attitude, and patient demands such as requesting injectable forms for faster relief might impact the practice of rational prescribing.[17,18] As for physician and patient-based issues, improved adherence to the guidelines as recommended by WHO might contribute to rational pharmacotherapy, as well as minimizing these regional differences.[3]

Around half of the injections on NCD prescriptions were analgesics (34.0%) or muscle relaxants (16.9%). Thiocolchicoside was present in one in six NCD prescriptions and also was the most common drug among all prescriptions, which indicates that primary care physicians tend to prescribe high numbers of injectable preparations of this drug. The potential safety issues of various muscle relaxants, including thiocolchicoside, had been questioned. Due to the concerns such as disruption in cell proliferation and increased risk of male infertility, teratogenicity, and cancer development, clinical usage of the drug was restricted in various countries, including Turkey, in recent years.[19,20] Although these safety precautions emerged after the data collection of the study, the results point out the risk of widespread usage of the drug. A Turkish nationwide drug utilization study from 2013 to 2016 showed that despite the declining trend, thiocolchicoside was generally among the most frequently prescribed drugs, which suggests possible overuse.[13] Guideline recommendations regarding the use of muscle relaxants for low back pain are conflicting and evidence of clinical benefit are uncertain.[21] Despite the lack of widespread recommendations, efforts of physicians to achieve faster patient relief or to address their demands might lead to irrational practices such as additional prescribing of a muscle relaxant when in fact unnecessary. Diclofenac was second after thiocolchicoside in almost all musculoskeletal system diagnoses, followed by other NSAIDs such as dexketoprofen and tenoxicam. Also, it was noteworthy that the most preferred injectable analgesic in CD prescriptions was metamizole (9.1%), which was approximately five times higher than diclofenac (1.9%). These results showed that primary care physicians mainly prescribed NSAIDs as injectable analgesics for musculoskele-

Figure 2a. Distribution of the most frequently encountered twenty drugs included in prescriptions for communicable diseases. Antibiotics in Others include streptomycin (0.5%), metronidazole (0.3%), amoxicillin (0.2%), rifamycin (0.2%), ampicillin (0.2%), amikacin (0.1%), thiamphenicol (<0.05%), benzylpenicillin (<0.05%), sulfamethoxazole and trimethoprim (<0.05%), moxifloxacin (<0.05%), amoxicillin and beta-lactamase inhibitor (<0.05%), cefotaxime (<0.05%), ceftazidime (<0.05%), and levofloxacin (<0.05%). Analgesics in Others include tenoxicam (0.3%), etofenamate (0.2%), meloxicam (0.2%), and lornoxicam (0.1%).

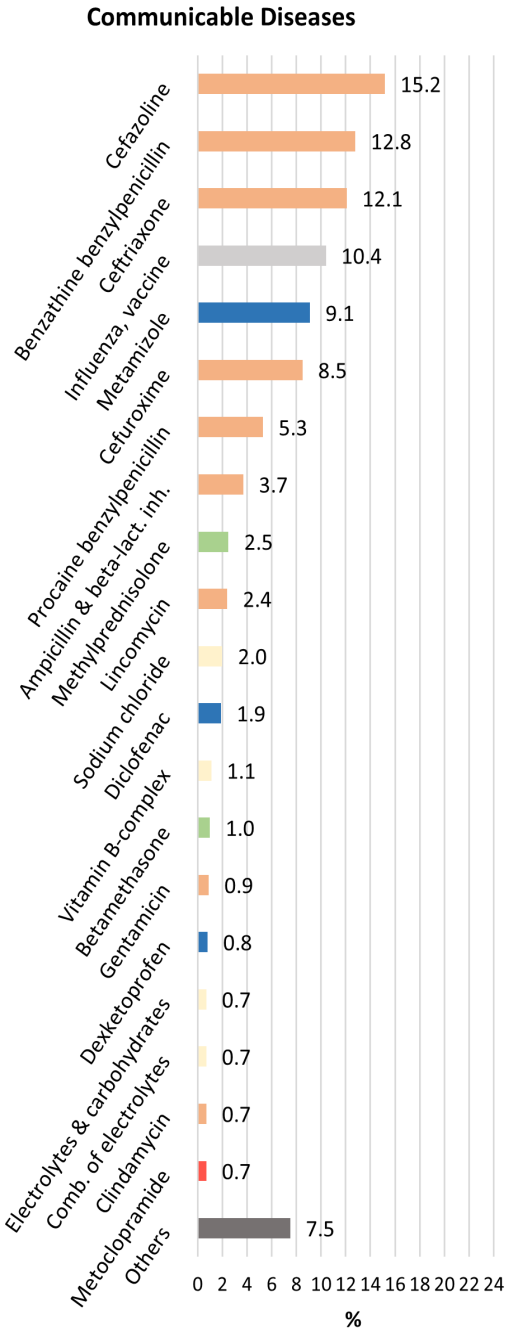
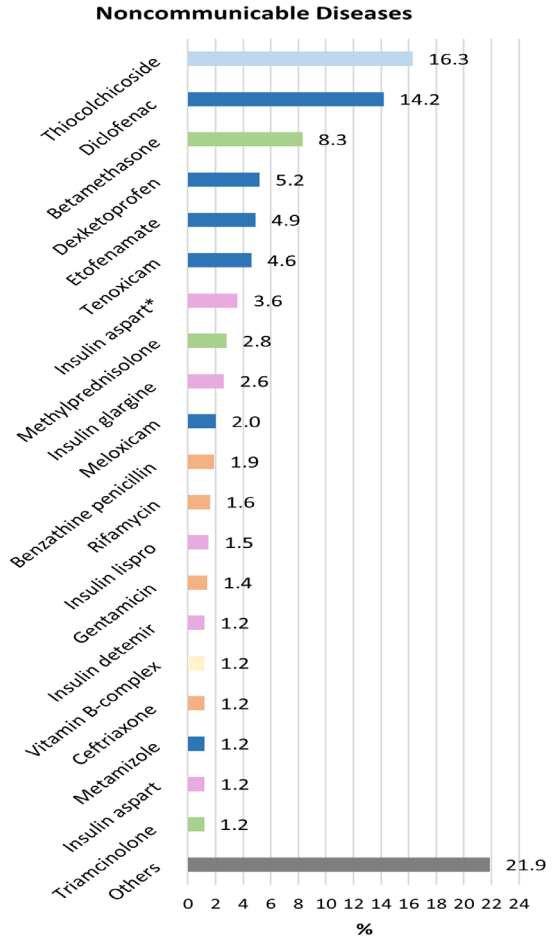


Figure 2b. Distribution of the most frequently encountered twenty drugs included in prescriptions for noncommunicable diseases. *: Insulin aspart and insulin aspart protamine. Analgesics in Others include lornoxicam (1.0%), ketoprofen (0.6%), pethidine (0.1%), tramadol (0.1%), morphine (<0.05%), and piroxicam (<0.05%). Muscle relaxant in Others was fenyramidol (0.6%).



tal system diseases, whereas other analgesic/antipyretics were mainly preferred for infectious diagnoses.

Cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes were reported among the major disorders responsible for 82% of deaths by NCDs.[22] In a study examining primary care prescriptions in Turkey between 2013 and 2016, hypertension was reported as the most common diagnosis, followed by myalgia and gastroesophageal reflux among other top ten NCDs.[13] In our study, the most common diagnosis was diabetes mellitus (14.5%). In 2010, prevalence of diabetes in adults was reported as 13.7%, and 13.6% of the patients with diabetes was using insulin.[23] Especially in the last decade, both insulin and the newer injectable agents such as glucagon-like peptide-1 analogues were reported to effectively provide glycemic control, and those agents were added to the recommendations for type 2 diabetes management in earlier stages.[24] In our study, the most commonly used injectable drug for diabetes treatment was insulin, however, considering up-to-date recommendations of current treatment guidelines, future studies may provide to what extent the newer injectable components of diabetes treatment have influenced the primary care practice.

In CD prescriptions, the most commonly encountered injectable drugs were antibiotics (63.3%) and analgesics (12.6%). Ceftriaxone was the most frequently prescribed antibiotics in the diagnosis of lower RTIs, whereas benzathine benzylpenicillin and cefazolin were the most frequently prescribed antibiotics in the diagnosis of acute tonsillitis, acute pharyngitis and acute sinusitis. A study from India conducted in 2013 reported that ceftriaxone was the most commonly used injectable antibiotic in tertiary care, followed by cefotaxime and amikacin.[25] Contrary to our finding, cefazolin was not among the first fifteen drugs, which might be due to different levels of health care setting and the type of infectious disease. A study from Italy reported that injectable forms prescribed by primary care physicians constituted 15% of antibiotic prescriptions in upper RTIs. Besides, cephalosporins, ceftriaxone in particular, were the most commonly prescribed antibiotics.[26] Upper RTIs are one of the common reasons for primary care visits in Turkey.[13] Considering injectable use of third generation cephalosporins could significantly contribute to the development of antimicrobial resistance, widespread prescribing of narrow-spectrum injectable agents such as cefazolin and benzathine penicillin for upper RTIs might be regarded as relatively rational.[27] However, ceftriaxone, a broad-spectrum antibiotic, was the third most commonly prescribed drug in the CD group (12.1%), which might be potentially remarkable in terms of inappropriate antibiotic use.

Between April and November, monthly distribution of prescriptions in CD and NCD groups showed discrete trends. Incidence of the common infectious diseases generally increases in the winter months.[28] Accordingly, the use of antibiotics also reported to peak in that season.[29,30] Our study revealed that injectable drug prescribing for CDs followed a similar trend. On the other hand, the decrease in NCD prescriptions during the autumn season needs further evaluation.

The findings of the study should be interpreted with their limitations. First, although the diagnoses in the prescriptions were assumed to be registered into the electronic system correctly, the physicians who generated the prescriptions might have preferred to register a more relevant diagnosis available in the system, instead of the actual diagnosis. Absence of any method to confirm the diagnosis on the prescription registry might be considered as a limitation. Second, we only analysed the prescriptions with a single diagnosis to assess drug choice for a specific diagnosis. Third, since the identity information of the patients cannot be accessed for ethical reasons, it was technically possible to have more than one prescription data of the same patient in the electronic database. Fourth, the study data covering the year of 2010 might not reflect the most recent changing trends, however, these potential differences might be limited as general clinical practice could be comparably less dynamic and slowly adapting field compared to many specialties of medicine

in terms of therapeutic approach. Lastly, the data reflected the practice in the first years of FMIS. The mass transition to electronic prescribing might have affected prescribing practices in different ways. On the other hand, the results of this study might give an insight for future studies considering the limited number of nationwide studies investigating injectable drug use.

CONCLUSION

This study revealed the details of injectable drug use in primary care in terms of prescribing preferences in CDs and NCDs. Muscle relaxants and analgesics were the most commonly prescribed injectable drugs for NCDs, musculoskeletal system diseases in particular. In prescriptions with CDs, broad-spectrum injectable antibiotics were commonly prescribed for lower RTIs and narrow-spectrum ones for upper RTIs. Thiocolchicoside, whose rationale for use in NCDs is in question, was remarkably the most commonly prescribed drug among all injectable drugs. On the other hand, considering high levels of excessive and inappropriate use of antibiotics in Turkey, preference of relatively narrow-spectrum injectable agents in this study suggests a rational prescribing practice. This study may shed light on the areas needed to be focused when proposing any interventions on limiting injection overprescribing, which is among the main principles of rational use of medicines.

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All authors read and approved the final version.

Ethical statement: The study described in this article was conducted within the framework of the Declaration of Helsinki. Per national legislation of Turkey, ethical approval was not required for this retrospective study using anonymized data.

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Original Research

Experience of Lung Surgery in the COVID-19 Pandemic

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ABSTRACT

Objective: During the pandemic, elective cases other than those requiring emergency thoracic surgery were postponed. Depending on the magnitude of the impact the pandemic posed on hospitals and clinics, there have been changes in the number and variety of cases of thoracic surgery. The intention behind conducting this study was to share the experiences gained by a thoracic surgery clinic during the pandemic period.

Methods: Altogether, 214 patients were included in the study. Patient data that were recorded included those on age, gender, lung pathology, duration of hospital stay, positivity for COVID-19, survival, and causes of death.

Results: Of the 214 patients operated on, 12 died during the postoperative period. Eight of these patients died due to their primary disease and one died due to gastrointestinal bleeding, whereas the remaining three patients died due to COVID-19 infection.

Conclusions: If opportunities and facilities favorable for the administration of surgical treatment are made available, surgical treatment services can be offered safely to all patients

Keywords: Lung cancer, covid-19, thoracic surgery

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 is a viral agent that can cause a broad spectrum of clinical symptoms ranging from cold-like symptoms to acute respiratory distress syndrome. COVID-19 originated in Wuhan, China, and spread throughout the world in 2019. It was later declared a pandemic by the World Health Organization. In Turkey, the first case of COVID-19 was detected in March 2020. At the beginning of the COVID-19 pandemic, various recommendations were published concerning thoracic surgery services. Delays and alternative therapies were proposed even for lung cancer surgeries worldwide [1-5].

During the pandemic, elective cases other than those requiring emergency thoracic surgery were postponed. Depending on the magnitude of the impact the pandemic posed on hospitals and clinics, there have been changes in the number and variety of cases of thoracic surgery.

Similar surgical restrictions and delays were experienced in our clinic during the pandemic. During the pandemic when the number of cases escalated, elective cases other than emergency surgical procedures and surgical treatment of cancer patients were postponed. As the staff at our clinic became more experienced in handling the pandemic, the variety of patients and surgical procedures increased and it was decided to resume surgeries in some elective cases.

This study therefore presents an evaluation of the thoracic surgeries performed at our clinic during the pandemic and their outcomes. The intention behind conducting this study was to share the experiences gained by a thoracic surgery clinic during the pandemic period.

METHODS

The cases of lung surgery operated during the pandemic at the Thoracic Surgery Clinic of Ankara Bilkent City Hospital were examined retrospectively. Ethical approval for this study was obtained from the ethics committee of Ankara Bilkent City Hospital (approval number E1-20-669). Altogether, 214 patients were included in the study. Patient data that were recorded included those on age, gender, lung pathology, duration of hospital stay, positivity for COVID-19, survival, and causes of death.

RESULTS

Overall, 214 patients were retrospectively examined in our study. Among all the patients, 63 (29.4%) were women and 151 (70.6%) were men. The patients had a mean age of 55.7 (12–82) years. Pathologies of the patients are shown in Table 1.

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Table 1: Distribution of Pathologies

Pathology	Number of Patients
Lung Malignancies	**133**
Pulmoner Metastasis	**15**
Others	**66**
Solitary Fibrous Tumor	2
Sclerosing Pneumocytoma	2
Synovial Sarcoma	1
Sequestration	1
Parenchymal Cavity	1
Organizing Pneumonia	2
Necrotizing Granulomatous Reaction/ Granulomatous Inflammation	3
Malignant Mesenchymal Tumor	3
Chondromyxoid Tumor	1
Lung Hydatid Cyst	11
Interstitial Lung Disease	7
Spindle Cell Mesenchymal Neoplasia	1
Hemoptysis (4 arteriovenous malformations, 1 Bronchiectasis)	5
Hamartoma	7
Hemothorax (Penetrating Injury)	
Fibrosis	1
Destroyed Lung	1
Bulla	12
Bronchiectasis	4

In our study, 62.1% (n = 133) of the patients who underwent surgical operation had primary lung malignancy, 7% (n = 15) had pulmonary metastasis, while 30.9% (n = 66) were operated for conditions other than malignancies.

The mean duration of hospital stay of the patients was 15.64 days (1–45 days).

Of the 214 patients operated on, 12 died during the postoperative period. Eight of these patients died due to their primary disease and one died due to gastrointestinal bleeding, whereas the remaining three patients died due to COVID-19 infection.

Two of the three patients who died due to COVID-19 infection were infected postoperatively during their stay at the thoracic surgery service, while the one remaining patient was infected in the early period after discharge.

DISCUSSION

During the COVID-19 pandemic, thoracic surgery clinics around the world developed various guidelines recommending different operational programs. Elective cases were postponed and emergency cases and cancer patients were prioritized. Guidelines have been published in the United States and Europe to assist physicians in deciding the lung cancer treatment to be administered during the pandemic [1,6-8].

Continuing thoracic oncological surgeries during the COVID-19 pandemic was found to be safe and feasible and it was not associated with an increased risk of postoperative complications or death [9]. In our clinic, depending on the severity of the pandemic conditions and the conditions at our hospital, many cases with

lung pathologies requiring surgical treatment were operated during the pandemic. Emergency cases and malignancies were prioritized. The perioperative COVID-19 testing and the isolated inpatient rooms and intensive care units helped prevent any potential increase in the risk of postoperative complications or deaths.

As more experience was mastered in managing the thoracic surgery procedures during the COVID-19 pandemic, the number of patients operated and interventions in terms of various lung pathologies increased over time. In Europe and Canada, the risk associated with COVID-19 appeared to be elevated; as a result, the number of surgically intervened cases was minimized, particularly in the field of oncology. In a multicenter prospective study that included 731 patients, it was concluded that maintaining surgical oncological activity during the COVID-19 pandemic was safe and feasible with an extremely low rate of postoperative morbidity or mortality 10. To ensure that all patients and pathologies are treated, our clinic applied triage in case of patients with lung pathology, primarily oncological and emergency patients. During the COVID-19 pandemic, hospitals gradually reduced the number of surgical procedures both to minimize disease transmission within the hospital and to protect the staff and available personal protective equipment and other resources needed for providing health-care services to patients with COVID-19. The same was noted in our hospital as well. While surgical procedures were initially performed only in prioritized cases, the rate of these procedures increased as more experience was mastered with the COVID-19 pandemic. Initially, patients to be operated were selected more rigorously. Even surgeries in patients with cancer were delayed. Depending on the inpatient and intensive care occupancy rates in our hospital due to the pandemic and the number of health-care staff available in COVID-19 clinics, our clinic decided to increase the number of patients to be operated whenever possible. It was also ensured that patients having non-malignant conditions who were supposed to receive surgical diagnosis/treatment received their treatment during this period, although it was not as early as in those patients with malignancies. During the pandemic, many recommendations were made regarding the preoperative period in patients with thoracic surgery [1,2,11]. It is aimed to protect patients from contracting the COVID-19 infection perioperatively. Preoperative polymerase chain reaction (PCR) testing for COVID-19 and PCR testing in the presence of suspicious clinical manifestations were recommended [12]. At our clinic, PCR testing for COVID-19 was performed both preoperatively in all patients and postoperatively in the presence of suspected clinical manifestation. Preoperative pulmonary function tests are considerably important in lung surgery. At the beginning of the pandemic, preoperative pulmonary function tests (PFTs) could not be performed at our hospital; the pulmonary function laboratory was in fact closed during this period. Other tests were employed to assess the respiratory performance of patients. Tests that were preferred included stair-climbing and 6-minute walk tests. Some studies have reported that the 6-minute walk test can correlate adequately with forced expiratory volume in 1 s and diffusing capacity of the lungs for carbon monoxide [12,13]. During this period when bronchoscopy units were closed, bronchoscopies for endobronchial lesion screening were performed in an operating room and only in selected patients whose PCR tests were negative. Overtime, during the pandemic, procedures, such as PFT, bronchoscopy, and preoperative echocardiography, were performed in patients whose PCR tests for COVID-19 were negative.

Among all the recommendations made, it was suggested that surgical treatment during the pandemic would pose a high risk to patients with locally advanced disease because they were predominantly of an advanced age and had comorbidities; therefore, non-surgical treatment methods should be applied to such patients [14]. We continued using the same approach as that previously applied to patients with locally advanced lung cancer at our clinic during the pandemic. Neoadjuvant treatments were administered in line with the oncological principles that were eventually established, and surgical treatment remained among the treatment procedures applied cautiously against the risk posed by the pandemic. To summarize, our clinical approach toward lung cancer has not changed during the pandemic and surgery was performed in patients who had a chance in surgery. In a study evaluating the safety of surgery during the pandemic, it was reported that all 93 patients operated for thoracic malignancies tested negative for COVID-19 in PCR tests conducted during the postoperative period [15]. Only 2 of the 214 patients in our study postoperatively tested positive for COVID-19 in PCR tests conducted during their hospital stay as inpatients. One of our patients contracted COVID-19 after discharge.

In our study, of the 12 patients who passed away, 3 died of COVID-19 infection. Two of these three patients were those who underwent surgery for lung cancer, whereas the remaining one patient was operated for non-malignant lung pathology.

CONCLUSION

Many reasons are involved in terms of difficulties experienced while applying surgical treatment methods during the pandemic. These include increased morbidity and mortality burden due to infection, difficulty in finding empty inpatient beds and intensive care unit beds in hospitals, and shortage of doctors and assisting staff.

If opportunities and facilities favorable for the administration of surgical treatment are made available, surgical treatment services can be offered safely to all patients, particularly to those with malignancies and emergency cases, provided that patients, relatives, doctors, and assisting health-care workers comply with all the measures in place during the pandemic.

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Original Research

The Change of Oral Presentations of National Anatomy Congresses Over the Years

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ABSTRACT

Introduction: In this study, it is aimed to examine the characteristics and publication rates of oral presentations presented in national congresses.

Methods: The data about the oral presentations in national anatomy congresses (from 2016 to 2021), including the number of contributing institutions, the institutions that contributed the most oral presentations each year, and the publication rates of the oral presentations in the journals scanned in different indexes. In the study, the titles and the keywords were scanned using Google Scholar and Web of Science, and journal index details were noted.

Results: Of the national congresses we examined in our study, the most oral presentations (193) were the congress in 2019 and the least oral presentations (47) were the congress in 2017. The publication rate of oral presentations was highest in the papers of the congress in 2016 (42%) and at least in the papers of the congress in 2019 (13%). None of the oral presentations in the last congress held in 2021 were published until the time of our study. Large proportions (34%) of the papers that are accepted as oral presentations and published are radiological studies. 70% of the publications were published in journals indexed in Web of Science.

Conclusion: For both new and senior academics, congresses offer a useful and distinctive setting. Congress papers showcase the scientific opportunities and interests of various universities while also providing opportunities for collaboration. Attendance at a conference is significantly influenced by its location, timing, and financial situation.

Keywords: National anatomy congresses, oral presentation, publication rate

INTRODUCTION

Congresses and symposiums are unique and important academic platforms where individuals or groups present their experiences and studies establish a cooperation and communication network with different people and institutions discover innovations and developments in the field, and thus new working ideas and projects emerge. [1-3] In particular, national meetings in the mother tongue of the participants are the events where the interaction takes place the most and the mutual benefit is the highest. National congresses also have a great advantage due to the fact that they are less costly and troublesome (possible problems with travel, accommodation and nutrition) compared to international congresses. [4]

On the other hand, within the framework of our country's academic policies[5-6], the higher scoring of international congresses, the increase in participation and accommodation fees due to the high cost of congresses held in the metropolitan cities of our country in recent years, the overlap of the dates of the congresses with the academic calendar or holidays, and the coronavirus (COVID-19) pandemic, which has affected our country as well as the whole world since March 2020, have adversely affected the participation to national congresses.[7-8]

Anatomical studies in Turkey have been examined bibliometrically in different researches before.[9-10] Bibliometric analysis of congresses is very important in terms of reflecting the scientific productivity of congresses and the preferences of researchers as well as providing an idea about the scientific characteristics of the congresses. This study is a kind of bibliometric examination of national anatomy congresses and a complement to the study in which the publications in our national Anatomy journal were examined.[9]

The aim of this study was how the negative impact of participation in national congresses affected the rate at which the papers presented in the congress, which symbolized the effectiveness and quality of the congresses, became publications in refereed journals. In this context, in our study, the national anatomy congresses of the last six years, including the pandemic period, and the oral presentations presented in these congresses were examined.

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Table 1. National anatomy congresses and oral presentations held between 2016-2021.

Congresses	Place	Number of Oral Presentations
2016 (17th National)	Eskisehir Osmangazi University, Eskisehir	48
2017 (18th National)	Taksim International Abant Hotel, Bolu	47
2018 (19th National)	Necmettin Erbakan University, Konya	176
2019 (20th National)	İstanbul Medipol University, İstanbul	193
2020 (21st National)	Ankara University, Online	94
2021 (22nd National)	Gazi and Yuksek Ihtisas Universities, Online	51

METHODS

PIn our study, six national congresses held between 2016 and 2021 and 558 oral presentations presented in these congresses were included. Although previous studies have stated that it takes five years for papers to turn into publication1, the average time to turn into publication in the same studies was found to be about twenty months. For this reason, congresses in 2020 and 2021 were also included in the study in order to examine the effects of the COVID-19 pandemic. Since an international congress (International Symposium of Morphological Sciences) was held in our country in 2015, the year 2016 was taken as the beginning because no national congress was held in 2015.

Each congress was evaluated in terms of accepted oral presentations and general characteristics (location, opportunities), and oral presentations were examined by factors such as field of study, publication rate, speed of return to publication and index of the journal in which it was published. We obtained the imprints of the papers presented in the congresses from the supplementary issues of Anatomy, an international journal of experimental and clinical anatomy, which is the publication organ of the Turkish Anatomy and Clinical Anatomy Association, and the ones for 2021 from the congress program.

Main Points:

- National congresses, where participation has decreased due to their low value in academic evaluations and the difficulties brought about by physical participation, can be re-popularized by internationalizing and conducting them online.
- The publication rate of oral presentations presented in Turkish National Anatomy congresses decreases every year. The fact that this is despite the increasing number of papers shows that academics should be encouraged to publish their work.
- The fact that 70% of the articles published after being presented in our national congresses are indexed in Web of Science shows that the studies are of high quality.

RESULTS

The national anatomy congresses we examined within the scope of our study and the number of oral presentations presented are given in Table 1. While approximately 48 oral presentations were included in the national congresses held in 2016 and 2017 in Table 1, the number of oral presentations increased two-and-a-half times due to the 1st International Mediterranean Congress of Anatomists held simultaneously with the national congress in 2018. In the congress held by Istanbul Medipol University in 2019, the highest number of oral presentations were seen with the acceptance of papers from clinical branches as well as facilities such as accommodation in student dormitories. Although the first congress of the pandemic period was held online within the framework of the measures, it was reduced by half compared to the previous congress, twice as many oral presentations were presented compared to our classical congresses (Table 1).

When the study areas of the oral presentations presented in national congresses were examined, it was seen that a large part (on average 30%) consisted of radiological studies (Figure 1). While radiological studies have maintained their popularity during the pandemic period, the rate of experimental studies has decreased from 27% to 5%. Reports on anatomy education reached 10% with the pandemic period (Figure 1).

Figure 1

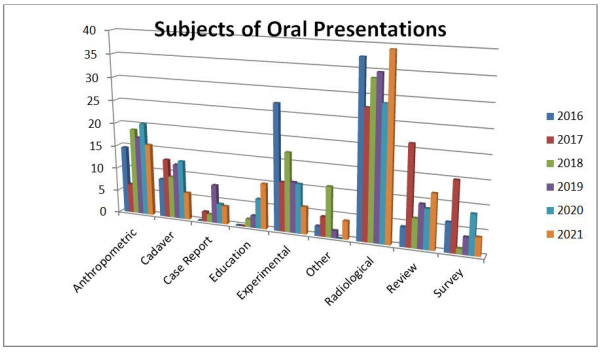

Table 2. Indices of the journals published by the oral presentations presented at the national anatomy congresses.

	2016	2017	2018	2019	2020
SCI-E	15	8	23	16	14
ESCI	3	2	9	1	2
Turkish Citation Index	0	2	14	4	4
EBSCO-SCOP-US-Medline	0	2	3	1	4
Google Scholar-DOAJ-Cross-Ref	1	0	4	1	0
Index Copernicus	1	0	2	3	0
TOTAL	20	14	55	26	24

SCI-E, Science Citation Index Expanded; ESCI, Emerging Source Science Citation Index

The rate of publication of oral presentations presented at national anatomy congresses has declined greatly over the years (Figure 2). While 42% of the oral presentations in the first congress we examined (held in 2016) were published in journals, none of the presentations in the last congress were published until the time our study was carried out (taking place in 2021). In the congress in 2019, where the most papers were presented, the publication rate was lower (13%) compared to other years (around 30%).

Figure 2

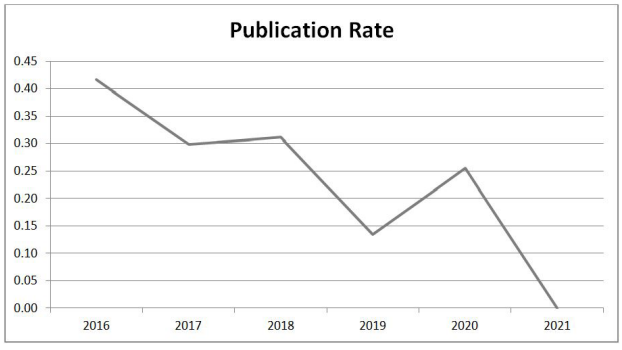

Figure 3

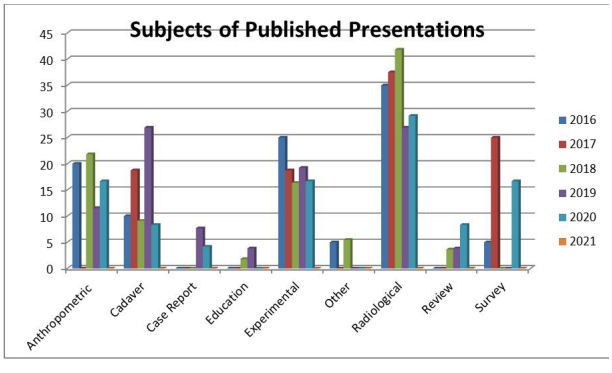

Table 3. The proportion of the indices (SCI) of the journals in which the papers presented at national congresses are published.

	2016	2017	2018	2019	2020	Average
SCI-E(%)	75	57.1	41.8	61.5	58.3	58.8
SCI-E+ESCI(%)	90	71.4	58.2	65.4	66.7	70.3

SCI-E, Science Citation Index Expanded; ESCI, Emerging Source Science Citation Index

When we examine the published oral presentations from the presented papers, radiological studies similarly constitute the vast majority (five-year average 34%). This was followed by experimental studies with an average of 19%, with the lowest rate of publication of educational studies (1.2%, Figure 3).

The indexes of the published papers and the journals in which they were published were examined and given in Table 2. It has been observed that most of the studies have been published in journals indexed in Science Citation Index –Expanded (SCI and SCI-E). Publication in Turkish Citation Index journals has become the second choice of authors.

When the proportions of the publications presented in the congresses and scanned in Web of Science were examined according to the years, it was seen that SCI-E journals had an average of approximately 59% and reached up to 70% with the Emerging Source Science Index (Table 3).

DISCUSSION

National congresses are unique and necessary environments for the sharing and development of academics who speak the same language. The quality of these congresses is determined by the quality of the papers presented in the congresses and, indirectly, by the transformation of these papers into publications in refereed journals. Gurses et al. (2017)[1] examined the national anatomy congresses held in 2007 and 2008, but it became necessary to carry out this study due to various developments in the intervening period and the limitations in the study.

Our study is also a complimentary work of Adanır et al.[9] which examines the published articles in the Anatomy journal bibliometrically. They investigated the articles published in the regular issues of the journal while we examined the abstracts usually published in the special issues of the journal.

Especially as a result of the changes made in the Academic Incentive Practice[5] that started in 2015, the preferences of academicians shifted to international congresses with the exclusion of national congresses from evaluation. In our study, it is seen in the "1st International Mediterranean Anatomy Congress" held simultaneously with the "19th National Anatomy Congress" held in Konya and the number of oral presentations increased from 47 in previous years to 176 (Table 1). As a result of the changes made by the Higher Education Institution in the criteria related to international congresses, it is not possible to continue international congresses for a certain field. As a result, academics have

found a solution in organizing and participating in international multidisciplinary congresses.[11]

Another obstacle to the participation of Turkish academics in the congresses is economic reasons.[11] The fact that universities do not able to provide congress participation support, transportation opportunities to the place where the congress is held, travel and accommodation costs affect the participation in the congress. While the participation in the national congress held in Abant in 2017 and the number of oral presentations presented relatively were low, the fact that the number of oral presentations in the 20th National Anatomy Congress in Istanbul in 2019, where Medipol University provided accommodation and transportation facilities, was 193 shows the importance of these factors.

Another important aspect of our study is the online activities that started with the coronavirus pandemic, which emerged in China at the end of 2019 and caused restrictions and closures in Turkey in March 2020. These activities, which were carried out using digital platforms to prevent the spread of the epidemic, allowed to overcome elements such as time and space and also significantly reduced economic problems and carbon footprint.[12] We observed the impact of this at the first online national anatomy congress in 2020 (94 oral presentations). The likely reason we won't see it at the convention in 2021 is that remote work slows down or interrupts scientific research.[13]

The subject distribution rates of the oral presentations in the congresses we examined in our study are given in Figure 1 and it is seen that the majority of the congresses consisted of radiological studies. Case report type papers are usually presented as posters and are seen in very small numbers as oral presentations. While the number of studies on anatomy education was low in the first years, it started to increase during the pandemic period. On the other hand, the number of papers of experimental studies has decreased over the years. The distribution in oral presentation rates was also parallel in the publications and the most publications came from the studies in the radiological field of study.
When the rate of conversion of oral presentations into publications, which is the main purpose of our study, is examined, the decrease has started with 42% in 2016, contrary to the previous study, has shown a tendency to decrease over the years (Figure 2). This rate, which ranged from 34% to 75% in previous studies, was found to be an average of 24% in our study, and was found to be 28% on average in case of exclusion of this congress due to the fact that none of the 2021 papers were published (Table 4). The fact that the publication rate is so low can be interpreted as a decrease in the quality of the papers presented in the congress. On the other hand, as emphasized in previous studies, it was proposed to pass five years from the date of the congress to determine the rate of publication of the papers[2], but since the publication rate was 20.94±16.66 months and the life of the scientific information was three years[14], it is thought that sufficient time has passed, including the 2020 congress. If the congresses in 2016 and 2017 are taken as a basis, the publication rate has increased to 36% and it is still less than half of result of the observation of Gürses et al. (2017)[1]. In our study, the publication time of the papers was found to be 18.04±4.1 months and it was found to be faster compared to other studies.

When the indices of the journals in which the papers published in our study were examined, an average of 58.8% was found in Science Citation Index-Expanded journals and 70.3% when Emerging Source Science Index journals were included. This result shows that the studies carried out and published by our academicians are very valuable.

Table 4. Publication rates of papers presented in national congresses of different disciplines

	Number of meetings included	Number of abstracts presented	Publication rates	Reference
Psychiatry	5	187	34%	Kahve et al (2020)[15]
Orthopaedics	1	770	44%	Yalcinkaya and Bagatur (2013)[16]
Endocrinology	3	161	68.4%	Sahin Ersoy et al (2015)[17]
Anatomy	2	342	75%	Gurses et al (2017)[1]
Anesthesiology and Reanimation	4	319	42.3%	Tok Cekmecelioglu (2019)[3]
Present study (Anatomy)	6(5†)	558	**24(28†)% (2016–2017, 36%‡)**	

† Since oral presentations in 2021 have not been published, the recalculated average.

‡ According to Gursel et al. (2017), the average publication rate of oral presentations from congresses five years ago.

When we look at the constraints related to our study, at first there is the difficulty of classification due to the fact that the study areas are intertwined. In addition, the fact that the Abstract Book of the National Anatomy Congress in 2021, the second national congress in the pandemic period, has not been published has caused difficulty in researching the papers examined.

Our study is original and important in that it includes online national congresses held during the pandemic period and shows the current status of oral presentations presented in national anatomy congresses. On the other hand, since the publication of papers as an article is a time-consuming process, re-examination can be made in the following years.

CONCLUSION

Examination of abstracts presented in national congress and their publication in later period gives an insight to the organising committees and the reviewers of the congress about choosing abstracts for oral presentations. The findings of this study are important for improving the scientific quality of national Anatomy congress of Turkey.

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Author's contributions: MO and YMD, design of the study; MO, collection of the data and performing analysis; MO and YMD, writing the article, reviewing and editing the final draft.

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Original Research

Digital Analysis of Soft Tissue Nasal Anatomy for Individual Treatment Planning

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ABSTRACT

Objective: Changing contour lines of the external nose following traumatic, aesthetic and tumour surgeries have become very trendy. The goal of this research is to study the several soft tissue landmarks, measurements (linear distances, ratios, angles) of the external nose and its nasal indicis using a computer program.

Methods: Face region were taken a photographs of the two hundred adults. Analyses of linear (the lengths of nares, nasal bridge, and columella and nose height, nares width) and angular analyses (angles of nasofrontal, nasolabial and nasal tip) were computed and averaged for gender with age.

As for the shape of the nose, it was categorized as subunits: nasal tip (sharp, normal, wide, protrusive and asymmetric), nasal base (normal, wide, asymmetric) nasal alae (normal, thick, thin, asymmetric), nares (normal, horizontal and asymmetric) and columella (normal, wide, short and bifid) nasal base, nares, nasal alae, columella and classified subunit as normal, protrusive, sharp, asymmetric, and wide.

Results: The nose height have to 49.05 ± 3.48 mm in young male adults, 50.37 ± 2.33 mm in young female adults. Distance lengthwise the nasal bridge have to 48.60 ± 3.24 in males, 37.09 ± 5.49 females. The two mean measured nasal lengths were significantly greater in men. At the same time, angular measurements for nasolabial and interalar were higher in males. Nasal tip angle was 127.47 ± 82.9° in males, 75.8° in females. On average, young male adults had larger nasal linear distances such as nasal bridge length, nares lengths and nares widths relation of height than young female adults ($p<0.01$); No gender differences were observed for columella widths and to nose height ratio ($p<0.01$). The nasofrontal, nasal tip, nasolabial and interalar angles showed statistically significant differences among young male adults and young female adults ($p<0.05$). The nasolabial angle exhibited considerable variability. The shape details of nares was showed large variability. Nasal base, nasal tip and nasal alae shapes were similar, nares asymmetry was more frequently compared with other features.

Conclusions: The Anatolian people's nose exhibits wide nasal tip, has a wider nasal base, and is more thicker at the alae, with wider definition of the columella. The significant gender differences of nasal shapes were found. The wide and sharp features of nasal tip were related to an important features in men, whereas asymmetric nares were dominant in young female adults. Using digitized reference details, this study helped define the best cosmetic surgery recreate the nose and increase the success of customized therapy. Also, our findings facial alteratios, facial reconstruction, personal identification, Trauma assessments may also have data banks based on age and gender.

Keywords: aesthetic surgery, columella, facial aesthetics, facial analysis, nose, nasal tip, nares, nasal alae.

INTRODUCTION

The nose is the most characteristic feature of the face and it can create a beauty of the face and attractive appearance to the face.[1,2] Since interest in cosmetic surgical procedures has increased in recent decades, a great deal of research has been done to examine and refine the cannons through which beauty can be measured.[2,3] The appearance of nose such as the size, protrusive shape, and asymmetry are important features of facial beauty (Fig. 1).[3,4-5] Definition of ideal nose changes as age, gender, ethnic, culture, and current fashions. The ideal definition is complete with a well-defined nasal end and with the right balance between the two nares.[6,7-8] The upper face anatomy has various relationships among of the cartilaginous and the osseous overlying skin.[9,10-11] Nasal reconstruction including congenital defects and secondary defects such as tumor resection or traumatic injury require redesigning with aesthetic and reconstructive approach.[12,13-14] Knowledge of nasal dimensions and form is essential for repairing and reconstructing an aesthetic nose. Various authors have included soft tissue parameters in photogrammetric analyses and various soft tissue facial analyses based on a standard photogrammetric approach. [15,16-17]

Quantitative practice as computerized photogrammetry and di-

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Figure 1. Views of frontal and basal soft tissue of nose.

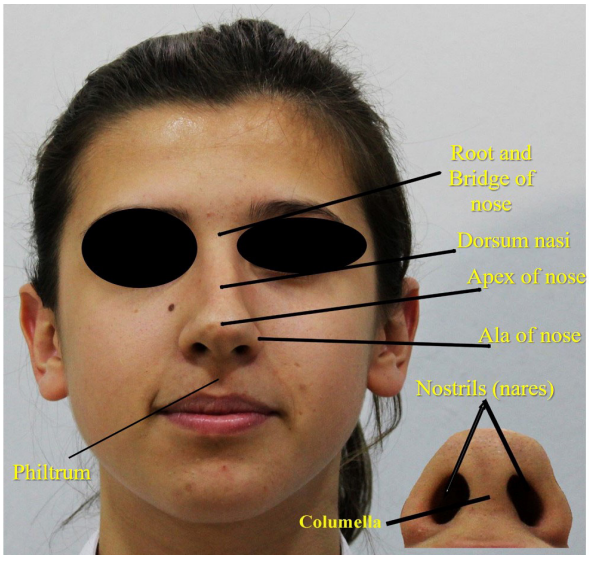


Figure 2. Frontal (**A**), lateral (**B**) views of some linear and angular measurements with soft tissue landmarks. glabella (**g**), nasion (**n**), pronasale (**prn**), subnasale (**sn**), ala (**al**).

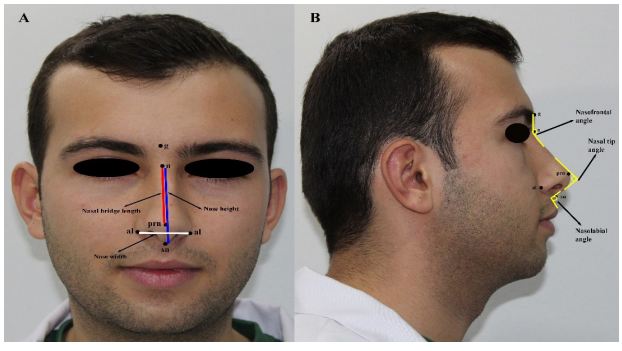
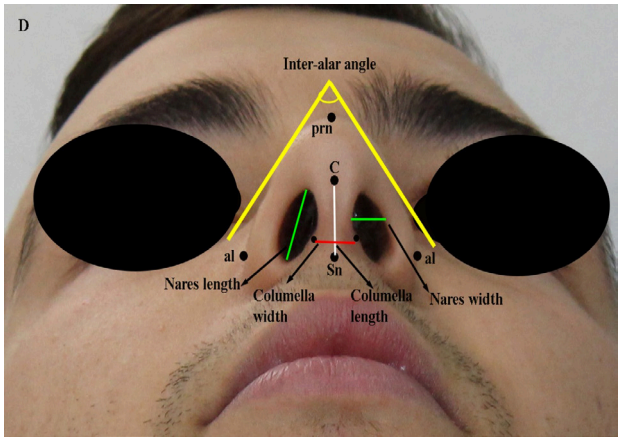


Figure 3. The nasal tip and columella in relation to the selected reference points: Linear and angular measurements. Columella ©, subnasale **(Sn)**, pronasale (**prn**), ala (**al**).



mensional imaging are utilized for analysis soft tissue of the nose with guide landmarks as the linear and angular measurements defining the patterns of nose.[18,19] It is important to save detailed nose measurements in order to plan an individual treatment protocol for each patient. The goal of this research (1) to apply digitalized reference norms from standardized photographs, (2) to find gender differences, and (3) to check against features of Anatolian patterns with that of other researchers studying nasal beauty.

METHODS

2.1 Subjects

100 young male adults and 100 young female adults (19 - 21 years old) no nose surgery, no traumatic effects and congenital syndromes to the nose were selected. The resulting demographics included age, birth rate and parental inheritance. This study was endorsed by duly incorporated individuals Ethical Committee at Research (Date: May 16, 2014, Decision no: 659/311). Each individual who participated in the study voluntarily declared to participate in the study by filling out the informed consent form.
2.2. Collection of nasal landmarks

Personal photographs were taken from the standard distance by same researcher. Photographs of each individual's face (frontal, left side, right side and basal side) were taken (Figs. 2 and 3). The pictures were used to calculate the distances and angles using Image J 1.48v software (Fig. 4). All measurements were obtained by the same investigators. All nasal points used for measurements have been described in detail as indicated in Figures 2, 3 and Table 1. The linear parameters (nose height, nasal bridge length, nose width, columella width, columella length, nares length and nares width) were extracted. Angles of nasofrontal, nasolabial, nasal tip, inter alar were calculated (Figs. 2,3 and 4) , (Table 1).

2.3. Statistical Analysis

The statistical analysis was carried out using SPSS 22.0 for Windows (IBM Corporation, New York, USA). The results were analyzed using the mean value, the standard deviation, the estimate of the population average with a 95% confidence interval, and the Student test t with meaning was established at P;< 0.05).

Main Points:

- Nares asymmetry was more frequentin young male adults compared with other features. However, the same group of young female adults reported lower values for nasal bridge length, nose width, and nares measurements.
- On average, young male adults had larger nasal linear distances such as nasal bridge length, nares lengths and nares widths and height ratio than young female adults.
- With assistance from digitized reference details, in our study has helped determine the best aesthetic design remedy for the nose in addition to Improve successful reconstruction treatment.

Tablo 1. Linear and angular distances measured between two different points are shown below: (n:200, mm: millimeters).

Linear Analysis	AVE	STD	MAX	MIN	p value
Nose height	M= 49.08 F= 50.37	M= 3.48 F= 2.34	M= 53.65 F= 53	M= 34.77 F= 38.27	0.025
Nasal bridge length	M= 48.6 F= 37.09	M= 3.24 F= 5.49	M= 53.22 F= 49	M= 36.12 F= 27.55	0.014
Nose width	M= 33.23 F= 32.57	M= 2.8 F= 2.18	M= 41.22 F= 39.22	M= 23.44 F= 23.66	0.026
Columella width	M= 7.94 F= 7.82	M= 1.59 F= 1.62	M= 11.4 F= 11.3	M= 4.1 F= 4.8	0.033
Columella length	M= 9.15 F= 8.64	M= 1.62 F= 1.43	M= 14 F= 11.3	M= 5.4 F= 4.8	0.038
Nasofrontal angle	M= 133.73 F= 130.94	M= 6.24 F= 5.71	M= 145 F= 141.43	M= 112 F= 118.53	0.049
Nasal tip angle	M= 82.91 F= 75.81	M= 12.08 F= 8.49	M= 109 F= 97.32	M= 60 F= 62.99	0.028
Nasolabial angle	M= 83.53 F= 91.91	M= 12.65 F= 5.76	M= 113 F= 105.77	M= 57 F= 77.44	0.016
Inter-alar-angle	M= 89.15 F= 91.97	M= 8.73 F= 5.77	M= 108.5 F= 105.77	M= 71 F= 77.44	0.029
Nares length ®	M= 9.33 F= 8.09	M= 1.68 F= 1.32	M= 14.5 F= 12	M= 6.4 F= 6	0.045
Nares width ®	M= 6.06 F= 5.80	M= 1.12 F= 1.35	M= 8.7 F= 8.7	M= 3.9 F= 3.3	0.019
Nares length (L)	M= 9.27 F= 7.98	M= 1.55 F= 1.19	M= 13.7 F= 11.4	M= 6.4 F= 5.4	0.015
Nares width (L)	M= 6.1 F= 5.89	M= 1.07 F= 1.02	M= 8.6 F= 8.3	M= 4.3 F= 4.1	0.032

Significant P level of < 0.05.

Tablo 2. Nose shape analysis and values (n: 200, mm: millimeters).

Shape nose	Nasal tip	Nasal base	Nasal alae	Nares	Columella
Normal	M= 43 F= 54	M= 70 F= 70	M= 53 F= 60	M= 62 F= 61	M= 29 F= 39
Wide	M= 31 F= 26	M= 28 F= 29	M= 0 F= 0	M= 0 F= 0	M= 40 F= 32
Sharp	M= 17 F= 11	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0
Protrusive	M= 7 F= 6	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0
Asymmetric	M= 7 F= 3	M= 2 F= 1	M= 1 F= 7	M= 22 F= 25	M= 9 F= 0
Thick	M= 0 F= 0	M= 0 F= 0	M= 37 F= 23	M= 0 F= 0	M= 0 F= 0
Thin	M= 0 F= 0	M= 0 F= 0	M= 9 F= 10	M= 0 F= 0	M= 0 F= 0
Horizontal	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 16 F= 14	M= 0 F= 0
Short	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 22 F= 14
Bifid	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0	M= 9 F= 15

Figure 4. Measurement of the nasofrontal angle using Image J 1.47 version.

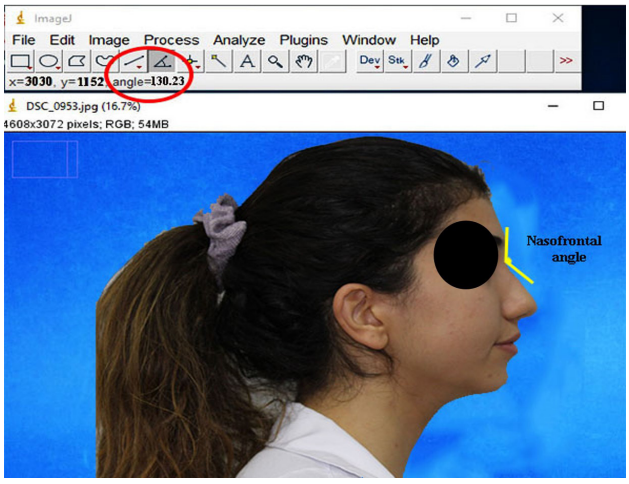


Figure 5. Specimens of nasal tip as normal, sharp, asymmetric, protrusive and wide cases.

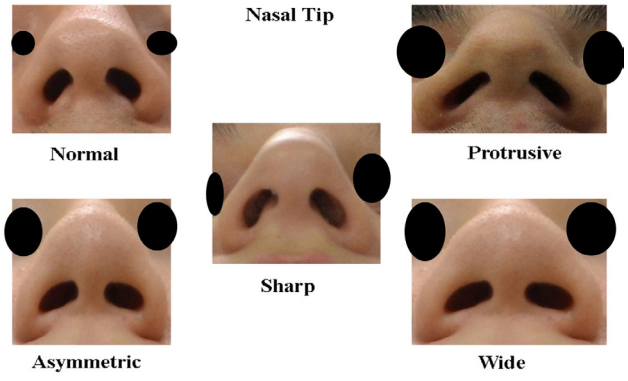
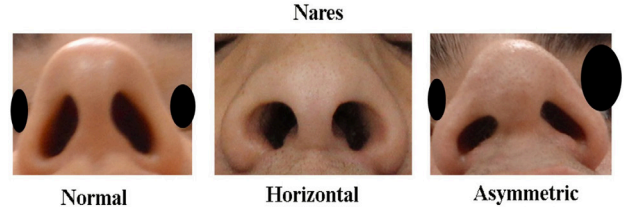


Figure 6. Specimens of nasal base as normal, asymmetric and wide cases.



2.4. Shape analysis

Nasal shape analyses were controlled by another researcher. Shapes of nasal tip (sharp, normal, wide, protrusive and asymmetric) (Fig. 5), nasal base (normal, wide, asymmetric) (Fig. 6), nasal alae (normal, thick, thin, asymmetric) (Fig. 7), nares (normal, horizontal and asymmetric) (Fig. 8) and columella (normal, wide, short and bifid) were determined (Fig. 9), (Table 2).

RESULT

Demographic Data

All subjects were of purely Anatolian descent.

Nasal Soft Tissue Distances

The distances of the external nose in connected to the marked reference landmarks were presented in Table 1. The nose height was 49.05 ± 3.48 mm in males, 50.37 ± 2.33 in young female adults. Length of nose bridge 48.60 ± 3.24 in males, 37.09 ± 5.49 in females. The two measured mean nasal reference lengths computed a much greater value in young male adults. The angle of the nasal tip was much more pronounced for females than for males. (males = 82.91° ± 12.08, female = 75.80° ± 8.49, p;<0.001). On average, young male adults had larger nasal linear distances such as nasal bridge length, nares lengths and nares widths and height ratio than young female adults (p;<0.01); no gender differences were found in the columella widths and nose height ratio (p;<0.01). The nasofrontal, nasal, nasolabial and interalar angles exhibited statistically significant gender differences (p;<0.05). The nasolabial angle exhibited great variability of 57 to 113 degrees. It was much more common among females than males (young male adults = 83.53 12.65, young female adults = 91.91 5.76, p;<0.05). The details of nostril shape also showed large variability. Nasal base, nasal tip and nasal alae shapes were similar. Nares asymmetry was more frequent in young male adults compared with other features. However, the same group of young female adults reported lower values for nasal bridge length, nose width, and nares measurements.

Analysis Results of Nose Shape

The shape of the nose in 200 adults was analysed with respect to the nasal tip, nasal base, nasal alae, nares and columella (Figs. 3,4). The Anatolian people's nose exhibits wide nasal tip. It has a wider nasal base, and is thicker at the alae, with wider definition of the columella. Significant gender differences were observed in the beauty classifications of nasal forms. Wide and sharp features of nasal tip were related to a substantial fraction in young male adults, whereas asymmetric nares were predominant in young female adults (Table 2).

DISCUSSION

Asymmetries, deformities and irregularities of the nasal region have a basis symbol on the sensation of face beauty (Figs. 5-8). The majority of women reported to change a disliked nasal feature mostly thought of as unattractive (54%).[20,21] Probability of irregularities has been detected to play a critical role in its reconstruction in personalized procedure for facial beauty (Figs. 5-8). It is possible to shorten and reshape nose with operative efforts or with non-surgical techniques We know that nose surgery is the most common procedure.[17,22]

Nasal morphology and morphometry are valuable in establishing treatment expectations and identifying a primary for monitoring evaluation of algorithm effectiveness.[23] In addition, the achievement of cosmetic goals with minimal damage to adverse events requires knowledge of the morphology of the nose, clinical experience in the use of various surgical and non-surgical injection techniques. Various materials as cartilage, expanded

Figure 7. Specimens of nasal alae as normal, thick, thin and asymmetric cases.

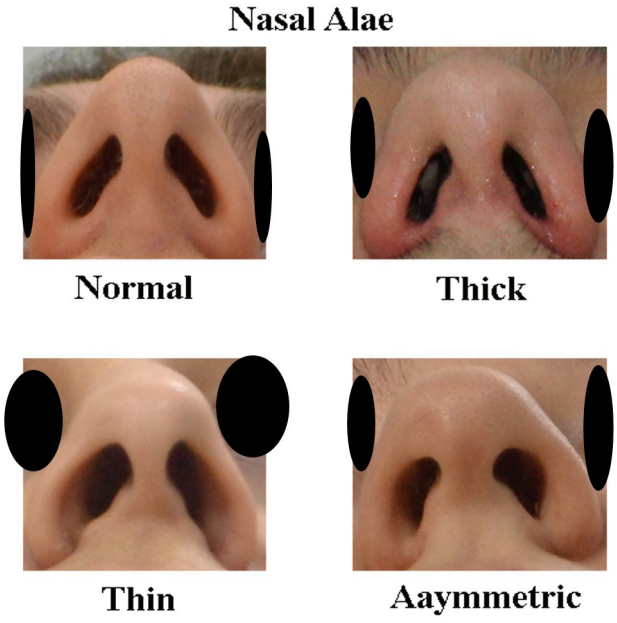


Figure 8. Specimens of nares as normal, horizontal and asymmetric cases.

Figure 9. Specimens of columella as normal, short, wide and bifid cases.

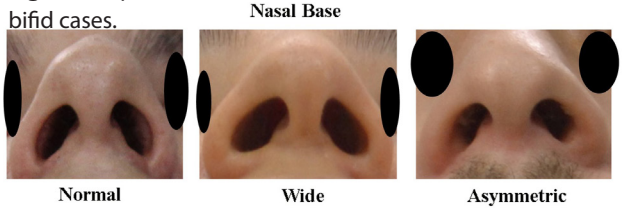
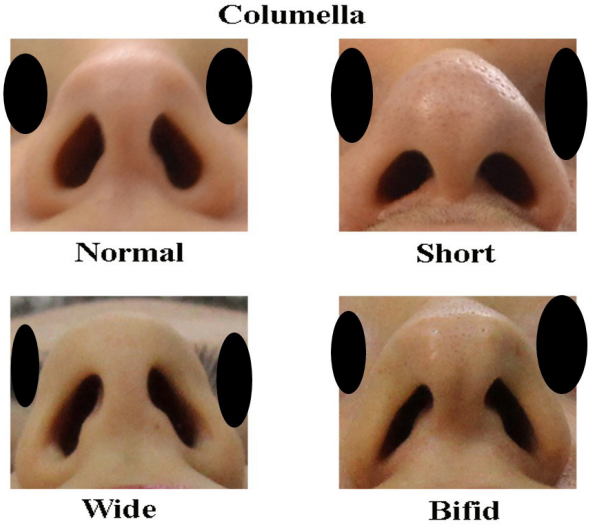


Figure 9. Specimens of columella as normal, short, wide and bifid cases.



polytetrafluoroethylene and silicone are commonly used for dorsal nasal enhancement. Hussein et al. reports that sub-period nasal implants have been practiced on a large scale for dorsal nasal augmentation and nasofrontal angle shift.[18,23] As a result, non-surgical and surgical nasal redesign procedures should be based on recording patient-specific morphometric calculations and evaluating them on ethnic models.[24]

Along with many other racial or ethnic populations, plastic surgery will take into account racial or ethnic differences in planning and performing rhinoplasty.[19,22-25] An anthropometric nasal analysis is investigate among various populations as groups of Koreans, Chinese, Mexicans, Africans, Americans, Indians and Persians.[26,27] Korean women show a greater angle to measurements of the nasal, nasofacial and nasofrontal extremities.[27,28]

Women in Korea have higher values for nasal index, nasal root width and height index, and Simon ratio. Published standards for young North American white adult women show greater glabellonasal, nasolabial and nasomental angles.[29] The white group of North American also exhibits a more pronounced wing slope and inclination of the nostril axis.

This study examined many anthropometric measures of the nose of young adult Anatolians. The two average measured lengths of the nasal markers were much greater in young male adults. On average, men had more linear nasal distances such as the length of the nasal bridge, the length of the nares and the width of the nares in relation to the height, than young female adults (p;<0.01); no gender differences were found between the width of the columella and the height ratio of the nose (p;<0.01).

The nasolabial and interalar angular measurements were larger in young male adults (Table 1). The nasofrontal, nasal tip, nasolabial and interalar angles displayed statistically significant gender differences (p;<0.05). The nasal tip angle was narrower in females than in males. The shape details of nares showed large variability. Nasal base, nasal tip and nasal alae shapes were similar (Figs. 5-9). Nares asymmetry was more frequent compared with other features (Table 2).

The Anatolian people's nose exhibits wide nasal tip, has a wider nasal base, and is thicker at the alae, and the columella is wider (Table 1). Aesthetic classifications of nasal forms exhibited significant gender differences. The broad, acute features of the nasal end were associated with a substantial fraction in young male adults, while asymmetrical nares predominated in young female adults. The morphometric results of the nostril symmetry were as follows: The normal aspect of the nostrils is reflected across the hole. In light asymmetry, one side of the nose measured a difference of 1 mm in length or width of the standing nares. In moderate asymmetry, one side of the nose differed from the remaining nares by 1.1 to 2 mm in length or width. In severe asymmetry, one side of the columella displayed a >2mm difference in width or in length of the nares at repose.[30] Nares asymmetry with 24% of frequency was present in every one fourth, which is a high rate.

In present research, digitalized morphometrical and morphological patterns of the nose with standardized photographs determined from normal subjects. Our results also showed that facial alterations, facial reconstruction, personal identification and trauma assessment can also benefit from age- and gender-based databases.

In nasal surgery, it is important to check and standardise the modification of copies of previous measurements and after the operation.[19,28-31] This research shows that detailed measurements of the nose play a significant role in algorithmic planning concerning the aesthetic profile. In men, nose would occupy a much greater proportion. It might have a bigger aesthetic impact. Present research, not only focused on the nasal morphometrical findings to obtain the gender differences but also proposed an algorithm for morphological details. The Anatolian people's "ideal/attractiveness nose" manifests wide nasal tip, and nasal base, and is thicker at the alae with wider columella.

Using computerized methods is more useful in controlling nasal angles and nares.[32,33] One of them is to provide repeating nasal measurements anytime, and add new landmarks. Another advantage is desired in standard parameters.[34,35] The first point is to carry out a detailed knowledge of the geometric shapes and the mathematical value of the nasal zone for the reconstruction specific to the patient. Secondly, the identification of the surface analysis would provide an advantageous overview of how to reach the ideal nasal anatomy in terms of customized nasal measurements and the attractiveness of the patient's face. The use of computer technology also representing the nasal form of Anatolian men and women was investigated. The nasofrontal, nasal, vertical nasal, and nasal dorsum angles demonstrated statistically significant gender differences.

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Conflict of interest: All the authors certify that they have no potential conflicts of interest with any entity mentioned in this manuscript and that they received no specific financial support for this work.

Ethical Committee: The scan protocol was conducted in accordance with guidelines from The study was approved by the suitably constituted Ethical Committee at Research of Ege University (Date: May 16, 2014, Decision no: 659/311).

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Original Research

An Investigation of the Effectiveness of the 577-nm Pro-yellow Laser in Patients with Vascular Disorders

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ABSTRACT

Background: Vascular disorders severely impair the psycho-social status of individuals. Various laser and light systems, which have advantages and disadvantages, including the pro-yellow laser are used for the therapy of these disorders.

Aim: The goal of the present study was to investigate the effectiveness and feasibility of the 577 nm pro-yellow laser for a broad range of indications including erythematelangiectatic rosacea, facial erythema, post-acne erythema, facial telangiectasis, hemangioma, genital angiokeratoma, and port wine stain nevus.

Methods: A total of 98 patients (25 male, 73 female) older than 15 years who were treated with the pro-yellow laser for vascular disorders at the cosmetology unit between 2017 and 2019 were retrospectively included in the study.

Results: The mean rate of recovery was 100% in genital angiokeratoma, 94.4% in spider angioma, 83.3% in facial telangiectasis, 74.8% in erythematotelangiectatic rosacea, 72% in facial erythema + facial telangiectasis, and 68.3% in facial erythema. Over 60% of improvement was observed in most patients with vascular disorders. There was no significant link between the Fitzpatrick skin type and treatment success. Treatment success was significantly low in cases with nasal involvement.

Conclusion: The current study concluded that pro-yellow laser is an efficient and safe laser modality that may yield satisfactory outcomes regardless of the different skin types.

Keywords: Pro-yellow laser, vascular disorders, laser treatment, vascular laser

INTRODUCTION

Vascular disorders affecting the skin, particularly the rosacea, facial telangiectasia, facial erythema, port-wine stain nevus, and many others influence negatively the psychological status of individuals, these vascular skin problems are more common in women (1). Light systems were first used for the therapy of cutaneous vascular disorders by Goldman et al. in the 1960s (2). Many laser systems including Nd:YAG lasers (1064 nm), KTP-lasers (532 nm), pulsed dye lasers (585 and 595 nm), intense pulsed light (IPL), alexandrite lasers (755 nm), and diode lasers (800–900 nm) continue to be utilized today (3). The pro-yellow (577 nm) laser has recently been introduced as an alternative (4,5).

In vascular lesions, the goal chromophore is mostly oxyhemoglobin followed by deoxyhemoglobin and methemoglobin. The energy is first transferred to the target chromophore oxyhemoglobin, the warmth is transferred to the vascular wall and ultimately the vascular wall is injured. Absorption peaks of oxyhemoglobin are in the green and yellow light spectrum, the wavelengths are 410-429, 541,577 nm, and 700 and 1200 nm (4).

The goal of the present study was to investigate the usefulness and feasibility of pro-yellow laser for a broad range of indications including erythematotelangiectatic rosacea, facial erythema, facial telangiectasia, post-acne erythema, hemangioma, spider angioma, genital angiokeratoma, and port-wine stain nevus.

METHODS

98 patients older than 15 years that were treated with the pro-yellow laser for vascular disorders at the cosmetology unit between 2017 and 2019 were retrospectively included in the study. Ten patients were excluded because they were lost to follow-up or due to missing information. A total of 98 patients (25 male, 73 female) were evaluated.

Diagnoses included facial erythema, facial telangiectasia, erythematotelangiectatic rosacea, post-acne erythema, facial erythema + facial telangiectasia, hemangioma, spider angioma, genital angiokeratoma, and port-wine stain nevus. Data about the anamnesis of the patients, the lesion locations, the pro-yellow laser application doses, the duration of treatment, comorbidities, and the other dermo-cosmetic procedures and treatments were obtained from patient files. Exclusion criteria were active cutaneous infection, pregnancy, photodermatoses, and systemic or local retinoid treatment.

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Figure 1. Post acne erythema, pre and post treatment

Figure 2. Immediately after the procedure of a single session in a patient with erythematelangiectatic rosacea, pre and post treatment

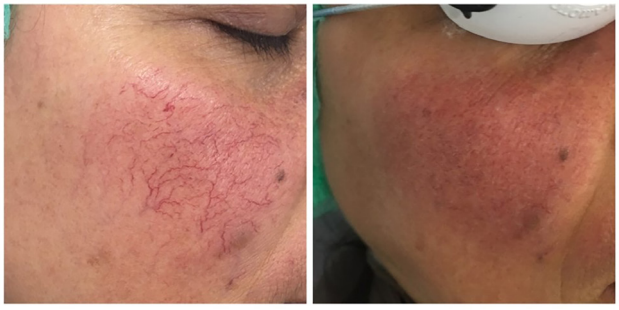


Figure 3. Port-wine stain nevus, pre and post treatment

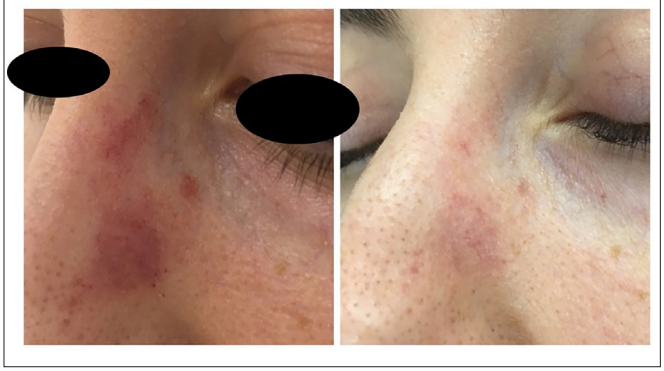
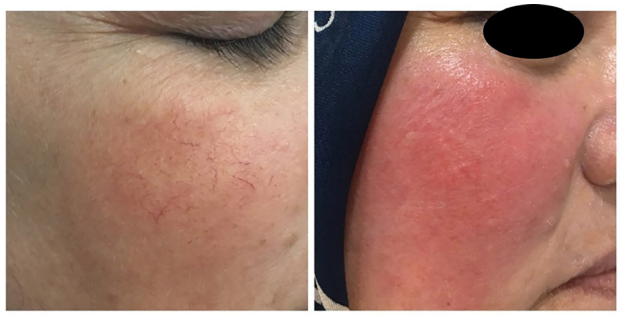


Figure 4. Erythema immediately after the procedure in a patient with facial telangiectasis



Before the treatment, the skin was cleaned and made readied. The therapy was performed with a 577-nm pro-yellow laser (Asclepion Laser Technologies QuadroStar PRO YELLOW®) using a wavelength of 577-nm. The therapy was ordered at four-week intervals. The first session was begun with 20j/cm^2, and the dosage enhanced an average value of 2j/cm^2 at each session. The dose was elevated up to 26j/cm^2. If the lesion had telangiectasia, the basic mode was used first. The scanner mode was used if the lesion did not include telangiectasia, or it was larger than 0.5 cm. A cold implementation was applied and/or a topical steroid was done if the patient had a burning sensation or erythema after the procedure. Sunscreen was applied after the procedure in all patients. All patients were photographed before and after the procedure.

Main Points:

- The study is the first in the literature to investigate the pro-yellow laser effectively used for the therapy of vascular disorders in the highest number of patients and the largest indication spectrum.
- In the study, nasal involvement was observed more in males, and in addition to the current literature, treatment success was significantly lower in cases with nasal involvement.
- The study concluded that pro-yellow laser is an influential and safe laser modality that may yield satisfactory outcomes regardless of the different skin types.

The evaluation of the therapy relied on clinical examination and standardized digital photographs using a camera at baseline and after the treatment sessions. Treatment success of patients was evaluated by using a visual analog scale (VAS) that 0 indicated the minimum value, and 100 indicated the maximum value. Improvement was rated by the same dermatologist as follows: "excellent" (90%-100%), "very good" (70%-89%), "good" (25%-69%), "slight" (1%-25%), and "ineffective" (0%). In the monthly follow-up, the adverse effects that had developed in the patients were recorded in their files.

Ethics Approval

Ethics approval: All the procedures followed the Helsinki declaration and the Necmettin Erbakan University Meram Faculty of Medicine local ethics committee approval was received for the study (Decision date and number: 2019/2036).

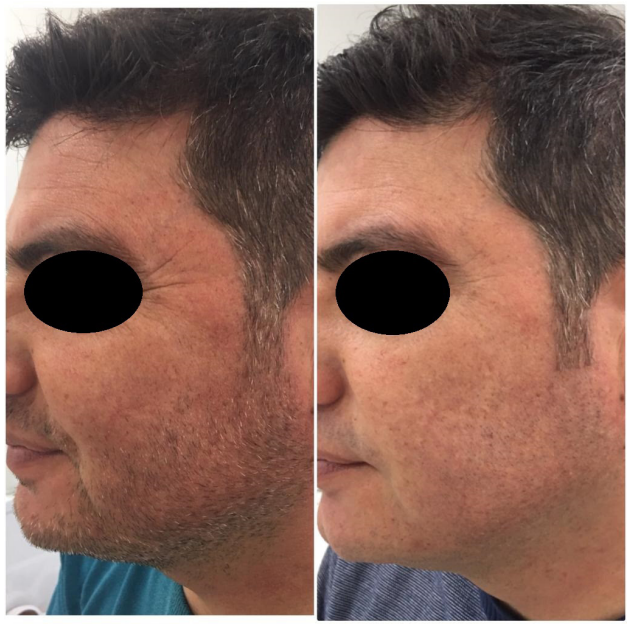
Statical Analysis

Data were investigated by using the SPSS 22.0 (SPSS Inc., an IBM Company, Chicago, IL, USA) statistical software. The normality distribution of the variables was evaluated with one sample Kolmogorov Smirnov test. Variables that showed normal distribution were shown as mean and standard deviations (mean ± SD). Oneway ANOVA, student t, and chi-square tests were used for statistical analyses. Written and oral informed consent form was received from all patients prior to the study.

Table 1. The distribution of the patients according to gender and indications

Disorder type	Female	Male	Total
Facial erythema	8	1	9
Facial telangiectasias	18	9	27
Erythematotelangiectatic rosacea	24	7	31
Postacne erythema	3	1	4
Facial erythema + Facial telangiectasias	6	2	8
Hemangioma	2	2	4
Spider Angioma	8	1	9
Genital angiokeratoma	2	0	2
Port-wine stain	2	2	4
	73	25	98

Figure 5. Facial erythema, pre and post treatment



RESULTS

A total of 98 patients (73 females and 25 males) were investigated in the study. The dispersion of the patients according to gender and indications is presented in Table 1. The distribution of the patients according to age, gender, skin type, and number of sessions is presented in Table 2.

The ratio of excellent and very good outcomes were as follows: 66.6% for facial erythema, 96.3% for facial telangiectasia, 83.9% for erythematotelangiectatic rosacea, 100% for post-acne erythema, 62.5% for facial erythema + facial telangiectasia, 100% for hemangioma, 100% for spider angioma, 100% for genital angiokeratoma, and 25% for port-wine stain nevus. Age, gender, skin type, and number of sessions are presented in Table 3.

We observed higher than 60% success in the vast majority of the cases. The mean success rates and the number of sessions are presented in Table 4.

Table 2. The characteristics of the patients

	Facial erythema (n=9)	Facial telangiectasias (n=27)	Erythemato-telangiectaticro-sacea (n=31)	Postacne erythema (n=4)	Facial erythema + Facial tel-angiectasias (n=8)	Hemangi-oma (n=4)	Spider Angioma (n=9)	Port-wine stain (n=4)
Age/years Range (Mean±SD)	15–39 (30.3±9.1)	8–70 (31.2±15.4)	23–56 (40.9±8.2)	16–27 (22.3±5.2)	37–60 (43.6±7.2)	18–48 (32.7±13.7)	9–59 (27.2±17)	14–19 (16.5±2)
Gender, n (%)								
Female	8 (88.9)	18 (66.7)	24 (77.4)	3 (75)	6 (75)	2 (50)	8 (88.9)	2 (50)
Male	1 (11.1)	9 (33.3)	7 (22.6)	1 (25)	2 (25)	2 (50)	1 (11.1)	2 (50)
Skin type, n (%)								
II	6 (66.9)	13 (48.2)	12 (38.7)		5 (62.5)	2 (50)	1 (11.1)	1 (25)
III	3 (33.3)	11 (40.7)	18 (58.1)	4 (100)	3 (37.5)	2 (50)	8 (88.9)	2 (50)
IV		3 (11.1)	1 (3.2)					1 (25)
Number of sessions Mean±SD	2±0.7	1.7±0.9	2.7±0.9	1.75±1	3.1±1	3.2±0.9	1.4±0.7	3.75±0.5

Table 3. The success according to disorder type

Disorder type,n (%)	Excellent	Very good	Good
Facial erythema		6 (66.6)	3 (33.3)
Facial telangiectasias	14 (51.9)	12 (44.4)	1 (3.7)
Erythematotelangiectatic rosacea	1 (3.2)	25 (80.7)	5 (16.1)
Postacne erythema		4 (100)	
Facial erythema + Facial telangiectasias	2 (25)	3 (37.5)	3 (37.5)
Hemangioma	1 (25)	3 (75)	
Spider Angioma	9 (100)		
Genital angiokeratoma	2 (100)		
Port-wine stain		1 (25)	3 (75)

Table 4. The success rate according to disorder type

Disordertype	The mean success rate (%)	Average session
Facial erythema	68.3	2
Facial telangiectasias	83.3	1.7
Erythematotelangiectatic rosacea	74.8	2.7
Postacne erythema	78.7	1.7
Facial erythema + Facial telangiectasias	72	3.1
Hemangioma	80	3.2
Spider Angioma	94.4	1.4
Genital angiokeratoma	100	2
Port-wine stain	65	3.7

Table 6. The relationship of nasal involvement with gender and treatment success

	Nasal involvement (-)	Nasal involvement (+)	p value
The mean success rate (%)	81.8	74.3	**0.01**
Average session	2.14	2.54	0.26
Female	47	26	**0.03**
Male	10	15	

The patient skin types were mostly skin type II and III of Fitzpatrick's. There was no significant link between Fitzpatrick's skin type and treatment success (p=0.64). The success rate according to skin types is presented in Table 5.

The nasal involvement was higher in the males, and the number of sessions was higher in patients with nasal involvement but with no statistical difference. Treatment success was significantly low in cases with nasal involvement (p=0.01). The relationship of nasal involvement to gender and treatment success is shown in Table 6.

Images of the patients before and after pro-yellow laser in different indications may be seen in Figures 1-5. We observed temporary erythema that regressed maximum within one to two days maximum after the procedure in a small number of patients (Figure 4). No patient had any permanent adverse effects.

DISCUSSION

This is the first study in the literature to investigate the pro-yellow laser effectively used for the therapy of vascular disorders in the highest number of patients and the largest indication spectrum. Two studies are available in the literature that investigates the effectiveness of the pro-yellow laser in a smaller number of patients and indications (5,6). Kapicioglu et al. reported 80-100% improvement in a total of 40 patients with erythematotelangiectatic rosacea, facial erythema, and facial telangiectasia (5). Mohamed et al. investigated the effectiveness of the pro-yellow laser in 95 patients with port-wine stain nevus, papulopustular rosacea, facial telangiectasia, and facial erythema and observed a significant healing in more than 50% of the patients (6). Our study included a total of 98 patients with a larger indication including facial erythema, facial telangiectasis, erythematotelangiectatic rosacea, post-acne erythema, facial erythema + facial telangiectasia, hemangioma, spider angioma, genital angiokeratoma, and port-wine stain nevus. We observed significant improvement, greater than 60%, in our study.

In the literature, no significant link has been found between Fitzpatrick's skin type and treatment success (5,6). Similar to the literature, the achievement of treatment in terms of skin type was not significantly different in our study. In the study by Kapıcıoğlu et al., nasal involvement was found more in men (5). In our study, nasal involvement was observed more in males, and in addition to the literature, treatment success was significantly lower in cases with nasal involvement.

Pulsed dye laser, one of the lasers used for the treatment of vascular disorders, has been shown to be effective in various studies;

Table 5. The success rate according to skin types

	Fitzpatrick skin type II	Fitzpatrick skin type III	Fitzpatrick skin type IV	p value
Number of the patients	40	51	7	**<0.001**
The mean success rate (%)	78.1	78.4	82.9	0.64
Average session	2.4	2.3	2	0.63

however, it has some disadvantages in addition to the necessity of needing dye including leading to hypo/hyperpigmentation, scarring, post-laser effect-related purpura, and discoloration of the lesion (7,8,9). The KTP laser produces green light at a wavelength of 532 nm and does not lead to post-operative purpura, a reason that KTP is superior to PDL. The main disadvantage of KTP is the higher absorption of energy by the epidermal melanin with a higher risk of pigmentary changes, especially in darker or tanned skin types. Near-infrared lasers (700-1200 nm), diode lasers (800–900 nm), alexandrite lasers (755 nm), and Nd:YAG lasers (1064 nm) have deeper penetration with lower absorption by the melanin, so it is safe for all skin types. However, higher joules may be required for real photocoagulation due to low hemoglobin absorption and high water absorption. This is a disadvantage because it means more pain. Intense pulsed light (IPL) is a large band light between 500 to 1200 nm. This large band range allows a different depth of oxyhemoglobin absorption; however, adverse effects like post-inflammatory hyperpigmentation, bullae, and crust may be observed particularly, in dark individuals, as the light may be absorbed easily by the melanin (10). The pro-yellow (577 nm) laser has a yellow light wavelength with the substantial advantages of high hemoglobin absorption, low absorption of melanin, low water absorption, and a low pain level. It also has real photocoagulation. The 577 nm pro-yellow laser can see the lesions 40% better than the KTP and 70% better than the 585 nm PDL (4). No adverse effects such as discoloration, crusting, or permanent scar formation happen, and irritation or erythema are rare. We did not detect any adverse effects other than mild irritation in dry-sensitive skin, temporal erythema, and mild pain in high joules. No persistent irreversible adverse effects emerged in our patients.

Although the exact cause of facial erythema and telangiectasia is not known, genetic factors, having the Fitzpatrick skin type I-II, topical or oral corticosteroid use, sun exposure in childhood, and rosacea are usually blamed (11). These are reported to be important cosmetic problems by the patients and are effectively treated with laser and light systems (4,9,10,12). Effectiveness and adverse effects were found to be equal in a study in which PDL was applied to one side of the face and IPL to the other (13). In a study comparing 532 nm and 940 nm, no difference was found with regard to effectiveness; however, 940 nm was found to have fewer adverse effects like erythema, crusting, swelling, bullae, and pain (14). In our study, we found good effectiveness in more than 60% of the patients with facial erythema, telangiectasia or facial erythema + telangiectasia although mild erythema and crusting were noticed by a small number of patients. Uebelhoer et al. applied KTP to one side of the face and PDL to the other. In their study conducted with the patients with telangiectasia and erythema, KTP was found to be more effective although it led to more adverse effects, Complete clearance was found to be 85% in the third session with KTP and 75% with PDL (12).

The pro-yellow laser was used for the first time in the literature in the treatment of post-acne erythema by Saraç et al. in 40 patients, and a success rate of 76-100% was reported (15). In our study, nearly 70% treatment success was found in facial erythema.

Rosacea is a common skin disease that negatively affects the social life of the patients and reduces the quality of life by leading to anxiety and depression (16). Medical treatment is usually insufficient in erythematotelangiectatic rosacea. Various light and laser systems including IPL, PDL, KTP, and Nd:YAG are utilized for therapy. Various success rates have been reported in various studies (7,17). We have observed good improvement in more than 80% of rosacea patients; however, we have observed erythema lasting for a maximum of one to two days in some of the patients with a sensitive skin type (Figure 4). Overall, we did not encounter any adverse effects other than mild pain and temporal erythema with 24-26 joules and above screening mode or 16 joules or above with a pencil probe. We consider its low adverse effect profile as an advantage of the pro-yellow laser.

CONCLUSION

The pro-yellow laser is a very influential method for the treatment of vascular disorders. This is the first study to investigate pro-yellow laser in a large patient group and with a wide spectrum of diseases including facial erythema, facial telangiectasia, erythematotelangiectatic rosacea, post-acne erythema, facial erythema + facial telangiectasia, hemangioma, spider angioma, genital angiokeratoma, and port-wine stain nevus. We consider that the 577 nm pro-yellow laser is a useful treatment choice with pleasing outcomes for both patients and physicians in the treatment of vascular lesions or other vascular-based lesions.

Limitations: The limitations of the study were the retrospective nature and the imbalance in the female/male ratio.

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Ethics approval: All the procedures followed the Helsinki declaration and the Necmettin Erbakan University Meram Faculty of Medicine local ethics committee approval was received for the study (Decision date and number: 2019/2036).

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Original Research

Agents Isolated from Vaginal Cultures in the Reproductive Period and Their Antibiotic Sensitivities (Vaginal Culture and Antibiotic Sensitivitie)

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ABSTRACT

Objective: In our study, we aimed to examine the strains isolated from vaginal swab samples sent to our laboratory from various clinics with a pre-diagnosis of vulvovaginitis and antibiotic resistance rates, retrospectively.

Methods: ethods: 90 vaginal swab samples of patientswere included into this study. Two samples were taken from each patient and one sample was used for Gram staining. The other sample was inoculated in 5% sheep blood, Eozine Methylene Blue agar and Saboraud dextrose agar. TIdentification of isolated colonies and antibiotic susceptibility studies were carried out with Vitek 2 Compact automated system. Result of the susceptibility testing are reported according to EUCAST criteria. Nugent scoring was used for bacterial vaginosis.

Results: Normal vaginal flora elements were found in 66 (73.3 %) of the specimens, and 28 organisms were found in 24(26.7 %). The distribution of the organisms is as follows: 6 (21.4%) Escherichia coli, 5(17.9%) Streptococcus agalactiae, 5(17.9%) Gardnerella vaginalis, 3 (10.7%) Candida spp., 3(10.7%) Klebsiella pneumoniae, 3(10.7%) Enterococcus faecalis. Two of the E.coli strains and one of the K.pneumoniae strains are ESBL positive. Of the agents, 21 were isolated from outpatients and 7 from inpatients. All S. agalactiae strains were susceptible to penicillin and ampicillin. E.coli and K.pneumoniae strains, which are the most frequently isolated Gram (-) organisms, are most susceptible to amikacin, carbapenems and tigecycline.

Conclusion: In patients with vaginal discharge and itching complaints, determining the organisms with microbiological culture and agent-directed treatment instead of empirical treatment will be more beneficial for cure.

Keywords: Antibiotic sensitivity, vaginitis, vaginal discharge

INTRODUCTION

Approximately 10 million patients a year present to gynecology clinics due to vaginal discharge, pruritus, and purulence (1). The three most frequently observed agents in the diagnosis of vaginitis are *Candida* spp., (CC), bacterial vaginosis (BV), and Trichomonas (TCH). However, the agent involved in vaginitis remains undiagnosed in between 7% and 72% of patients, and these soon re-present to clinics with the same symptoms (2-5). Appropriate treatment directed toward the agent should therefore be prioritized in terms of diagnosis and treatment. Various different methods are available for identifying agents of vaginitis, including vaginal discharge microscopy, examination with 10% potassium hydroxide (KOH), Gram staining, vaginal pH examination, and vaginal culture (6). Micro-organisms detected in cultures from patients of reproductive age presenting to our hospital with vulvovaginal discharge and itching and the antibiotic sensitivity and resistance rates of those micro-organisms were examined retrospectively in this research.

METHODS

Approval for the study was granted by the Clinical Research Ethical Committee on 28.01.2021 (Decision No: 03/31). Ninety vaginal culture specimens sent from patients with vaginal pruritus and discharge to the Erzincan Mengücek Gazi Training and Research Hospital, Türkiye, between January 2016 and January 2020 were included in this study. Vaginal discharge specimens collected using two sterile swab sticks from patients presenting to various clinics were placed into Stuart transport medium (Fıratmed, Türkiye) and sent to the laboratory without loss of time. One of the specimens was inoculated onto 5% sheep's blood (bioMerieux ®, France), Eosin Methylene Blue (EMB) agar, and chocolate medium (bioMerieux ®, France). The other speci-

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Table 1. Service and polyclinic distribution of agents reproduced in vaginal culture

Bacteria	Policlinic	Service	Total
	Number (%)	Number (%)	Number (%)
Escherichia coli	4	2	6 (21.4)
Streptococcus agalactiae	4	1	5 (17.9)
Gardenerella vaginalis	4	1	5 (17.9)
Candida spp.	3	0	3 (10.7)
Klebsiella pneumoniae	2	1	3 (10.7)
Enterococcus faecalis	2	1	3 (10.7)
Enterobacter aerogenes	1	0	1 (3.6)
Acinetobacter baumannii	0	1	1 (3.6)
Proteus mirabilis	1	0	1 (3.6)
Total	**21 (75)**	**7 (25)**	**28 (100)**

Results were presented as numbers (n) and percentages (%).

men was suspended with sterile saline solution and placed onto a slide. After drying, it was then Gram-stained. The blood and EMB media were left to incubate for 24-48 h at 37° C in an aerobic environment, and the chocolate medium at 37° C in an environment with 5-10% CO_2. Identification and antibiotic susceptibility studies of colonies with morphology compatible with Gram results and dominant according to flora bacteria but regarded as causative agents based on the presence of inflammatory cells were performed using a Vitek 2 Compact (Biomerieux, France) automated system. EUCAST criteria were employed in reporting the sensitivity results (7). The presence of clue cells at Gram staining, the dominance of Gram labile coccobacilli, a decrease in lactobacilli, and the absence of an increase in inflammatory cell numbers were taken into account for bacterial vaginosis.

Main Points:

- The purpose of this study was to examine micro-organisms detected in cultures from women of reproductive age with vulvovaginal discharge and pruritus and the antibiotic sensitivity and resistance rates of those micro-organisms in a retrospective manner.
- Ninety vaginal culture specimens from patients with vaginal itching and pruritus sent to the Erzincan Mengücek Gazi Training and Research Hospital microbiology laboratory between January 2016 and January 2020 were included in this retrospective study.
- The most frequently detected agents, in descending order, were E. coli (21.4%), S. agalactia (17.9%), G. vaginalis (17.9%), Candida spp. (10.7%), K. pneumoniae (10.7%), E. faecalis (10.7%), E. aerogenes (3.6%), A. baumannii (3.6%), and P. mirabilis (3.6%).
- The identification of agents using the culture method in women with vulvovaginitis and the initiation of treatment aimed at the agent rather than empiric therapy appears to be more potentially useful in terms of complete cure and preventing antibiotic resistance.

Statistical Analysis

The distribution of agents by years was analyzed using the chi-square test designed for single measurement variables. The year from which a difference derived was analyzed using the Bonferroni method with corrected p values. p values < 0.05 were regarded as significant.

RESULTS

Normal vaginal flora elements were isolated in 66 (73.3%) of the 90 vaginal smear specimens sent to our laboratory during the four-year study period, and vaginitis agents in 24 (26.7%). The patients' ages ranged between 19 and 50 years (average 37.5). Two agents grew in two patients, and 28 agents were identified in the 24 patients. Six (21.4%) of the 28 growing agents were *Escherichia coli*, five (17.9%) *Streptococcus agalactiae*, five (17.9%) *Gardnerella vaginalis,* three (10.7%) *Candida spp*., three (10.7%) *Klebsiella pneumonia, and three* (10.7%) *Enterococcus faecalis*. Lower numbers of other agents (*Enterobacter aerogenes, Acetinobacter baumannii,* and *Proteus mirabilis*) were detected. Agents detected together were *E. coli* + *S. agalactiae, E. faecalis* + *K. pneumoniae, E. coli* + *E. faecalis,* and *Candida* spp.+*A. baumannii*. Twenty-one (75%) agents were isolated from outpatients and seven (25%) from patients admitted to the ward. The distribution of agents growing in specimens from the ward and clinics is shown in Table 1. In chronological terms, the largest number of agents was detected in 2018 (n=10) and the lowest in 2016 (n=5). Examination of distribution by years using chi-square analysis revealed no statistically significant variation (p=0.249). Although more agents were detected in 2018(n=10), the difference was not significant.The distribution of agents by years is shown in Table 1.

The most frequently isolated Gram(+) bacteria were *S. agalactia* (Group B Streptococcus), followed by *E. faecalis*. The mean age

Table 2. Antibiotic resistance rates of Gram (+) bacteria (%)

Antibiotics	Bacteria (n)	
	S.agalactia (n=5)	E.faecalis **(n=3)**
Penicillin	0	–
Ampicillin	0	0
Erythromycin	2 (40)	–
Clindamycin	2 (40)	–
Vancomycin	0	0
Teicoplanin	0	0
Trimethoprm/ sulfamethoxazole	3 (60)	3(100)
Linezolid	0	0
Tetracycline	3 (60)	–
Levofloxacin	2 (40)	–
Daptomycin	0	0
Tigecycline	0	–
Ciprofloxacin	–*	1(33.3)

Results were presented as numbers (n) and percentages (%).

*-: Not tested

Table 3. Antibiotic resistance rates of gram (-) bacteria (n/%)

Antibiotics	Bacteria (n)	
	E.coli **(n=6)**	K.pneumoniae **(n=3)**
Ampicillin	5(83.3)	3 (100)
Amoxicillin/ Clavulonic acid	3(50)	2 (66.7)
Piperacillin/ Tazobactam	1(16.7)	0
Cefuroxime	2(33.3)	1(33.3)
Ceftazidime	2(33.3)	1(33.3)
Ceftriaxone	2(33.3)	1(33.3)
Amikacin	0	0
Gentamicin	1(16.7)	1(33.3)
Ciprofloxacin	1(16.7)	0
Trimethoprim/ sulfamethoxazole	2(33.3)	1(33.3)
Tigecycline	0	0
Ertapenem	0	0
İmipenem	0	0
Meropenem	0	0

Results were presented as numbers (n) and percentages (%).

of the patients in whom *S. agalactia* was isolated was 38. All the Group B streptococci were sensitive to penicillin, vancomycin, teicoplanin, linezolid, tigecycline, and trimethoprim/sulfamethoxazole. No resistance to vancomycin, ampicillin, or teicoplanin was encountered in *E. faecalis*. The antibiotic resistance results are shown in Table 2. The most frequently isolated Gram (-) bacteria were *E. coli* and *K. pneumoniae*. Two of the *E. coli* strains (33.3%) and one *K. pneumoniae* strain (33.3%) were broad spectrum β lactamase (ESBL)-positive. The antibiotics to which the Gram (-) strains were most sensitive were amikacin, carbapenems, and tigecycline (antibiotic resistance rates are shown in Table 3).

DISCUSSION

Genital infections in women can lead to local discomfort during sexual relations and pain or pelvic inflammatory disease by causing vaginal discharge and mucosal ulceration. Persistent infection of the upper genital system can even result in infertility, ectopic pregnancy, and chronic pelvic pain. Physical examination must be performed after history has been taken from patients presenting with symptoms of vaginitis. Inspection of the vulva and speculum examination can be indicative in terms of several vaginal agents. Mild, transparent discharge may be observed in asymptomatic patients with a normal vaginal flora. Changes occur at physical examination as a result of vaginitis agents. An off-white vaginal discharge with a fishy odor may be expected in bacterial vaginosis. Vaginal discharge resembling cottage cheese, erythema, and vulvar edema may be present in Candida infections. A foam-like, yellow discharge, erythema, and petechiae may be observed in Trichomonas infections (5). Investigation in terms of the vaginal agent may be required in addition to vaginal examination in complicated and refractory infections (8). One of the methods applied to identify the vaginal micro-organism is vaginal culture. Although many micro-organisms in human vaginal flora do not grow in culture, molecular studies have been used as an addition to molecular methods in some studies (9-10). FISH and PCR methods have also been employed to identify vaginal infections in recent studies (11-13). The most frequently detected agents in this study, in descending order, in this study were *E. coli* (21.4%), *S. agalactia* (17.9%), *Gardnerella vaginalis* (17.9%), Candida spp. (10.7%), *K. pneumoniae* (10.7%), *E. faecalis* (10.7%), *E. aerogenes* (3.6%), *A. baumannii* (3.6%), and *P. mirabilis* (3.6%). The agents identified by Mohhamed Kadir et al. using a similar method were *S. aureus* (12.4%), *E. coli* (11.6%), *C. albicans* (8.0%), β hemolytic streptococci (2.8%), *Klebsiella* spp. (1.2%), *N. gonorrhoeae* (0.8%), and *Pseudomonas* spp. (0.4%) (14). In another study, coagulase negative staphylococci (n=11), enterococci (n=8), *S. aureus* (n=5), β hemolytic streptococci (n=5), *S. viridans* (n=1), and *P. mirabilis* (n=1) were isolated in vaginal cultures from 505 pregnant patients with no symptoms (6). *G. vaginalis, E. coli*, group B streptococci, *Mycoplasma* spp. and *C. albicans* are frequently seen in normal vaginal flora (5). In the light of the results of these studies, not all growth in culture may require treatment. However, positive culture results in addition to a patient history

and physical examination findings can be a useful guide in terms of treatment options. The detection of growth in culture alone in patients with no complaints and with no significant examination findings does not require treatment directed toward the agent in question. The most common Gram (+) bacterium in vaginal culture specimens in this study was *S. agalactia*. In terms of antibiotic resistance rates, this was 100% sensitive to penicillin, vancomycin, teicoplanin, and linezolid. Bolukaoto et al. reported similar results (15). Another study involving different patient groups also detected similar antibiotic sensitivity rates (16). The Gram (-) bacteria most frequently isolated in this study were *E. coli* and *K. pneumoniae*. Two (33.3%) of the *E. coli* isolates and one *K. pneumoniae* isolate (33.3%) were broad spectrum β lactamase (ESBL)-positive. ESBL positivity reduces the therapeutic options since it bestows the ability to hydrolyze broad spectrum cephalosporins and monobactams. Several studies have shown that ESBL positivity has an adverse effect on therapeutic options (17-19). The antibiotics to which Gram (-) bacteria were most sensitive in this study were carbapenems, amikacin, and tigecyclines. Similar studies have also reported comparable antibiotic resistance rates (17). Another study described carbapenems as the antibiotic to which *K. pneumoniae* isolates were most sensitive (20). Recurring urinary tract infections are also frequently seen in women with refractory vaginitis infection (21).

CONCLUSION

In conclusion, the number of patients presenting to gynecology clinics with vaginal discharge is high. Identifying the agents concerned using the culture method and the initiation of treatment aimed at the agent rather than empiric therapy, in addition to history and examination findings in these patients, appears to be more useful in terms of complete cure.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erzincan Binali Yıldırım Üniveristesi (January 28.2021, Decision No: 03/31).

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Original Research

Measurement of the Body Physical Parameters by Bioelectrical Impedance Method in Individuals Survived after Covid-19

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ABSTRACT

Objective: The coronavirus disease 2019 (Covid-19) has significantly affected human health around the world, causing many complications. However, it is not fully understood how the body compositions of individuals affected in the short or long term after disease. In this study, we aimed to show the effects of Covid-19 on body composition and phase angle values, using Bioelectrical Impedance Analyzer.

Methods: Subjects were selected from individuals in the 18-60 age group, who had survived COVID-19 disease. 33 individuals who had survived it 1-3 months ago, and 30 individuals who had survived it 3-6 months ago were included in the study.

Results: Effects of COVID-19 on basal metabolism and body composition and the ratio of damaged cells in the body after the disease were determined. Basal metabolic rate, lean body mass, body cell mass, total body fluid, intracellular fluid, and phase angle values were found to be significantly changed in the 3-6 months range compared to that of 1-3 months.

Conclusions: These results indicate that the basal metabolism and body composition parameters of the body become better, and the proportion of damaged cells decreases as time goes on after suffering COVID-19, reaching values close to normal in 1-3 months and quite better values in 3-6 months. It can be concluded that, although covid-19 influences body composition parameters and cell integrity in survivors of Covid 19 disease, these effects are limited to 3-6 months.

Keywords: Bioelectrical impedance analyzer, phase angle, coronavirus disease, fat mass.

INTRODUCTION

In December 2019, unknown viral pneumonia appeared in the Chinese province of Wuhan, which later turned into an epidemic that spread worldwide (1, 2). The Chinese authorities have identified a new type of coronavirus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (3). On February 11th, 2020, the infectious disease caused by this viral virus has been officially designated COVID-19 (Coronavirus Disease 2019) by the World Health Organization (WHO) (4). To date, COVID-19 has spread over at least 180 countries and caused the death of millions of people, and the World Health Organization has officially declared a pandemic of the viral disease COVID-19 (4).

According to the analysis of nearly 45,000 confirmed cases, 19% of the COVID-19 patients has been identified as severe cases and critical illness cases involving severe pneumonia and metabolic disorders, developing into acute respiratory distress syndrome (ARDS) and multi-organ disorder (5–8). In another study, basal metabolic rate (BMR) levels were measured, and BMR was observed to change in male and female migrant workers, quarantined during the COVID-19 pandemic. However, no study has been conducted in the literature on the phase angle parameter, which gives the ratio of damaged cells in the body and physical strength of the body, as well as Basal Metabolic Rate and body composition values (body fat mass, muscle mass, extracellular fluid, intracellular fluid) in individuals who survived COVID-19.

In a study conducted in Shenzhen (China), obesity was associated with a 142% higher risk of developing severe pneumonia (9). The National Audit and Research Center for Intensive Care in the United Kingdom observed that 72.1% of confirmed COVID-19 cases were overweight or obese, and 60.9% of patients with a body mass index (BMI)>30 died in intensive care (10). Among the 4103 patients in New York City, BMI> 40 kg/m² was observed to be the second strongest independent predictor for hospitalization following old age (11). In a retrospective and single-centered study, evaluating 124 consecutive patients in France, 47.6% of the cases had obesity (BMI> 30 kg/m²) and 28.2% had severe obesity (BMI> 35 kg/m²). The need for invasive mechanical ventilation was associated with a BMI of 35 kg/m² (11). Previously,

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Figure 1. Significantly altered parameters were found between those who had the disease between 1-3 months and those who had it between 3-6 months.

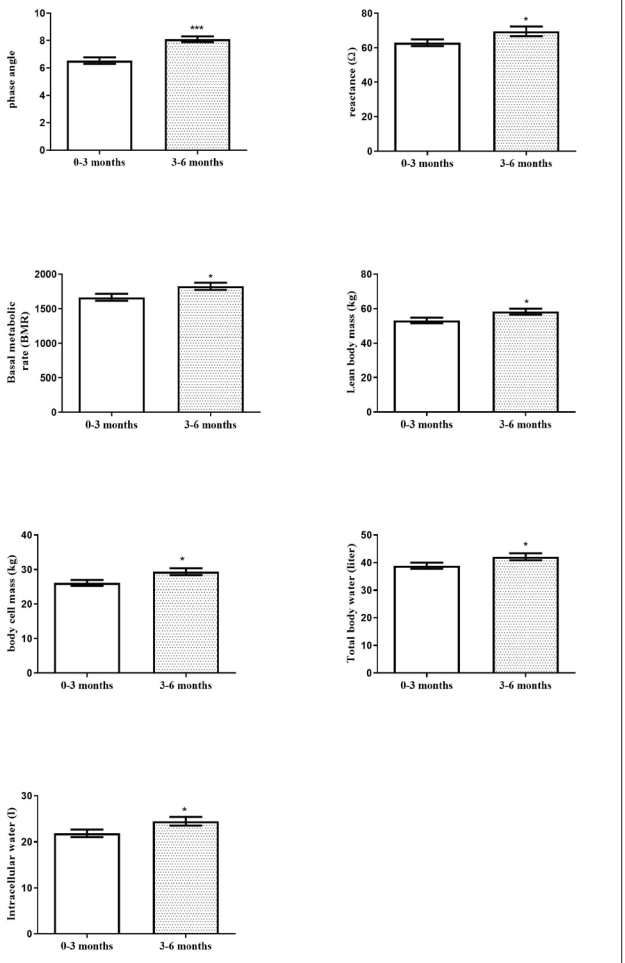
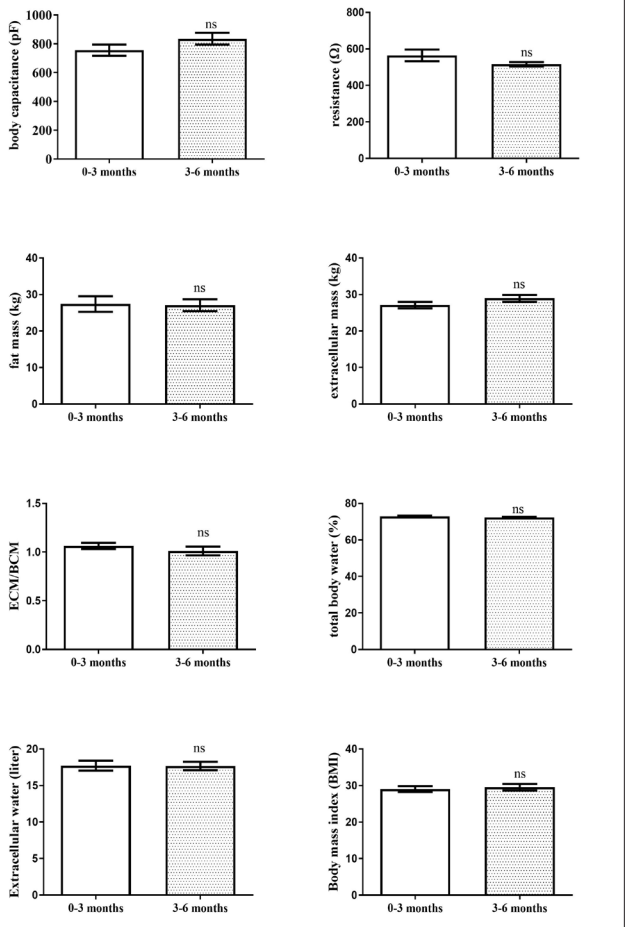


Figure 2. Parameters that did not change significantly between those who had the disease between 1-3 months and those who had it between 3-6 months.



numerous reports around the world have defined obesity and severe obesity as risk factors for hospitalization and mechanical ventilation in the H1N1 influenza virus (12).

An increased glucose level in a study in which data of mild and severe COVID-19 cases, as well as young children suffering from COVID-19, were analyzed to explore their metabolic changes and immune profiles. The increased glucose is partly due to the cells' reduced glucose consumption. This, in turn, indicates that the metabolism has changed. The findings show that acute respiratory distress syndrome (ARDS) I-III and glucose, lipid, uric acid, etc. metabolic disorders, even multiple organ dysfunction (MODS) and disseminated intravascular coagulation (DIC) is common in severe cases (13,14).

In one study, extraordinarily low serum uric acid was observed in severe cases [176μmol / L (IQR, 131-256)], and extremely low CD4 + T-cells and CD8 + T cells, but unusually high neutrophils [6.5 × 10^9 / L (IQR, 4.8-9.6)] procalcitonin [0.27 ng/mL (IQR, 0.14-1.94)], C-reactive protein [66 mg/l (IQR, 25-114)] and extremely high interleukin-6 levels were observed in the late stages and in severe cases of COVID-19. Diabetes comorbidity was observed in three patients, and high blood glucose was observed in 18 patients who had no diabetes mellitus [7.4 mmol/L (IQR, 5.9-10.1)]. In severe cases (71%), glucose was found in the urine and urinary ketone in nine (43%) out of 21 patients. The increased glucose was partly due to the cells' reduced glucose consumption. The findings suggest that severe cases have acute respiratory distress syndrome (ARDS) I-III and glucose, lipid, uric acid, etc. metabolic disorders, even multiple organ dysfunction (MODS) (15).

Regarding the immune response, there is a clear, and innate association between obesity and chronic inflammation, which can alter adaptive immune responses and make the immune system more vulnerable to infections. Obesity is associated with low-level inflammation due to adipocyte hypoxia and dysfunction. This, in turn, results in a strong secretion of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL) 1β and interleukin 6, as well as adipokines, which leads to the aggregation of immune cell macrophages, T cells, and B cells (16).

The aim of this study is to determine the physical strength of the body, basal metabolism change, body composition change, and damaged cell ratio by measuring all these parameters.

METHODS

In this study, parameters such as phase angle, which gives the ratio of damaged cells, basal metabolism, and body composition values (body fat mass, muscle mass, extracellular fluid, intracellular fluid) were determined by measuring through BIA (Bioelectrical Impedance Analyzer, BIA-450) in individuals who had COVID-19 infection. Subjects were selected from individuals in the 18-60 age group, who had survived COVID-19 disease 1-3 months and 3-6 months ago, 33 individuals who had survived it 1-3 months ago, and 30 individuals who had survived it 3-6 months ago were included in the study. The study groups were composed of different individuals. The measurement is performed by connecting an electrode to one hand and one foot of an individual. The measurement takes about one minute, and nothing is administered to the patient, and the BIA shows the results digitally in real-time. This study was approved by Gaziantep University Medical School Medical Ethics Committee with the decision numbered 2016/276 (Date: 17 October 2016, Protocol Number: 276) and supported by Gaziantep University Scientific Research Projects Unit (TF.DT.17.11).

The BIA technique measures the whole-body impedance, that is, the body's resistance to an alternating current consisting of two components: resistance (R) and reactance (Xc). The resistance refers to the voltage drop caused by conduction through ionic solutions. Reactance refers to the delay of the current flow, which is measured as a phase shift, reflecting the dielectric properties of cell membranes and tissue interfaces. The most commonly used and clinically relevant impedance parameter obtained with BIA is the phase angle. In the past, the measurement of the phase angle has been applied to many clinical settings with evidence of good reliability as a marker of nutritional status and a predictor of poor clinical outcomes (17). However, changes in hydration and obesity have been shown to limit the reliability of phase angles in everyday clinical practice (18).

Statistical Analysis

Graph Pad Prism Software had been employed for statistical analyses. Differences between means were expressed by Student's t-test. All results were expressed as means ± standard error of mean (SEM). Statistical differences were set at $p < 0.05$.

Main Points:

- It has been observed that Covid-19 disease significantly affected the percentage of healthy cells in the short term and in those who survived after disease.
- Covid-19 drastically changed the basal metabolism, body resistance, body cell mass and phase angle values in short-term.
- It has been found that these devastating effects of the Covid-19 are only short-term, and that whole-body composition parameters returned to normal in the long term.

RESULTS

Effects of COVID-19 on basal metabolism and body composition, and the ratio of damaged cells in the body after the disease were determined. Basal metabolic rate, lean body mass, body cell mass, total body fluid, intracellular fluid, and phase angle values were found to be statistically significantly increased in 3-6 months compared to that of the 1-3 months' range (Figure 1 and 2).

These results show that the basal metabolism and body composition parameters of the body become better, and the proportion of damaged cells decreases in time, after suffering COVID-19, reaching values close to normal levels in 1-3 months and much better levels in 3-6 months (Figure 1 and 2).

DISCUSSION

At the first encounter with a new pathogen, the energy requirements of the immune system of an individual increase significantly. For every 1 °C increase in body temperature, a more than 10% increase is seen in metabolic rate. Then, the activation of adaptive immunity leads to rapid and extensive cell growth, and the proliferation of virus-specific T and B lymphocytes, which intensifies energy metabolism in the cells according to their sizes (19).

Immune cells also need the energy to perform various special effector functions, such as migration, phagocytosis, etc. In total, the total energy cost of the entire immune system in a motionless state is approximately 20% of the daily average of the total metabolism rate at rest, whereas inflammation may lead to a 25-60% increase in energy consumption associated with the immune system (from mild inflammation up to sepsis) (20, 21). Physical fitness is necessary for our body to perform various daily activities properly and not to get sick easily. However, if the case of consumption of unbalanced foods, problems will arise and certainly increase the risk of becoming overweight. The level of energy needs is measured using the basal metabolic rate (BMR) method. Individual BMR is affected by some factors, such as body weight and gender (FAO, 2001). Basal metabolism is the minimum energy that an individual must have in order to maximize the basic functions of the body. Regarding the proper food intake, excess weight is triggered by unbalanced consumption of foods, such as high fat, plenty of carbohydrates, and low fiber, without a balanced expenditure of energy, such as physical activities. A person with a BMI (Body Mass Index) of more than 23 (23 - 24.9) is classified as overweight. On the other hand, according to the Asia Pacific criteria (P2PTM, 2018), a BMI value above 25 (> 25) is considered obesity. The WHO 2020 data show that more than 1.9 billion adults aged 18 and over were overweight, and more than 650 million of them suffered from obesity in 2016 (22).

Physical exercise is the best alternative natural treatment to increase body immunity against the COVID-19 virus. This virus is known to attack the immune system of the body. It is expected that physical exercise can increase immunity NSCA (2020). Performing outdoor sports carries a great risk of spreading COVID-19. However, it can still be performed by the protocol. An appropriate physical activity is necessary to increase immunity against COVID-19 (23).

Adequate physical activity will have a good relationship with the nutrients absorbed by the body. It has also been revealed that the immune function during obesity is associated with impaired immune response, which leads to excessive adiposity (24).

The physical activity and physical fitness performed are closely related to the bodyweight, which is associated with food intake. If the food intake into the body is not consumed through physical fitness and activity, it will probably result in obesity or overweight, which will negatively affect health (25). The habit of an individual to engage in physical activity is a method of improving his/her immune system or immunity. Performing a physical activity can promote a healthy lifestyle and promote healthy behaviours in society (27). As for the BMR values, the average calorie for the basal activities during the COVID-19 quarantine period is 1669 kcal/day for men and 1335 kcal/day for women. In a study on migrant workers, individuals were allowed to performed physical exercise during the quarantine period, and the average calorie burnout during the 14-day COVID-19 quarantine was found to be 2595 kcal/day for men, and 2031 kcal/day for women. The findings show that physical activity performed for immunity against bacteria and viruses also strengthens the body and will be important in protecting against viruses (26).

The immune system is an important factor against the physiological functioning of bacteria, viruses, and foreign substances through a complex and multi-layered mechanism. The human immune system has two parts, the adaptive and acquired immune systems. The immune system is also affected by nutrition, psychological factors, environment, physical exercises, or activities (27).

In cases of immediate danger (real or imaginary), the central nervous system can also have profound immunosuppressive effects through bioenergetic limitation. The psychomotor activity caused by acute psychological stress, sleep disturbance, pain, and anxiety can cause additional energy consumption of up to 30% of the basal metabolic rate (28). Fear of being infected by an "invisible virus", feeling helpless and isolated in pandemic conditions can also contribute to immune dysregulation (28).

In general, the energetic, structural, regulatory, and psychological negative factors can critically limit the ability of the immune system to remove the infection. Gradual energy depletion leads to functional depletion of immune cells and, ultimately, cell death (lymphopenia). Similarly, disruption of immune function is characteristic in chronic infections, autoimmune diseases, and oncological diseases, but this occurs over much longer periods (29).

In the COVID-19, the inability of the adaptive immunity to take control of the infection quickly causes uncontrolled viral spread, which in turn leads to secondary pathological hyperactivation of innate immunity (cytokine storm), acute respiratory distress syndrome, acute injuries in the heart, kidney, and other organs. So far, there are only a few studies investigating the body composition and prognostic relationship in hospitalized COVID-19 patients (29).

One study found that a lower phase angle in the care unit increases the likelihood of severe COVID-19 (22). Another recent study reports that the low phase angle (<3.95°), which was determined by BIA, is an important predictor of mortality regardless of age in hospitalized COVID-19 patients (median age 69, IQR 59-71) (23). On the contrary, in a retrospective study of 90 hospitalized COVID-19 patients (mean age 65±14 years), a low phase angle was not found to be associated with longer hospitalization, or intensive care unit admission and death (24). In our study, the phase angle was associated with an increased risk of death at 60 days in a univariate model, but the statistical significance disappeared after the adjustments made for age and gender. The phase angle is directly associated with lean body mass (LBM) and body cellular mass (BCM) but is inversely proportional to extracellular water (ECW) and intracellular water (ICW) in healthy adults (13). Disease-related malnutrition is characterized by an early shift of fluids from the ICW to the ECW cavity, an increase in ECW/ICW, and simultaneously a decrease in BCM and phase angle (29).

The changes in the physical resistance of the body, body fluid mass, body fat mass, basal metabolic rate, and damaged cell rate in individuals who have survived this disease are also of great importance.

CONCLUSION

In this study, basal metabolism, physical resistance of the body, body fat mass, intracellular and extracellular fluid mass, and damaged cell levels were investigated in individuals who had survived COVID-19. If there are significant deviations from the normality, its course over time is important. Therefore, they were investigated in this study. Parameters such as basal metabolism rate, reactance (cell membrane resistance), total body water, intracellular water, lean body mass, body cell mass were found to be significantly increased in patients who had COVID-19 1-3 months ago compared to those who had COVID-19 3-6 months ago. The phase angle parameter, which indicates the ratio of damaged cells, was also found to be increased. An increase in the phase angle indicates a decrease in the number of damaged cells. A phase angle value of 6-8° indicates very few damaged cells, 8-10° indicate no damaged cell and 3-6° indicate many damaged cells. This study shows that the parameters have improved over time, the physical resistance of the body has increased over time, and the proportion of damaged cells has decreased significantly.

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Conflict of interest: The authors declare that there is no conflict of interest.

Authors' contributions: Conception: CS, ŞK; Design: CS, ŞK; Materials: YG; Data Collection and/or Processing: YG; Analysis and/or Interpretation: CS, YG, ŞK; Writing: CS, YG, ŞK; Critical Review: CS.

Ethical statement: This study was approved by Gaziantep University Medical School Medical Ethics Committee with the decision numbered 2016/276 (Date: 17 October 2016, Protocol Number: 276) and supported by Gaziantep University Scientific Research Projects Unit (TF.DT.17.11).

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Original Research

Effects of Moderate Exercise Training on ApoE and ApoCIII in Metabolic Syndrome

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ABSTRACT

Objective: Metabolic syndrome (MetS) is an endocrinopathy with a combination of cardiovascular and metabolic compounds. In our study, it is expected to obtain results showing that mortality rate, loss of workforce, and treatment costs due to disorders caused by MetS can be reduced by physical exercise. The study analyses the effect of moderate exercise training on this Apolipoprotein E (ApoE), Apolipoprotein CIII (ApoCIII), adiponectin, resistin, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) which are thought to have a role in the deterioration of glucose and lipid metabolism in MetS.

Methods: This clinical experimental study consists of 3 groups. The MetS+E (n=24) group, which included the participants who agreed to participate in the exercise program in addition to their medical treatment, the MetS (n=23) group who received medical treatment but did not exercise, and the Control+E (n=25) group, which included healthy volunteers who had the same protocol as MetS+E. ApoE, ApoCIII, adiponectin, resistin, IL-6, and TNF-α plasma levels of all participants were measured both at the beginning of the study and at the end of the protocol.

Results: At the end of the study we reached the following findings; insulin and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) levels decreased in exercise groups (p=0,03). ApoCIII levels are increased all the groups after the study (p<0,01). IL-6 levels decreased in MetS+E (p<0,01) and Control+E (p=0,037). ApoE (p=0,01) and TNF-α (p=0,037) levels decreased only the Control+E group.

Conclusion: Training showed metabolic, anti-inflammatory and physical improvements independent of ApoE and ApoCIII in those with MetS.

Keywords: Adipokines, Apolioprotein CIII, Apolipoprotein E, exercise, metabolic syndrome.

INTRODUCTION

Many adipokines and cytokines with paracrine, endocrine and neural effects are synthesized, and released in adipose tissue. It has been reported that adipose tissue has important roles in inflammation, immunity, cardiovascular and neuronal homeostasis[1].

Metabolic syndrome (MetS), which was named syndrome X for the first time by Reaven[2], according to the World Health Organization (WHO) 1998 definition; Type II diabetes mellitus (DM) is a disease complex accompanied by hypertension, dyslipidemia, abdominal obesity, microalbuminuria, which is the basis of at least one of the disorders of insulin resistance and glucose intolerance. Most of the factors that cause the disease are preventable. The modern sedentary lifestyle is a potential risk factor for the development of MetS. Training programs that include aerobic exercise are involved in the treatment and prevention of MetS.

In this study, we aimed to find the effect of moderate aerobic exercise recommended in addition to medical treatment in patients with MetS, on the levels of Apolipoprotein CIII (ApoCIII) and Apolipoprotein E (ApoE), which are apolipoproteins that are claimed to have a role in the disorder of glucose and fat metabolism. Our hypothesis; was that the training we applied led to an increase in the ApoE / ApoCIII plasma ratio. Another hypothesis is; As a result of the aerobic training program, an increase in adipokine plasma levels and a decrease in resistin, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) levels. Thus we aimed to contribute to elucidating the mechanisms that have a role in the protective and therapeutic effect of exercise in MetS.

METHODS

Ethics committee approval was obtained from Ankara University Clinical Research Ethics Committee (25 January 2016, approval number 02-57-16) and conformed to the most recent version of the Declaration of Helsinki. All of the volunteers who agreed to

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participate in the study were given the necessary information before starting the study and their written consent was obtained.

Experimental Design

Participants included in the study were sedentary patients who were diagnosed with MetS (according to WHO 1999 MetS diagnostic criteria) between the ages of 18-40 years from Ankara University Faculty of Medicine, Department of Endocrinology and Metabolism outpatient clinic from 2016-2018. This study, which was designed as experimental clinical longitudinal research, consisted of an MetS intervention group (MetS+E), MetS control (MetS) group and a healthy sedentary control group (Control+E). Among the patients diagnosed with MetS, participants who agreed to participate in the exercise program were included in the MetS+E (n=24) group and those who did not want to exercise were included in the MetS group (n=23). Control+E (n=25) group consisted of healthy sedentaries. The same exercise protocol was applied to this healthy group as the MetS+E group.

Participants

A total of 134 volunteers were included in the study. The number of participants who completed the study was 72. Those participants who did not complete the study either left voluntarily or were removed because they could not adapt to the exercise regime and disrupted the program. All of the participants in our study were asked whether they were sedentary according to the International Physical Activity Questionnaires (IPAQ- short physical activity scale)[3]. Subjects who were not sedentary, and who had cardiovascular and lung diseases, pathological findings in the exercise test in the last two years, malignancies, psychotic disorders, muscle-nervous system disorders that may prevent exercise, hemoglobin level below 10 g/dl in women and 12 g/dl in men, infectious diseases, any chronic inflammatory disease, any hematological disease, history of taking lipid-lowering drugs, and usage of antidepressant drugs were excluded.

The demographic data including age, sex, and clinical data including co-morbidities and smoking habits (current smoker/non-smoker) were recorded and anthropometric data (height, weight, body mass index and waist circumference) were measured for all participants.

Main Points:

- Does a 12-week exercise program cause a change in ApoE and ApoCIII levels in individuals with metabolic syndrome?
- If there is a change, will this be reflected in the lipoprotein metabolism?
- While treadmill exercise caused a decrease in ApoE plasma levels in healthy sedentary people, it did not make a difference in individuals with MetS.
- Pro-inflammatory cytokines, body composition and metabolic improvements were noted in healthy exercisers and individuals with MetS.

Experimental Procedure

The body composition analyzes of participants (fat ratio, body mass index, basal metabolic rate) were determined using a Tanita BC420 MA device 1-3 days at the initiation of the study and the completion of the study. Blood samples were taken from participants in the morning after 12 h of fasting for the parameters to be evaluated in the study, 1-7 days before the initiation of exercise program and 1-3 days after the completion of the 12th weeks exercise program. Blood samples taken for ApoE, ApoCIII, adiponectin, resistin, IL-6 and TNF-α evaluated by ELISA method were centrifuged at 1,000 g x 15 min, and plasmas were separated and stored at -80 °C.

The electrocardiography recordings of the volunteers in the Control+E and Mets+E groups in the exercise program were taken with a Nihon Kodlen 6551-ECG before the exercise protocol and at the end of the 12th week to determine the fitness level of the cardio-circulatory and respiratory systems. In addition to medical treatment, the participants were monitored with the mobile phone application of the same company with Polar brand H7 model heart rate monitors under the supervision of a sports physician. The exercise protocol was performed in the form of aerobic treadmill exercises for 12 weeks, 4 days a week, 30 minutes a day at 50% VO_2max load for the first four weeks, 60% maximal oxygen consumption (VO_2max) load between the fourth and eighth weeks, and 70% VO_2max load for the final four weeks.

Measurements

Total cholesterol, triglyceride, VLDL, LDL, HDL, fasting and postprandial blood glucose levels were measured by autoanalyzers (Beckman Coulter DXI800, Beckman Coulter DXC 800). While calculating the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), the formula HOMA-IR= (Fasting glucose (mmol/L) x serum fasting insulin (mUI/L)/405 was used[4].

The Modified Bruce protocol was used to determine the VO_2max[5].

In ELISA measurements, Elabscience brand E-EL-H0470 for ApoE, Elabscience brand for ApoCIII, E-EL-H0467, ELISA Ebioscience brand for Resistin, BMS2040/BMS2040TEN, Ebioscience brand for Adiponectin, BMS2032-2TEN, Ebioscience brand BMS213 for IL-6- 2- For BMS213-2TEN, TNF-α, we used Ebioscience brand kit with catalog number BMS223/4 / BMS223/4TEN. All ELISA analyses were evaluated at a wavelength of 450 nm. ApoE, ApoCIII, adiponectin results were calculated as ng/mL, resistin, IL-6 and TNF-α results were calculated as pg/mL.

Data and Statistical Analysis

The descriptive statistics on numerical measurements, differences between independent groups from those whose variables did not conform to normal distribution were evaluated using Kruskal Wallis Analysis of Variance. Wilcoxon Sign Rank test was used for those who did not show normal distribution in the comparisons of preprotocol and postprotocol values. SPSS 21.0 package program was used for statistical analysis results (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Type-I error level for testing the two-way hypothesis tests was taken as α=0.05.

Table 1. Characteristics of the participants

	Control+E (n=25)	MetS+E (n=24)	MetS (n=23)	p
Age (years)	26,80±8,30	28,29±7,82	32,83±8,23	0,055
Sex (F/M)	(22/3)	(15/9) *	(12/11) *	*0,019
Dyslipidemic	1	15**	14**	**0,000
DM and/or insulin resistance	4	24**	23**	**0,000
Sedentery (IPAQ (Short))	24	24	23	0,391
Medications				
Metformin	-	6*	4*	*0,036
Statin	-	-	2	0,114
Hypertensive agents	4	11*	11*	*0,036

Control+E; Control + exercise group, MetS+E; metabolic syndrome + exercise group, MetS; metabolic syndrome group, F; female, M;male, DM; diabetes mellitus *Significant difference with Control+E (p<0,05). **Significant difference with Control+E (p<0,01). IPAQ=International Physical Activity Questionnaire

RESULTS

The groups were age-matched, but female predominance was seen in the Control+E group when compared to the MetS groups (p<0,05). All participants were sedentary according to the IPAQ (Table 1).

Data obtained from bioimpedance measurements are given in Table 2. Body mass index (BMI), total body water (TBW), visceral fat rate (VFR). Basal metabolism rate (BMR), BMI, VFR, and fat% values before and after the exercise programme; MetS and MetS+E groups was significantly higher than the Control Group (p<0,001) (Table 2). A significant decrease in BMI, VFR and BMR values was only observed in the MetS+E group (p<0,001).

At the end of the study, an increase in HDL, HOMA-IR and decrease in insulin were observed in the MetS+E Group. A decrease in insulin, HOMA-IR and LDL was observed at the end of the program in the Control+E Group (Table 3). IL-6 levels of the Control+E Group were lower both before and after the program compared to the MetS and MetS+E groups (p=0,048). MetS+E Group showed a decrease in IL-6 (p=0,000) at the end of the program (Table 4). In addition, at the end of the program IL-6 (p=0,014) and TNF-α (p=0,042) values of the MetS+E Group significantly lower than MetS Group (Table 4). When ApoCIII end-of-study data were compared between groups, MetS (p<0,01) and MetS+E (p<0,05) groups were found lower than the Control Group, and there was a significant increase in all groups (p<0,01). The only difference noted in ApoE is the decrease in the Control+E Group (p<0,05) (Table 4).

There was no difference in resistin between the groups before and after the exercise program. In addition, there was no differ-

Table 2. Body compositions (Mean±S.D)

Parameters	Control+E (n=25)		MetS+E(n=24)		MetS(n=23)		p
	Before	After	Before	After	Before	After	
BMI kg/m²	23,71±4,05	23,38±3,91	32,92±6,01**	31,59±6,15** ##	34,91±7,38**	34,21±6,64**	##0,000 **0,000
VFR	3,22±2,60	3,14±2,62	9,33±4,81**	8,52±4,41** ##	9,31±4,35*	9,94±4,21 **	##0,002
TBW %	51,73±5,61	51,06±6,58	45,80±4,38*	46,23±4,72*	47,09±4,66*	47,48±4,68*	*0,04
Muscle %	69,95±7,88	67,52±16, 39#	60,45±6,59 **	61,40±7,45**	62,90±6,07**	63,50±6,34**	**0,009 # 0,014
Fat %	26,41±8,16	26,00±7,39	36,34±6,96**	35,42±7,90**	34,56±7,37**	36,65±7,10 *	*0,02
BMR	1329,40±118,86	1341,8±0125,00	1887,20±347,74**	1870,80±345,58**##	1807,80±279,34**	1811,80±309,60**	##0,000 **0,000
BMR/BMI	61,68±10,15	62,11±9,96#	56,54±10,04	55,29±16,10	56,61±9,29	54,70±8,96	#0,040

Control+E; Control + exercise group, MetS+E; metabolic syndrome + exercise group, MetS; metabolic syndrome group, BMI; body mass index, VFR; visceral fat rate, TBW; total body water, BMR; basal metabolic rate. *Significant difference with Control+E (p<0,05). **Significant difference with Control+E (p<0,01). #Significant difference with before the protocol (p<0,05). ## Significant difference with before the protocol (p<0,01).

Table 3. Blood values and systolic and diastolic arterial blood pressure values of the participants

Parameter		Control+E (n=25)		MetS+E (n=24)		MetS (n=23)		p pairwise
		Before	After	Before	After	Before	After	
Insulin µIU/ml	Mean±S.D	11,00±2,32	9,15± 0,38	21,47±6,10 **	18,19± 5,12**	19,37±8,33**	18,50±13,11**	**0,000
	Median (min-max)	0,964(0,172-2,94)	8,52(3,13-14,79)	1,313(0,11-2,94)	16,445(2,7-34,18)	2,563(0,172-12,356)	12,81(8,99-62,31)	
	p group within	0,030		0,033		0,114		
HOMA-IR	Mean±S.D	2,24±0,96	1,81±0,58	4,79±2,73 **	3,90±1,98 **	5,18±3,44**	4,49±3,06**	**0,000
	Median (min-max)	2,32(0,62-5,37)	1,72(0,68-3,14)	3,93(0,87-13,47)	3,37(0,46-7,09)	3,72(1,53-18,72)	3,05(1,91-11,66)	
	p group within	0,040		0,027		0,028		
FBG mg/dl	Mean±S.D	81,68±8,00	81,08±5,50	88,75±1,00 **	85,17±6,00** δ	105,32±38,87**	99,86±36,58**	δ 0,027 **0,001
	Median (min-max)	82(67-92)	80(69-93)	87(75-141)	84(69-114)	92(76-226)	90(65-287)	
	p group within	0,879		0,034		0,480		
SBP mmHg	Mean±S.D	113,52±12,44	112,08±10,59	125,29±12,82**	123,83±11,09**	124,56±16,23**	123,86±15,44**	**0,001
	Median (min-max)	110 (100-140)	110 (100-140)	122,5 (110-160)	120 (110-155)	120 (99-160)	120 (99-160)	
	p group within	0,493		0,130		0,348		
DBP mmHg	Mean±S.D	71,48±9,75	70,83±8,20	82,38±12,38**	80,50±10,60**	81,70±11,08**	80,30±10,97**	**0,004
	Median (min-max)	70 (60-90)	70 (60-85)	80 (60-120)	80 (60-115)	85 (60-98)	80 (60-95)	
	p group within	0,483		0,077		0,072		

HDL mg/dl	**Mean±S.D**	53,54±10,44	56,04±8,84	45,84±11,30*	47,00±9,14*	47,68±16,45*	46,33±12,46*	*0,04
	Median (min-max)	49(37-74)	57(37-72)	43,5(31-60)	46(31-65)	45(0-92)	44,5(25-69)	
	p group within	0.112		0.036		0.669		
LDL mg/dl	**Mean±S.D**	100,66±25,57	94,45±27,31	120,58±45,95** δ	116,17±46,19** δ	114,05±29,08**	115,66±30,39**	** 0,000 δ 0,000
	Median (min-max)	97(52-147)	87(51-163)	112,5(65-280)	106,5(75-287)	124(41-154)	109,5(67-196)	
	p group within	0.044		0.605		0.548		
VLDL mg/dl	**Mean±S.D**	18,56±7,64	18,54±8,91	29,42±15,01*	26,83±9,98*	45,95±45,35**	32,86±27,19**	*0,06 **0,009
	Median (min-max)	17(8-38)	18(7-38)	25(12-70)	29(11-43)	35(7-224)	21,5(9-123)	
	p group within	0.827		0.513		0.064		
Cholesterol mg/dl	**Mean±S.D**	170,92±26,96	167,95±31,93	185,21±60,87*	190,79±45,62	200,59±27,30**	183,66±34,73	** 0,001
	Median (min-max)	173,5(115-231)	165,5(104-247)	191(124-355)	181,5(131-339)	205(114-246)	193(102-227)	
	p group within	0.175		0.485		0.010		
Triglycerides mg/dl	**Mean±S.D**	92,83±0,97	88,72±0,58	151,13±72,58**	138,13±52,41**	224,82±217,63**	165,86±142,66**	** 0,003
	Median (min-max)	84 (38-190)	78 (33-192)	127 (62-350)	130,5 (57-221)	166,5 (36-1068)	105 (43-637)	
	p group within	0,360		0,074		0,082		

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, Control+E; Control + exercise group, MetS+E; metabolic syndrome + exercise group, MetS; metabolic syndrome group *Significant difference with Control+E (p<0,05). **Significant difference with Control+E (p<0,01), #Significant difference with before the protocol (p<0,05). δ Significant difference with MetS (p<0,01).

Table 4. IL-6, TNF-α, Adiponectin, Resistin, ApoE and ApoCIII values of the participants before and after the study (Mean±S.D).

Parameters		Control+E (n=25)		MetS+E (n=24)		MetS (n=23)		p pairwise
		Before	After	Before	After	Before	After	
Adiponectin ng/ml		26831±18.252	24161±15.519	20168±17.636	17397±14.969	16769±10.490	17274±12.449	>0,05
	Median (min-max)	26450(4825-83825)	21890(2560-59500)	15507(1710-79300)	12325(1585-50970)	15672.5(2560-49380)	14437(1725-45825)	
	p value group within	0.619		0.475		0.864		
Resistin pg/ml		4075,74±2729	2958,06±843	4095,96±867	3737,66±1765	3245,35±1.352	4737,28±3405	>0,05
	Median (min-max)	3209(798-11739)	2367(315-7048)	3567(788-7502)	3476(460-10961)	2977(1468-5737)	3593(1187-13799)	
	p value group within	0.088		0.346		0.130		
ApoE ng/ml		717,76±116	558,66±11,84	812,57±315,24	822,67±164,56	744,40±559,97	988,96±824,62	>0,05
	Median (min-max)	733(104-2645)	450(41-1730)	791(126-2063)	767(11-2346)	724(97-2435)	689(128-2911)	
	p value group within	0.010		0.954		0.346		
ApoCIII ng/ml		58,81±5,18	258,46±88,52	41,87±0,44	229,37±125,96	33,18±50,80	143,34±113,04	**0,002
	Median (min-max)	14.15(8.25-241.88)	192.82(10.18-624) ##	11.16(8.06-241.88)	215(13.56-576) ##*	10.73(8.70-181.06)	114.94(6.88-391.72) ##**	
	p value group within	0.002		<0.001		<0.001		
IL-6 pg/ml		1,60±0,49	1,06±0,27	2,61±0,87	1,40±0,40	3,72±3,86	3,61±3,45	* 0,02 δ 0,014
	Median (min-max)	1.41(0.234-3.774)	0.96(0.172-2.94)	2.45(0.484-8.968) *	1.31(0.110-2.940) δ	2.38(0.696-15.64) *	2.56(0.172-12.356) *	
	p value group within	0.037		<0.001		0.710		
TNF-α pg/ml		6,66±13,22	4,80±9,87	4,35±0,81	3,36±0,21	5,74±5,85	4,19±2,77	δ 0,042
	Median (min-max)	2.99(0.56-64.32)	1.834(0.018-47.394)	2.065(0.332-33.936)	2.214(0.562-25.74) δ	3.453(0.562-19.774)	3.148(0.28-10.334)	
	p value group within	0.037		0.855		0.475		

Control+E; Control + exercise group, MetS+E; metabolic syndrome + exercise group, MetS; without exercise metabolic syndrome group *Significant difference with Control+E (p<0,05). **Significant difference with Control+E (p<0,01), #Significant difference with before the protocol (p<0,05). ##Significant difference with before the protocol (p<0,01). δ Significant difference with MetS (p<0,05). ApoCIII; Apolipoprotein CIII, ApoE; Apolipoprotein E, Interleukin-6; IL-6, Tumor necrosis factor-α; TNF-α.

Table 5. VO2max and total effort time values of the participants before and after the study (Mean±S.D).

Parameters	Control+E (n=25)		MetS+E (n=24)		p
	Before	After	Before	After	
VO_2max (ml/kg/min)	40,33±6,19	56,12±6,38 ##	35,66±7,76	53,57±7,13 ##	##0,000
Total Effort Time (sec)	609,96±94,32	834,52±99,29##	567,58±115,82	821,42±104,59##	##0,000

Control+E; Control + exercise group, MetS+E; metabolic syndrome + exercise group. #Significant difference with before the protocol (p<0,05).
##Significant difference with before the protocol (p<0,01

ence in the initial state of the MetS+E Group in terms of gender, but there was a significant difference in terms of gender after the training group (Table 4). In the MetS+E group, by the end of the program there was a decrease in the resistin in men and an increase in the resistin in women (p=0,03).
In the exercise groups, both VO2max and total effort times increased at the end of 12 weeks (Table-5).

DISCUSSION

This study looks to contribute to the elucidation of of the mechanisms of the protective and therapeutic effect of moderate aerobic training, which is recommended in addition to medical treatment in MetS patients.Our expectation was an increase in the ApoE / ApoCIII ratio and adipokine plasma levels and a decrease in resistin, TNF-α and IL-6 levels at the end of the program. The average muscle percentage of the participants in the MetS+E group increased following the end the training program (p=0,009). One of the study's aims was to see participants lose weight through exercise. Confirming this, there was a significant decrease in BMI and VFR in the MetS+E group. However, no change was observed in MetS and Control+E (Table-2). This may be due to the increased catecholamine synthesis during exercise. Visceral fats are more sensitive to catecholamine-induced lipolysis, while subcutaneous fats are more sensitive to insulin-induced lipolysis[6].

Studies have shown that exercise at 50-60% VO_2max intensity provides the highest fat burning, reduction in body fat ratio, BMI and waist circumference and the same studies have shown that there is a negative relationship between VO_2max and body fat ratio[7,8]. As in the studies mentioned, we programmed our training load as 50% VO_2max for the first 4 weeks, 60% for the next 4 weeks, but we applied it as 70% VO_2max to see the effect of the increased oxygen consumption capacity from the eighth week, and as a result, we saw an increase in effort capacity in our training groups (Table-5). According to the literature, the fat burning rate on the treadmill is higher than on the bicycle[9]. For this reason, we applied the treadmill exercise for 12 weeks as an exercise type.

The significant increase in VO_2max and effort duration in the exercise groups compared to the baseline indicates that this exercise program increased the compliance of the cardiopulmonary system with the exercise (Table-5). This data is consistent with that in the literature that aerobic capacity increases with weight loss, decrease in BMI, and decrease in fat ratio[10].

In our study, a significant decrease was observed in the exercise groups in insulin and HOMA-IR values at the end of the program (Table-3). These findings are consistent with the literature[11]. It was thought that the training might have created an effect that could prevent hyperglycemia and decrease insulin resistance in the MetS+E group. In addition, at the end of the study, the fasting blood glucose (FBG) of the MetS+E group was found to be different from the MetS group. Insulin resistance is a condition that occurs as a result of overnutrition, includes multiple factors, and triggers inflammation[12]. The most important hormones regulating lipoprotein lipase (LPL) activation and expression are insulin and cortisol, and adipose tissue capillaries are rich in LPL[13]. Insulin inhibits lipolysis and stimulates adipocyte differentiation. It causes triglycerides to remain as storage in adipocytes. The training applied in this study appears to have a corrective effect on the impaired fat storage mechanism in patients with insulin resistance.

One of the protective effects of adiponectin from MetS may be that it activates the AMP-activated protein kinase (AMPK) pathway in skeletal muscle and increases GLUT4 translocation in an insulin-independent manner, as well as increasing fatty acid oxidation[14]. Although not significant, the increase in adiponectin recorded in our study may have had positive effects on the metabolism of cytokines and fatty acids. If we had taken a sample at the end of the first week in our study, perhaps we would have seen the increase in adiponectin. The results of some studies suggest that the 12-week exercise program we preferred in our study may be insufficient to show the change in adiponectin[8].
This study showed no observable difference, in terms of ApoE levels between MetS and the control group such as Boiko et al.[15] (Table-4). However, there was a significant decrease in the Control+E group (Table-4). Shiina and Homma[16] found no correlation between ApoE and MetS components. Onat et al.[17] found a relationship between serum ApoE concentration and ApoB, ApoA-I, waist circumference and MetS, excluding genetic factors. Onat et al.[18] suggest that high concentrations of ApoE play a role in making HDL dysfunctional. In other words, high ApoE levels alone are not protective for atherogenic dyslipidemia. Our Control+E group consisted of healthy young individuals. The decrease we observed in ApoE level after the training program in this group is in line with the results of Onat et al.[17]. We could not find any study

showing how ApoE plasma levels are affected by aerobic exercise in people with MetS. Studies that analyse the relationship between ApoE gene polymorphism and MetS have increased in recent years [19,20]. Son et al.[20] state that the polyformism they found in the rs769450 region of the single nucleotide ApoE gene is associated with MetS. In addition, they note that the triglyceride change caused by the same polymorphism is affected by physical activity. However, Reas et al.[21] could not determine a relationship between carrying the ε4 allele and exercise. There is no consensus in the literature between ApoE and MetS. In a case study involving a 10-week exercise program, a 38-year-old male Apoε4 carrier with MetS had significant improvements in MetS criteria at the end of the exercise program[22]. The closest findings ours are in this study; however, this study include data from only one individual and do not provide information about the plasma ApoE level.

While it is known that ApoCIII also inhibits hepatic lipase, there are publications stating

that it increases the hepatic uptake of VLDLs via cholesteryl ester transfer protein (CETP)[23]. There is a study that show the ratio of ApoE/ApoCIII to be lower in individuals with hyperlipidemic and hypertriglyceridemia when compared with the healthy control group[24]. In addition, Boiko et al[15]. found ApoCIII to be higher in MetS patients than in the healthy control group. We hypothesized that ApoCIII would decrease as a result of exercise and could increase triglyceride hydrolysis by improving the LPL function. Surprisingly we found a significant increase in ApoCIII levels in all groups. ApoCIII gene expression was found to be negatively correlated with insulin and positively correlated with glucose[24]. Caron et al.[25] argue that this increase may also suppress lipolysis and therefore peripheral fatty acid intake may remain low. However, there is a also study stating that insulin does not affect ApoCIII gene regulation[26]. This may be because the promoter regions of the ApoCIII gene for insulin and glucose are different. In addition, the relationship between ApoCIII and MetS may be due to DM[27]. The reason we could not find a difference between groups may be that most of the participants with MetS had insulin resistance, not DM. The lack of correlation between the increase ApoCIII and the baseline indicates that our exercise protocol affects ApoCIII through pathways independent of LPL, CETP, or hepatic lisape (HL).

Prior to the study, we observed that IL-6 levels were higher in MetS groups than in the control group and IL-6 level decreased significantly after the training program in the both exercise groups (Table-5). In addition, a moderate correlation was found between IL-6 and HOMA-IR (r=0.298). Subcutaneous adipose tissue and visceral adipose tissues can synthesize proinflammatory cytokines like IL-6 under stress[28]. IL-6 is a proinflammatory cytokine known to reduce insulin-dependent sugar intake, affect fat oxidation and lipid conversion[29]. Balducci et al.[29] showed that inflammatory biomarkers such as IL-6 were decreased as a result of long-term training in individuals with metabolic syndrome. IL-6 is also an inhibitor of LPL, which is an important element of fat metabolism. Due to the high level of IL-6, blood fats cannot be broken down. Fats cannot pass to the tissues where they will perform lipolysis and remain at high levels in the blood, and increased blood fats increase IL-6 synthesis. Thus, IL-6 causes a vicious circle in obese humans. Antioxidant capacity is low in with visceral obesity and fatty liver disease. One of the significant reasons for the decrease in IL-6 level in our results may be decreased visceral adipose tissue and BMI. The decrease in IL-6 level, especially with the effect of training consisting of aerobic exercise, is in line with our findings.

In obese individuals, hypertrophic adipocyte cells are hypoxic because their diffusion distances for oxygen are increased[13]. In addition, since the oxidative capacity of the muscles of sedentary individuals is lower, the breakdown of fats is also low. The increased exercise capacity and VO_2max with the training program suppressed oxidative stress and decreased IL-6 secretion from adipose tissue, thus the inhibitory effect on LPL may have been weakened.

There was no difference in TNF-α plasma levels between groups at the start of the study (Table-4). The Control+E group was the only group in which we found a significant difference compared to the pre-training program (Table-4). TNF-α, like IL-6, is an important proinflammatory cytokine that is an inhibitor of LPL. Another study found this cytokine to be significantly higher in patients with MetS than in healthy controls[30]. Giannopoulou et al.[11] found no difference in the resistin, adiponectin and TNF-α data obtained as a result of the diet+exercise and diet-only program. The exercise protocol they applied (the walking exercise they performed at a VO_2max level of 65-70% for 60 minutes a day 3-4 days a week) is a similar protocol to the treadmill exercise we chose.

In this study, we have obtained results such as increase in anti-inflammatory agents, weight loss, decrease in BMI and visceral obesity, decrease in insulin resistance and increase in oxygen consumption capacity. Some of the limitations we identified in our study are as follows; Most of the participants were housewives, students, and the unemployed, and the majority of the Control+E group consisted of women. The fact that women show more interest in the determination of health status and health promotion studies than men can be the subject of a separate behavioral study. Extending the training program to 16-24 weeks by making intermediate measurements may contribute to the findings of this study. In addition to the dietary recommendations in national and international health programs to build the bridge between our biological evolution and our cultural evolution, increasing the amount of physical activity should become an important goal.

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Original Research

Evaluation of the Knowledge and Practices About Drug Prescribing and Adverse Reaction Reporting Among Turkish Dentists

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ABSTRACT

Objectives: The purpose of this study was to assess dental care professionals' drug prescription knowledge, practices, and reporting of adverse drug reactions (ADRs).

Methods: A cross-sectional exploratory study was conducted by using a face-to-face survey administered to a sample of dentists from tertiary care hospitals in Adana, Türkiye. A questionnaire consisted of six sections with closed-ended items including sociodemographic characteristics, knowledge about drugs, patient history information, counseling practices during prescribing, source of information and ADR reporting.

Results: The study included 180 dentists, with a 95.3% response rate. More than half of the dentists (50.6%) stated their level of knowledge about drug price/cost as low. Most of the dentists (50.6%;n=91) claimed that they never/rarely asked about health insurance during patient history information. Moreover, most of the participants reported that they never/rarely and sometimes counsel the patients regarding drug mechanism of action, side effects of the drug, interaction of prescribed drug with other drugs/nutrients. It was determined that the dentists learned about the drugs from Vademecum (Turkish Medication Guidebook: 70%;n=126) and the internet (55%;n=99). A higher proportion (85.5%) of the dentists indicated that they did not report ADR during their clinical practice.

Conclusion: This study showed a general improvement in dentists prescribing knowledge and practices, although they reported some lack of knowledge regarding drug cost, discussion about the possible side effect of a drug/interaction with other drugs/nutrients with patients and under-reporting of ADRs. Periodic education and training for dentists are critical to overcoming any problem related to prescribing errors and potential ADRs.

Keywords: Prescribing; adverse drug reactions; dentists; hospital, oral health; survey.

INTRODUCTION

The World Health Organization (WHO) states that prescriptions should include the identity of the professional, the patient receiving a drug, administration mode, the pharmaceutical form, dosage details, the frequency of usage, treatment duration, and any instructions or information for the patient(1). Errors in medical prescriptions should be avoided at all costs, as they can lead to not only difficulties and errors in medication dispensing, and improper drug use that can reduce the efficacy and safety of treatments and also increase the risks and healthcare costs(2).

Dentists typically prescribe medications to alleviate pain and/or combat infection(3). Dentists treat any condition that affects oral health by making specialized clinical decisions that combine surgical/operative interventions and medications(4). They play a crucial role in delivering high-quality dental care and individualized high-quality medication use(3,4). As a result, to choose the best treatment choice for each patient, dentists must constantly advance their understanding of medications, including side effects, contraindications, and interactions(2-4).

Patients' health and quality of life may suffer as a result of dentists' lack of knowledge about side effects, indications, and contraindications(5,6). It is reported that previous studies carried out around the globe documented many prescription errors including incorrect drugs, dosage regimens, duration of treatment leading to antimicrobial resistance, prolong hospitalization, and adverse patient outcomes(6). Additionally, dental doctors pre-

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scribe a variety of therapeutic interventions, including allopathic medicines like local anesthetics, analgesics, antibiotics and anti-inflammatory drugs. It is indicated that the use of antibiotics and analgesics is one of the most typical causes of adverse events. Therefore, the risk of adverse drug reactions (ADRs) in dentistry cannot be ignored, and dentists' contributions to improving the spontaneous reporting of ADRs cannot be undervalued(7).

Increased unnecessary pharmaceutical spending and costs are thought to be primarily the result of irrational prescribing practices, especially in countries with already overburdened healthcare systems like Türkiye(8). Despite the dentist's critical role in patient safety, pharmacovigilance and ADR reporting are the least understood and practiced in dentistry(9). To the best of our knowledge, studies assessing knowledge, counseling practices regarding prescribing, and adverse drug reaction reporting among Turkish dental care providers are scarce. Therefore, the main objective of our study was to evaluate the drug-prescribing knowledge and practices, as well as adverse drug reaction reporting among dental care practitioners in Adana, Türkiye.

METHODS

A cross-sectional exploratory study was conducted between 10 July to 10 November 2018 using a face-to-face survey administered to a sample of dentists from two tertiary care hospitals in Adana, Türkiye. Full-time registered dentists with at least one year of work experience in two tertiary care hospitals were included in this study. Healthcare students on a traineeship and part-time registered dentists, who had less than 1 year of work experience and were unwilling to participate were excluded.

As per hospital data, there were 105 working dentists in Fatma Kemal Timuçin Oral and Dental Health Hospital and 84 in Cukurova University Dental Faculty Hospital during the study period. The study was approved by the Ethical Committee of Cukurova University, Adana, Türkiye (Meeting number 79, decision number 43, dated 06-07-2018) ***(Supplementary file 1).*** We entered this information into the Epi Info™ software (Centers for Disease Control and Prevention, Epi Info™) and calculated a minimum of 127 participants, considering a hypothesized % frequency of outcome factor in the population of 50%, a confidence interval of 95%, and a design effect of 1(10). To ensure reliability, the sample size was increased to all 189 participants to account for any missing data or non-response rate.

A self-reported questionnaire was created for this study based on a review of prior literature (2,4,7). The questionnaire was first developed in Turkish (the official native language of Türkiye), then it was translated into English and then back into Turkish by two academic researchers. Before beginning data collection, Turkish versions were pilot tested on a sample of ten dentists. The few translation inconsistencies that were found in the pilot study were corrected by the investigators. Participants were interviewed face-to-face to gather data.

The final questionnaire consisted of six different sections: 1) socio-demographic and general information (gender, age, professional title, experience and prior training on rational drug use); 2) questions related to drug knowledge was calculated by using "very bad, bad, medium, good, very good" type choices 3) questions about patient history taking information and these questions were measured by using Likert scale "never, rarely, sometimes, often, always " types options; 4) questions related to patient counseling also measured by using Likert scale "never, rarely, sometimes, often, always" types options; 5) multiple choice questions about the source of information, and 6) questions related to adverse drug reporting was measured by using multiple-choice items ***(Supplementary file 2).*** The final data were collected and transferred to Microsoft Excel 365 (Version 2016, Microsoft Corporation, United States) and the findings were recorded in number and percentage form. The final results were presented in tabulated form.

RESULTS

In this study, a total of 189 dentists were approached. Nine (4.7%) were excluded due to lack of time (4; 2.1%) and less than 1-year of working experience (2.6%). Finally, a total of 180 dentists were included with a response rate of (95.3%). Females (n=97; 53.4%) were more than males (n=83; 46.2%) with a mean age of 35.4 years. The majority of the dentist were general dental practitioners (n=101; 56.1%) followed by the assistant dentist. Out of the total, 68.3% (n=123) claimed to obtain prior training on "rational drug use".

In this study, various knowledge-related variables to drugs were asked from the participants. This study showed a general improvement in dentists prescribing knowledge. However, 50.6% (n=91) indicated their knowledge level as a bad and very bad category regarding drug price/cost (Table 1).

The majority of the dentists reported that they always asked about the drug(s)/medicinal product used by patients, drug-related allergy, chronic disease, previous or current bleeding problems, pregnancy, or breastfeeding. However, more than half (50.6%; n=91) of the dentists claimed that they never/rarely asked about health insurance. In terms of counseling-related techniques, most of the participants reported that they often/always asked about important information. However, drug mechanism of action, the possible side effect of the drug, drug price, the interaction of prescribed drug with other drugs and nutrients, and any other drug warnings were never/rarely and sometimes explained by the dentists (Table 2).

The majority of the participants (95%; n=171) claimed that the drug information provided by them to patients is adequate and satisfactory. Vademecum (Turkish Medication Guidebook: 70%; n=126) followed by the internet (55%;n=99) was the main source of information used by the dentist for drug information purposes. Vademecum is a Book, which has been supporting healthcare professionals since 1987 and is a limited edition of the Vademecum database. Vademecum Medication Guide, published every year, is updated by adding newly licensed drugs from the Ministry of Health(11). About 2.8% (n=5) and 19.4% (n=35) of the respondents agreed that they prescribe medications (yes and partially) that are requested by patients (Table 3). Most of the dentists (57.8%) indicated that the adverse effects/reactions due

to the medicines and medical products should be reported. The majority of the participants reported that hospital pharmacovigilance officers (57.7%) followed by the pharmacovigilance center of Türkiye (24.4%) are responsible for ADR reporting and monitoring. However, a higher proportion (85.5%) of the dentists indicated that they did not report ADR during their clinical practice. Further details are listed in (Table 3).

DISCUSSION

Good drug-prescribing knowledge and practices are the most important indicators of quality healthcare service(12). Scientific evidence-based correct drug indications and a properly filled out prescription are critical parameters of quality pharmacotherapy delivery(2). In the current study, most of the claimed that they had sufficient knowledge related to drug pharmacology and pharmacotherapy items. A similar finding was also reported by the study conducted in Lebanon(4). However, more than half of the participants indicated that they had poor knowledge regarding drug price/cost. This finding was consistent with a United States study(13). A study conducted in Ireland observed that 88% of the prescribers stated that they were frequently unaware of actual drug costs(14). Another study conducted in Nigeria found that only 6.2% and 12% of respondents had accurate estimated costs for generic and originator brands, respectively(15). It is reported that inadequate prescribing knowledge of drug costs may harm patient outcomes and national drug budgets(14). It is recommended that the topic of cost awareness should be better covered in pharmacotherapy education because it is crucial for therapeutic reasoning and cost-effective prescribing(16).

In this study, most of the dentists reported that they always asked for important patient history information before prescribing medication. However, more than half of the dentists had never/rarely asked about health insurance. According to a Nigerian study, physicians' prescribing practices are influenced to some extent by their patients' health insurance status(17). Healthcare insurance is a type of healthcare financing that encourages the prudent use of resources and ensures the cost-effectiveness of interventions through the use of low-cost drugs(17). In Türkiye, healthcare is a mix of public and private health services. In 2003, Türkiye implemented universal health care. It is known as Universal Health Insurance [Genel Sağlık Sigortası (SGK)] and is funded by a 5% tax surcharge on employers. Approximately 75.2% of health expenditures are covered by the public sector(19). Additional training on the rational use of medications is required for all prescribers, particularly also for those who care for this group of patients.

In terms of counseling-related techniques, most of the participants reported that they never/rarely and sometimes counsel the patients regarding drug mechanism of action, the possible side effect of a drug, interaction of prescribed drug with other drugs and nutrients. A similar finding was also reported in a recently published study(4). Oral health is an important component of general health because it influences physical health, mental health, quality of life, and overall well-being(19). Clinical professionals must be up-to-date on their knowledge regarding new medications, drug interactions, and practical therapeutic trends due to the rapid advancement of dental pharmacotherapeutics(20). Dentists must need to have a deeper understanding of the relationship between themselves and their patients as well as a conscious awareness of the potential role of counseling to be able to provide truly holistic care(21). It is advised that dentists continually increase their understanding of drugs' side effects, contraindications, and interactions as well as their clinical proficiency to determine the best course of action for each patient.[4] Therefore, research that focuses on evidence-based practice in the use of counseling related to dentistry is urgently needed(21).

Table 1. Participants' Knowledge of Drugs

Knowledge Variables	Very bad	Bad	Medium	Good	Very Good
Indications	10 (5.6)	4 (2.2)	38 (21.1)	90 (50)	38 (21.1)
Posology and method of application	10 (5.6)	14 (7.8)	43 (23.9)	85 (47.2)	28 (15.6)
Pharmacological properties	11 (6.1)	21 (11.7)	78 (43.3)	59 (32.8)	11 (6.1)
Contraindications	11 (6.1)	10 (5.6)	63 (35)	78 (43.3)	18 (10)
Side Effects	12 (6.7)	9 (5)	85 (47.2)	59 (32.8)	15 (8.3)
Interactions of drugs (medicine, nutrients, etc.)	12 (6.7)	21 (11.7)	83 (46.1)	51 (28.3)	13 (7.2)
Warnings, Precautions	12 (6.7)	8 (4.4)	71 (39.4)	72 (40)	17 (9.4)
Special situations (pregnancy, breastfeeding, special age groups, etc.)	9 (5)	5 (2.8)	39 (21.7)	87 (48.3)	40 (22.2)
Bioequivalence	19 (10.5)	25 (13.9)	69 (38.3)	51 (28.3)	16 (8.9)
Drug price/cost	37 (20.6)	54 (30)	49 (27.2)	22 (12.2)	18 (10)

Table 2: Patients' history information and counseling techniques taken by dentists before prescribing medications

Patients' History Information	Never	Rarely	Sometimes	Often	Always
Inquire about other drugs (s)/medicinal product	6 (3.3)	1 (0.6)	9 (5)	33 (18.3)	131 (72.8)
Drug allergy	4 (2.2)	0 (0)	3 (1.7)	26 (14.4)	147 (81.7)
Liver, kidney, heart disease	1 (0.6)	5 (2.8)	5 (2.8)	34 (18.9)	135 (75)
Previous/current bleeding problem	8 (4.4)	6 (3.3)	22 (12.2)	39 (21.7)	104 (57.8)
Chronic disease	0 (0)	4 (2.2)	3 (1.7)	23 (12.8)	150 (83.3)
Surgery	12 (8.9)	11 (6.1)	34 (18.9)	37 (20.6)	82 (45.6)
Pregnancy/breastfeeding	7 (3.9)	6 (3.3)	9 (5)	32 (17.8)	126 (70)
Age	11 (6.1)	3 (1.7)	22 (12.2)	32 (17.8)	112 (62.2)
Gender	30 (16.7)	36 (20)	33 (18.3)	30 (16.7)	51 (28.3)
Health insurance	54 (30)	37 (20.6)	29 (16.1)	23 (12.8)	37 (20.6)
Counseling techniques					
Examine the patient before prescribing a medicine	4 (2.2)	0 (0)	0 (0)	8 (4.4)	168 (93.3)
Causes of illness.	0 (0)	4 (2.2)	14 (7.8)	29 (16.1)	133 (73.9)
Possible course of illness	5 (2.8)	2 (1.1)	14 (7.8)	41 (22.8)	118 (65.6)
Reasons for the treatment	1 (1.6)	5 (2.8)	11 (6.1)	30 (16.7)	133 (73.9)
How the disease can respond to treatment.	6 (3.3)	2 (1.1)	16 (8.9)	30 (16.7)	126 (70)
Possible complications of the disease.	8 (4.4)	2 (1.1)	19 (10.6)	41 (22.8)	110 (61.1)
Name of the drug	11 (6.1)	6 (3.3)	26 (14.4)	51 (28.3)	86 (47.8)
How to apply	11 (6.1)	2 (1.1)	14 (7.8)	47 (26.1)	106 (58.9)
Daily dose	11 (6.1)	3 (1.7)	17 (9.4)	36 (20)	113 (62.8)
Duration of use	14 (7.8)	2 (1.1)	4 (2.2)	38 (21.1)	122 (67.8)
Drug's mechanism of action	40 (22.2)	46 (25.6)	56 (31.1)	22 (12.2)	16 (8.9)
Possible side effects of the drug.	22 (12.2)	34 (18.9)	60 (33.3)	39 (21.7)	25 (13.9)
Price	119 (66.1)	27 (15)	18 (10)	8 (4.4)	8 (4.4)
Interactions with other drugs/nutrients.	38 (21.1)	41 (22.8)	50 (27.8)	31 (17.2)	20 (11.1)
Activities that should be avoided	23 (12.8)	28 (15.6)	53 (19.4)	43 (23.9)	33 (18.3)
Other warnings about drugs.	29 (16.1)	36 (20)	49 (27.2)	41 (22.8)	25 (13.9)
Recommend non-drug treatments to the patient	12 (6.7)	24 (13.3)	52 (28.9)	35 (19.4)	57 (31.7)
Call for a post-treatment check-up	7 (3.9)	10 (5.6)	31 (17.2)	59 (32.8)	73 (40.6)

Table 3. Source of information and adverse drug reaction reporting by the participants

Variables	Frequency	Percentage
Do you believe the drug information you provide the patient is adequate?		
Yes	87	48.3
Partially	84	46.7
No	8	4.4
Sources of information		
I do not benefit from information sources	16	8.9
Türkiye Drug Treatment Guide (TİK)	22	12.2
Diagnosis and Treatment Guidelines	41	22.8
*Vademecum	126	70
Pharmacology Books	30	16.7
Pharmaceutical Information Software Programs	18	10
Research and Promotion Studies of Pharmaceutical Companies	30	16.7
Colleague	72	40
Internet	99	55
Others	4	2.2
Prescribe medications that are requested by patients		
Yes	5	2.8
Partially	35	19.4
No, it's not. I never prescribe medication without examining it	140	77.8
After giving information to the patient about the drugs, would you check if the patient understands		
Yes	105	58.3
Sometimes	66	36.7
No	9	5
Do you think adverse effects caused by the use of medicines and medical products should be reported?		
Yes	104	57.8
No	76	42.2
Did not know	0	0
Which of the following is responsible for ADR reporting and monitoring		
Hospital pharmacovigilance officer	104	57.7
Pharmacovigilance Center of Türkiye (TÜFAM)	44	24.4
Ministry of Health	17	9.4
Pharmaceutical company	9	5
General Directorate of Medicine and Pharmacy	6	3.3
How many adverse events have you reported during clinical practice		
0	154	85.5
1	8	4.4
2	3	1.7
3	1	0.6
4	2	1.1
7	1	0.6
9	1	0.6
14	1	0.6
15	1	0.6

The majority of the participants believed that ADRs due to the use of medicines and medical products should be reported. Most of the participants indicated the hospital pharmacovigilance officer followed by TÜFAM responsible for ADR reporting. However, a high proportion of dentists (85.5%) did not report ADR during clinical practice. Under-reporting behaviors of dentists were also reported in previous studies (7,8,22). Such findings are troubling and need immediate attention. Dental doctors write prescriptions for a variety of therapeutic procedures, including allopathic medications like local anesthetics, antibiotics, analgesics, and anti-inflammatory drugs(7,23). One of the main causes of ADRs is the use of antibiotics and analgesics(7,8,24). Therefore, to enhance patient health care, continuous training modules on the subject of PV and activities such as highlighting the purpose and value of ADR reports are important.

Like any other research, this study had also limitations. First, the present findings may not be generalizable, especially since our study was based on a sample of dentists recruited from a two-hospital in Türkiye. Second, there is the possibility of respondent bias, as in any survey, where respondents choose to have a socially favorable opinion rather than actual answers. Finally, we did not use advanced statistics to make more accurate conclusions about the study variables. However, to reach a more accurate conclusion, we used descriptive statistics as part of our study objectives. Despite these limitations, there had some strengths in this study. This is the first study to assess Turkish dentists' prescribing knowledge and practices with ADR reporting in our healthcare settings. This study also provides baseline local data on dentist prescribing knowledge, practices, and ADR reporting behaviors, and the findings may be useful for clinical settings, healthcare professionals, and policymakers.

CONCLUSION

This study showed a general improvement in dentists prescribing practices knowledge and practices, although they reported some lack of knowledge regarding drug cost, discussion about possible side effects of a drug, interaction with other drugs/nutrients with patients and under-reporting of ADRs. Periodic education and training for dentists are critical to overcoming any problem related to prescribing errors, drug interaction, and potential ADRs.

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Original Research

Effects of Gluteus Maximus Muscle Strength on Ataxia, Gait, and Equilibrium in Multiple Sclerosis

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ABSTRACT

Objective: Multiple sclerosis (MS) is an autoimmune disease that causes scar tissue in the nervous system and seriously affects the quality of life of people. Muscle weakness, spasticity and coordination problems are seen primarily in the lower extremities. Strengthening exercises improve muscle strength in people with multiple sclerosis, but there is no consensus on their effect on walking capacity.

Methods: To determine the relationship between gluteus maximus muscle strength, ataxia, balance and walking capacity in Multiple Sclerosis. An experimental study design was applied. Gluteus maximus muscle strength was measured using a dynamometer. Walking capacity was determined by the 6-minute walk test (6MWT) and dynamic gait index (DGI). Balance was evaluated with the one-leg standing test (SLS). The severity of ataxia was measured with the International Ataxia Rating Scale (ICARS). Fatigue was evaluated with VAS and quality of life of all patients with SF36 short form.

Results: EDSS mean of the study = 3.39 ± 1.4; 2 men and 16 women with mean age = 37.17 ± 9.16 years were included. 6MWT, DGI, ALS, ICARS, VAS were different before and after treatment ($p<0.05$). There was no significant difference other than physical function and general health among the sub-parameters of SF36 ($p>0.05$). Correlation of muscle strength with ataxia, gait and balance was not significant ($p>0.05$)

Conclusion: In individuals with MS, the fact that the treatment program consists of modalities that include balance and sensory exercises as well as muscle strengthening exercises increases the success of rehabilitation.

Key words: multiple sclerosis, muscle strenght, balance, gait

INTRODUCTION

Multiple Sclerosis (MS); It is a chronic, autoimmune, demyelinating and degenerative disease that occurs in the central nervous system. MS affecting the central nervous system is a highly heterogeneous disease and may present with very variable clinical signs and symptoms, including motor, sensory, autonomic, and cognitive impairments, depending on the region of the central nervous system[1]. Motor symptoms are generally seen in the form of muscle weakness, coordination problems, spasticity, spasticity-related pain, cramps and spasms in the lower extremities and cause a decrease in mobility and physical activity. Walking problems are one of the most frequently reported problems in MS[2]. In addition to symptoms such as muscle weakness, spasticity, and ataxia, visual and oculomotor disorders may also cause walking difficulties[3]. The contribution of muscle weakness to walking, seen even in the early stages of the disease, is undeniable. Muscle weakness is a modifiable factor[4]. There are important studies examining gait patterns in MS patients[5]. Previous evidence has shown that foot and knee muscle function is associated with gait performance[6]. However, just strengthening the foot and knee muscles is not enough to normalize gait in MS[7].

Strengthening exercises for people with MS are typically part of a comprehensive physical therapy plan[7]. However, while strengthening exercises have consistently been shown to improve muscle strength and endurance in people with MS, they have not consistently resulted in significant improvements in gait performance[7]. Although conditions such as insufficient exercise dose and poor quality study design have been cited as the reason[8,9], another reason that is not overemphasized is that the muscles that may have the greatest impact on gait performance are not prioritized[8].

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Table 1. Exercise Training Program

Hafta / **Setler**	1st week	2nd week	3th week	4th week	5th week	6th week
1st set	10 max. repeat	12 max. repeat	12 max. repeat	15 max. repeat	15 max. repeat	15 max. repeat
2 minutes rest						
2nd set	10 max. repeat	12 max. repeat	12 max. repeat	15 max. repeat	15 max. repeat	15 max. repeat
2 minutes rest						
3th set	10 max. repeat	12 max. repeat	12 max. repeat	15 max. repeat	15 max. repeat	15 max. repeat
2 minutes rest						
4th set				15 max. repeat	15 max. repeat	15 max. repeat

Table 2. Participant characteristic

Characteristics	**MS patients (N=18)**
Age, years (mean±SD)	9.167 ± 37.17
Gender Female/male, n (%)	18/2.18(88.89/11.11)
EDSS	3.39±1.42

EDSS: Expanded Disability Status Scale, SD: Standard Deviation, MS: Multiple Sclerosis, $p < 0.05$.

There are also different studies investigating the effect of muscle strength on gait performance in MS. However, only a small number of muscle groups were examined at the same time in these individual studies[10]. Although proximal stabilization muscles are very important for normal lower extremity in people with MS, little is known about the relationship between gait performance and hip extension[10,11].

The gluteus maximus (Gmax), which makes up 16% of the cross-sectional area of the hip, is the largest muscle of the hip[12]. Gmax, which takes part in many functional activities such as walking, running and weight lifting, takes part in providing pelvic stability. Gmax muscle weakness causes a decrease in pelvic rotation and hip extension. In this study, gluteus maximus muscle strength will be associated with ataxia, gait and balance.

Main Points:

- Balance and gait problems in MS affect the quality of life of individuals.
- Balance and gait disturbances are associated with muscle weakness.
- Muscle strength should be evaluated for the effectiveness of treatment in balance and gait problems in MS

METHODS

Approval for this study was obtained from our local ethics committee (Decision No: 2021/3408) and written consent was obtained from individuals with MS, and it was conducted in accordance with the Declaration of Helsinki.

Subjects

Thirty remission MS patients who were diagnosed according to the 2010 McDonald criteria and presented to the MS outpatient clinic of a university hospital were evaluated. Ten patients did not meet the inclusion criteria. Two patients refused to particiipate13,14. Eighteen ambulatory type (16 female, 2 male) patients received exercise training between 16 March and 27 April 2022.

Table 3. Comparison of lower extremity muscle strength and ataxia, gait and balance in patients with Multiple Sclerosis

Assesments	**Right** **Mean±SD**	**Left** **Mean±SD**	
Muscle Strenght Pre-treatment Post-treatment P value	15.72±3.64 20.39±4.50 **.000**	14.83±2.97 20.67±5.64 **.000**	
Ataxia Pre-treatment Post-treatment P value	2.44±1.29 1,33±1.08 **.000**	2.17±1.33 1.06±0.99 **.000**	
The Single leg Stance Test (open eyes) Pre-treatment Post-treatment P value	14.11±9.05 19.50±9.76 **.000**	12.94±8.36 18.89±9.56 **.000**	
The Single leg Stance Test (close eyes) Pre-treatment Post-treatment P value	2.51±2.97 6.06±5.56 **.000**	2.61±3.23 5.50±4.95 **.000**	
Assesments	**Pre-treatment**	**Post-treatment**	P value
DYİ	16.28±5.39	19.17±4.89	**.000**
6MWT	403.64±106.182	465.67±79.01	**.000**
VAS	4.28±2.10	1.33±1.57	**.000**
SF-PF P value	51.39±26.27	60.83±24.57	.123
SF-RP P value	39.44±4566	72.22±33.08	**.007**
SF-RF	62.11±15.49	65.33±18.15	.507
SF-BP P value	66.61±2246	74.83±20.18	.058
SF-GH P value	45.83±14.87	69.72±17.94	**.001**

SF-SF P value	63.00±24.81	70.11±21.48	.265
SF-RE P value	44.17±36.06	70.11±34.15	.507
SF-VT P value	48.89±21.25	48.89±20.69	.981
*p < 0.05, **p < 0.001. VAS: Visual Analog Scale, SF: Short Form 36, PF: Physical Functioning, RP: Role Physical, BP: Bodily Pain, GH: General Health, SF: Social Functioning, RE: Role Emotional, VT: Vitality			

Inclusion Criteria
without any orthopedic disability,

Exclusion Criteria
-diabetes mellitus,
-defect of vision,
-cognitive and orthopedic problems,
-Patients with acute MS attacks (3 months before the study) or who were treated with corticosteroids (1 month before the study) were excluded from the study.

Progressive Resistance Training
Participants were instructed to perform all exercises in a moderate pace concentric and eccentric phase (Table 1)

Outcome Measures
Gluteus Maximus Isometric Muscle Strength
Six clinical measures were used to assess strength, coordination, gait, and balance performance. Gluteus maximus Lafayette -01165 (Lafayette Instrument Company, Lafayette IN, USA) was measured with a manual dynamometer [15]. Subject's position and dynamometer placement were standardized according to Bohannon[16]. Subjects were recorded for the weight of the exercise by measuring a maximum repetitions in Newtons.

Coordination
The validity and reliability of the International Cooperative Ataxia Rating Scale (ICARS), which has psychometric properties for balance assessment, has been proven. It contains a kinetic function subscale that assesses coordination[17]. The total score of the lower extremity kinetic functions section is 8. Evaluation was made out of 8 points.

Gait
Walking performance has commonly been evaluated in people with MS using 6-minute walk test (6MWT). Individuals with MS who are successful in the 6MWT have a more successful ambulation in the community[18].

Dynamic Gait Index (DGI)
It is used in the evaluation of functional walking. It was created to evaluate postural control during walking. It consists of eight steps including walking at different walking speeds, walking with horizontal and vertical head movements, turning during walking, walking over obstacles, turning around the obstacle, and going up and down stairs. The activity in each section is evaluated between 0 (severe disorder)-3 (normal)[19].

The Single Leg Stance Test (SLS, EC-EO)
Which lower extremity is dominant, which foot would you use to hit the ball?" was determined by the question. How long the patients could stand on one leg was recorded in seconds. When the patient lost his balance and the foot touched the ground, the chronometer was stopped. No verbal stimulus was given during the evaluation[20,21].

Quality of life (SF-36)
The SF-36 has been validated for use in individuals with neurological diseases and the Turkish translation has been validated[22]. Te Short Form Health Survey (SF-36) questionnaire measures health status, taking into account physical functioning and exercise, emotional and physical role functioning, mental health in general, social role functioning, body pain and general health.

Statistical Analysis
All data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, version 21, SPSS Inc. Chicago, IL). Data were expressed as mean ± standard deviations for numerical variables and numerically for categorical variables. Wilcoxon test was performed to determine the changes that occurred after treatment. Significant differences in the values of EDSS 4 above and below the tests were made with the Mann-Whitney U Test. Statistical significance level was accepted as p <0 .05.

RESULTS
There were 16 female and 2 male patients with a mean age of 9.167 ± 37.17 years between the range of 25 and 53 years (Table 2).

Ataxia, gait and balance were evaluated before and after muscle strengthening training.

Lower extremity ataxia in individuals with MS, the results before and after treatment are significant (pright=0.000, pleft=0.000) (Table 2). When gait is evaluated, the difference between DGI and 6MWT before and after treatment is significant (pDGİ=0.000,

Table 4. The relationship between EDSS 4 and above values ataxia gait and balance

Evaluation Parameters	EDSS Level	N	Mean	p value	(z value)
Muscle Strength	EDSS 4 below	13	21.26	0.251	–1.148
	EDSS 4 above	5	18.60		
$VAS_{fatigue}$	EDSS 4 below	13	1.01	0.108	–1.607
	EDSS 4 above	5	2.20		
ICARS	EDSS 4 below	13	0.80	**0.012***	–2.511
	EDSS 4 above	5	2.20		
6DYT	EDSS 4 below	13	494.77	**0.015***	–2.424
	EDSS 4 above	5	390		
DYİ	EDSS 4 below	13	14.20	**0.008***	–2.634
	EDSS 4 above	5	19.17		
SLS_{EO}	EDSS 4 below	13	23.03	**0.007***	–2.72
	EDSS 4 above	5	9.02		
SLS_{EC}	EDSS 4 below	13	7.61	**0.004***	–2.424
	EDSS 4 above	5	1.00		

p<0.05, z: Mann Whitney-U Test; ICARS:International Cooperative Ataxia Rating Scale, 6MWT:Six-Minute Walking Test; DYI:Dynamic Gait Index; SLSGA: The Single leg Stance Test (eyes open); SLSGK: The Single leg Stance Test (eyes closed)

p6MWT=0.000) (Table 3). The results were significant in the The Single leg Stance Test with eyes open and closed before and after treatment (peyes open=0.000, peyes closed=0.000) (Table 2). The difference in VAS fatigue evaluated before and after 6MWT walking was significant (p=0.000, p=0.000, respectively) (Table 3).

In order to evaluate the efficacy of the treatment, the SF36 test was applied to individuals with MS before and after the treatment. No significant changes were found in other parameters except mental function and general health, which are sub-parameters of SF36 (p<0.05).

As a result of the correlation analysis, no significant relationship was found between muscle strength and ataxia, gait and balance (p<0.05).

According to Wilxon analysis performed by dividing EDSS 4 into two groups as below and above, it was seen that EDSS and 6MWT, ataxia and balance were positively correlated (p>0.05), while there was no significant correlation with muscle strength and fatigue (p>0.05) (Table 4).

DISCUSSION

In this study investigating the effectiveness of isolated gluteus maximus exercises on ataxia, gait and balance in MS patients, positive improvements were obtained in ataxia, gait and balance after treatment compared to before. However, as a result of the correlation analysis, no significant relationship was found between muscle strength and ataxia, gait and balance. The reason for this is thought to be related to the low number of people in the evaluated MS group and the mild EDSS level. There are studies examining lower extremity gait pattern compensations for people with MS[23], and previous studies have shown that ankle and knee muscle function is associated with walking performance in people with MS[24]. However, improving ankle and knee muscle function through resistance training alone is probably not sufficient to optimize gait in people with MS[25]. Proximal muscle function should also be considered, as it provides the stability necessary for effective lower extremity movement during walking[26]. In a study by Broekmans et al. the relationship of knee flexion and extension muscle strengths at 45°, 60° and 90° with the 2 Meter Walking Test (2MWT) was examined in individuals with mild ambulatory dysfunction, and isometric knee extensor measured at 45°. no significant correlation was found except for the strength. The lack of robust associations between maximal muscle strength and walking tests may be related to the mild subgroup performing almost as well as healthy subjects[27]. The lack of a significant relationship between muscle strength and fatigue in the study can be explained by the fact that fatigue is not dependent on the EDSS level and the study group mainly consists of individuals who are mildly affected. In the study conducted by Schwid et al., it was stated that fatigue was not related to muscle strength, and that fatigue in individuals with MS was at 500 m. reported that it affects walking distance[28]. In the study, it was observed that exercise improved coordination. However, the lack of significant difference in the correlation can be explained by the small number of the study group. In the study of Salcı et al., individuals with MS were divided into three groups. While balance exercise was given to one of the groups, balance exercise and chorea stabilization exercises were given

to one of the other two groups, and task-oriented exercise was given to the other with balance exercises. While the ataxia kinetic functions section of ICARS improved in the group given chorea stabilization and balance exercises, no significant difference was observed in the group given only balance exercises[2]. In the study conducted by Brookmans et al., significant improvements were obtained in muscle strength and balance in the group given progressive resistance exercises compared to the group given electrical stimulation[29]. In the study, significant improvements in physical role and general health were obtained from the sub-parameters of SF-36. In the study conducted by Huisinga et al. 26 people with MS exercised with an elliptical device for 6 weeks and fatigue and quality of life were evaluated before and after the treatment[30]. Significant improvements in physical function, emotional state, energy, social function and general health were obtained after treatment. The difference between this study and ours can be attributed to the large number of people participating in the treatment and the elliptical exercise being a type of exercise that works the whole body.

Our results should be evaluated in light of the following limitations. Although the threshold values used in this study are supported by the previous literature, there is no standard threshold for mild to moderate disability in the EDSS. We did not have any follow-up to assess fatigue and quality of life and to monitor whether improvements were sustained weeks or months after the end of this study. The aim of this study was to present the effectiveness of isolated gluteus maximus exercises to MS patients independent of this research protocol. In addition, the short duration of this study, such as 6 weeks, was so short that significant changes in disease status were not expected in the MS group. However, a control MS group with a standard treatment such as physical therapy, with exercise performed for a longer period of time, for example 12 weeks, may also be important.

CONCLUSION

Isolated gluteus maximus exercise alone is not sufficient to improve ataxia, walking performance and balance in MS patients. The treatment approach should be multimodal considering the complex mechanism of MS. Isolated gluteus maximus exercise in addition to classical exercise provides better rehabilitation results. Randomized studies are needed to evaluate the effectiveness of this study.

Suggestions: More objective and precise results can be obtained with larger sample groups and randomized controlled studies in future studies.

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Original Research

Factors Predicting Febrile Urinary Tract Infection After Ureterorenoscopic Lithotripsy in Pediatric Patients

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ABSTRACT

Objective: To our knowledge, there is no study investigating the factors predicting postoperative febrile urinary tract infection (UTI) in pediatric patients. We aimed to determine the factors predicting postoperative febrile UTI in pediatric patients who underwent ureterorenoscopic lithotripsy (URS-L).

Methods: Pediatric patients who underwent URS-L due to ureter or kidney stones in our tertiary center between 2012 and 2019 were analyzed retrospectively. The demographic data, stone characteristics, and intraoperative and postoperative data of those with and without postoperative febrile UTI were compared. Multivariable binary logistic regression analysis was performed to determine predictors of postoperative febrile UTI.

Results: A total of 136 patients were included in the study. The mean age of the patients was 10±5.4 years and 78 patients (57.4%) were male, and 58 patients (42.6%) were female. Semirigid URS-L was applied to 72 (52.9%), and flexible URS-L to 64 (47.1%) patients. The mean operation time was 45.5±15.4 minutes. In the postoperative 1st month, 104 (76.5%) patients were found to be stone-free. Postoperative febrile UTI developed in 17 patients (12.5%). History of stone surgery, history of UTI, presence of nephrostomy / D-J stent, type of surgery, operation time, length of stay, and presence of complication except febrile UTI were found to be significantly different in patients with postoperative febrile UTI compared to those without. In multivariate analysis, only the history of UTI was identified as an independent predictive factor.

Conclusion: In pediatric patients, infectious complications constitute the majority of complications after URS-L. History of UTI is the only independent factor that predicts postoperative febrile UTI after URS-L.

Keywords: Pediatric urolithiasis, ureterorenoscopic lithotripsy, complication, urinary tract infection

INTRODUCTION

Globally urolithiasis is a very common disease and its incidence is increasing worldwide (1). Studies have shown that the incidence of pediatric urolithiasis has also increased in recent years (2–4). Pediatric urolithiasis has a high risk of relapse, and the primary goals in its treatment are ensuring the stone-free status and minimizing the risk of recurrence (5). In the past, while extracorporeal shock wave lithotripsy (ESWL) was the preferred treatment method in pediatric patients, thanks to technological advances ureterorenoscopic lithotripsy (URS-L) has been used more often in the treatment of ureter and kidney stones in recent years (6).

In pediatric patients, semirigid and flexible URS can be successfully applied with stone-free and complication rates similar to adults (7). Most of the postoperative complications are high fever and urinary tract infection (UTI) (8). In the literature, there are studies investigating the factors predicting postoperative febrile UTI in the adults (9–11). Although febrile UTI is the most common postoperative complication in pediatric patients (12, 13), as far as we know, there is no study investigating the factors predicting this complication. Only Doğan et al. investigated the factors predicting the overall complication rate, and in their multicentric study, operation time was indicated as the only independent predictor affecting the complication rate (14). In this study, we aimed to determine the factors predicting postoperative febrile UTI in pediatric patients who underwent URS-L.

METHODS

After obtaining the local ethics committee approval (Approval Number: 2021/165), the data of 140 pediatric patients aged 0-18 years who underwent URS-L due to ureteral or kidney stones in our tertiary center between 2012 and 2019 were obtained from our prospective URS-L database and were analyzed retrospec-

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tively. Age, gender, medical history, stone characteristics, presence of preoperative UTI, presence of D-J stent/nephrostomy, type of operation, degree of hydronephrosis, operation time, postoperative D-J stenting, stone-free status, complications, and follow-up data were investigated. Exclusion criteria were: patients with anatomical abnormality, patients with comorbidities that predispose to infection, such as diabetes mellitus or immunodeficiency, patients undergoing URS-L for residual stone, and patients with missing follow-up data. ESWL was not applied to any patient before surgery. As a result, a total of 136 patients were included in the study. The demographic data, stone characteristics, and intraoperative and postoperative data of those with and without postoperative febrile UTI were compared.

Urine culture was obtained in all patients preoperatively. All urine cultures were evaluated in a single laboratory, and less than 10^3 CFU was considered negative. Patients with positive urine culture were treated with antibiotics for at least 1 week according to the culture antibiogram results and a sterile urine culture was obtained before surgery. Abdominal low-dose computed tomography (CT) without contrast was performed in accordance with ALARA (As Low As Reasonably Achievable) principles for each patient to be operated on. Stone features and degree of hydronephrosis were determined preoperatively by CT. Stone size was calculated based on the longest dimension of the stone on CT. The journal article by Cietak and Newton was used as a reference to classify hydronephrosis (15). Simultaneous and moderate expansion of the pelvis and calyces (Grade 3 hydronephrosis) and simultaneous and severe expansion of the pelvis and calyces (Grade 4 hydronephrosis) were defined as the presence of high-grade hydronephrosis.

All operations were performed under general anesthesia with the lithotomy or frog-leg position. As antibiotic prophylaxis, second-generation cephalosporin was administered intravenously during anesthesia induction. Semirigid URS-L was performed using 4.5-6 Fr ultra-thin semirigid pediatric ureterorenoscope (Richard Wolf, Knittlingen, Germany) and flexible URS-L was applied with 7.5 Fr flexible ureterorenoscope (Flex-X2, Karl Storz, Tuttlingen, Germany). We applied semi-rigid URS-L to lower

Table 1. Comparison of patients with and without postoperative febrile UTI

Variables	Total (n=136)	Febrile UTI+ (n=17)	Febrile UTI- (n=119)	p
Age (Years) -Mean ± SD	10 ± 5.4	10.7 ± 4	9.99 ± 5.6	0.651
Gender (female) -n (%)	58 (42.6)	10 (58.8)	48 (40.3)	0.238
Stone Diameter (mm) -Mean ± SD	13.2 ± 4.8	15.0 ± 3.4	12.9 ± 5	0.108
Stone Number -n (%)	1.35 ± 0.5	1.24 ± 0.4	1.37 ± 0.5	0.280
Stone Density (HU) -Mean ± SD	1067 ± 421	993 ± 133	1077 ± 446	0.500
High-grade Hydronephrosis -n (%)	51 (37.5)	7 (41.2)	44 (37.0)	0.948
History of Stone Surgery -n (%)	35 (25.7)	8 (47.1)	27 (22.7)	**0.041**
History of UTI -n (%)	8 (5.9)	4 (23.5)	4 (3.4)	**0.009**
Presence of Nephrostomy/D-J Stent -n (%)	21 (15.4)	6 (35.3)	15 (12.6)	**0.026**
Type of Surgery -n (%)				**0.019**
Semi-rigit URS-L	72 (52.9)	4 (23.5)	68 (57.1)	
Flexible URS-L	64 (47.1)	13 (76.5)	51 (42.9)	
Flexible URS-L with UAS -n (%)	51 (79.7)	10 (76.9)	41 (80.4)	0.717
Operation Time (min) -Mean ± SD	45.5 ± 15.4	55.2 ± 8	44.2 ± 15.7	**<0.001**
Postoperative D-J Stenting -n (%)	119 (87.5)	13 (76.5)	106 (89.1)	0.229
Length of Stay (days) -Mean ± SD	2.6 ± 2.7	8.7 ± 3.4	1.8 ± 0.7	**<0.001**
Complications* -n (%)	12 (8.8)	4 (23.5)	8 (6.7)	**0.045**
Stone-free Rate -n (%)	104 (76.5)	11 (64.7)	93 (78.2)	0.231
D-J Stent Removal Time (days) -Mean ± SD	26.8 ± 8	30.4 ± 8.4	26.4 ± 7.8	0.085

SD: Standard deviation HU: Hounsfield Unite UTI: Urinary tract infection URS-L: Ureterorenoscopic lithotripsy *except febrile UTI

Table 2. Multivariate binary logistic regression analysis results for prediction postoperative febrile UTI

		Multivarite	
Variables	OR	95% CI	P value
History of Stone Surgery	0.699	0.196-2.496	0.581
History of UTI	0.152	0.029-0.809	**0.027**
Presence of Nephrostomy/D-J Stent	1.875	0.499-7.042	0.352
Type of Surgery (Flexible URS-L)	0.513	0.119-2.210	0.371
Operation Time	1.028	0.987-1.071	0.185
Complications*	0.630	0.132-3.010	0.562

HU: Hounsfield Unit, UTI: urinary tract infection, URS-L: ureteroscopic lithotripsy, *except febrile UTI

and middle ureteral stones and flexible URS-L to upper ureteral stones and kidney stones. In patients who underwent flexible URS-L, a 9.5/11.5 Fr ureteral access sheath (UAS) was advanced over hydrophilic guidewires under the guidance of fluoroscopy. In patients whose UAS could not be placed, a flexible ureterorenoscope was advanced over a hydrophilic guidewire. If this procedure was also unsuccessful, a D-J stent was placed, and a second session was performed 2-4 weeks later. Active orifice dilatation was not applied to any patient. The stones were fragmented with the holmium:yttrium-aluminum-garnet (Ho:YAG) laser. Postoperatively, a D-J stent was placed in case of need, according to the surgeon's preference.

During the hospitalization, the patients were given second-generation cephalosporin prophylaxis. The patients without complications were discharged with oral second-generation cephalosporin prophylaxis on the first or second postoperative day. One month after the operation, ultrasonography (US) was used to evaluate the stone-free status, and kidney-ureter-bladder radiography (KUB) was used in cases with opaque stones that could not be determined clearly by US. CT was used only in selected cases with high suspicion for residual stones. The absence of any stone or presence of a stone smaller than 2 mm in KUB or US was defined as stone-free status. Postoperative febrile UTI was defined as a fever above 38 °C within 1 month after surgery if symptoms related to fever were caused only by urinary tract abnormalities and the presence of UTI was confirmed in urine culture.

Statistical Analysis

Categorical data were presented as numbers and percentages. Data for continuous variables were presented as mean and standard deviation. The Shapiro-Wilk test was used to determine whether the distributions of continuous variables were normal. Mean differences between two related groups of normally distributed data were compared with the independent T-test, while the Mann-Whitney U test was used to compare non-normally distributed data. The frequencies of categorical variables were compared using Pearson's Chi-Square, Yates' Chi-Square, or Fisher's exact test, when appropriate. Statistical significance was considered when p value was <0.05. Multivariable binary logistic regression analysis was performed to determine predictors of febrile UTI. Statistical analysis was performed using Statistical Package of Social Sciences version 21 (IBM SPSS Statistics; IBM Corp., Armonk, NY).

RESULTS

A total of 136 patients were included in the study. The mean age of the patients was 10±5.4 years and 78 patients (57.4%) were male, and 58 patients (42.6%) were female. Of the patients, 45 had lower ureteral stones, 27 had middle ureteral stones, 22 had upper ureteral stones and 42 had kidney stones. Thirty-five patients (25.7%) had a history of stone surgery. Twenty-seven of them were semirigid URS-L and 8 were flexible URS-L. Before the operation, 20 patients had a D-J stent and 1 patient had a nephrostomy. Eight patients (5.9%) had a history of UTI (E. coli in six patients, K. pneumoniae in one patient, and P. mirabilis in one patient). Postoperative febrile UTI developed in 17 patients (12.5%) (E. coli in fourteen patients, P. aeruginosa in two patients, and P. mirabilis in one patient). The same microorganism was grown in 3 of 4 patients with a history of UTI with febrile UTI (E. coli in two patients and P. mirabilis in one patient) before and after the surgery. Semirigid URS-L was applied to 72 (52.9%), and flexible URS-L to 64 (47.1%) patients. UAS was used in 51 of 64 patients (79.7%) who underwent flexible URS-L. UAS could be placed in 46 (71.9%) of 64 patients who underwent flexible URS-L in the first session. In 13 patients (20.3%), the flexible ureterorenoscope could be advanced over the guidewire. In the remaining 5 patients (7.8%), the second session was performed by inserting a D-J stent. UAS was easily placed in all patients after passive dilatation. The mean operation time was 45.5±15.4 minutes. In the postoperative 1st month, 104 (76.5%) patients were found to be stone-free. The demographic data, stone characteristics, and perioperative data of the patients are shown in Table 1.

The rate of developing postoperative febrile UTI was significantly higher in patients with a history of stone surgery, history of UTI, presence of nephrostomy / D-J stent, and presence of complication except febrile UTI (p=.041, p=.009, p=.026 and p=.045, respectively). The application rate of flexible URS-L was significantly higher in patients with postoperative febrile UTI compared to those without (76.5% vs 42.9%, p=.019, respectively). The operation time was significantly longer in patients with postoperative

febrile UTI compared to those without (55.2±8 min vs 44.2±15.7 min, p<.001, respectively). The length of stay was significantly longer in patients with postoperative febrile UTI compared to those without (8.7±3.4 days vs 1.8±0.7 days, p<.001, respectively). Although stone-free rate (SFR) was lower in patients with postoperative febrile UTI, there was no significant difference in SFR between patients with and without postoperative febrile UTI (64.7% ve 78.2%, p=0.231, respectively). A comparison of patients with and without postoperative febrile UTI is shown in Table 1. In multivariate analysis, only a history of UTI was identified as an independent predictive factor. Multivariate regression analysis results of factors predicting postoperative febrile UTI are shown in Table 2.

Twelve (12.2%) complications developed apart from postoperative febrile UTI, including hematuria (Clavien I), which did not require additional treatment in 6 patients, and nausea and vomiting (Clavien I) in 4 patients which improved with the use of anti-emetics. Two Clavien III complications requiring additional intervention were observed. The first was ureteral wall damage due to impacted stone which was successfully managed by inserting a D-J stent. Secondly, on the first postoperative day, the D-J stent was seen to be migrated to the distal of the residual fragments in the proximal ureter, so the D-J stent was replaced.

DISCUSSION

The efficacy of ESWL in pediatric patients is well known. The fact that the ureter is both more elastic and flexible in children allows the passage of stone fragments and prevents impaction. Also, the small size of the child's body transmits most of the ESWL energy. However, there are concerns about the harmful effects of shock waves on the growing kidney and surrounding tissues, and the results are controversial (16). Advances in endoscopic equipment and laser technology have led to the increase in popularity of URS in the treatment of pediatric urolithiasis and consequently URS has been accepted as first-line treatment by many clinicians (17, 18). De Dominicis et al. found that URS in pediatric patients was more effective than ESWL in distal ureteral stones (SFR: 94% with URS, 64% with 2 sessions of ESWL) (19). In addition, in a meta-analysis, He et al. investigated treatment modalities used in the management of pediatric upper urinary tract stones and determined that retrograde intrarenal surgery (RIRS) had higher single session-SFR and lower retreatment rates compared to ESWL (5). In our clinic, we apply URS-L as the first-line treatment in pediatric patients due to the possibility of stone removal in one session, in addition to higher SFR and reasonable complication rates of URS. We prefer ESWL especially, in adolescents that do not require anesthesia.

Studies have shown that URS has high efficacy in the treatment of both ureteral and kidney stones in pediatric patients. In the literature, SFRs have been reported to range between 88-100% with semirigid URS in pediatric patients (14). In the systematic review, Ishii et al. evaluated flexible URS in pediatric patients and found an overall mean SFR of 85.5% (12). In our study, the number of patients treated with semi-rigid and flexible URS-L was almost half and half, and SFR was determined as 76.5%, similar to the literature.

Although URS is a minimally invasive procedure with a high success rate, it is not without complications. Most of the early postoperative complications in pediatric patients, as in adults, are infectious complications. In the study conducted by Doğan et al. with 642 children who underwent semirigid URS, the overall complication rate was found to be 8.4%, and the febrile UTI rate was 3% (14). A higher complication rate with RIRS relative to the semirigid URS is an expected condition, which is also supported by various studies. In the study conducted by Erkurt et al. with 65 patients of preschool age who underwent RIRS, the overall complication rate was 27.7% and the rate of febrile UTI was 15.4% (20). In our series, the overall complication rate was 18.4% and the rate of febrile UTI was 12.5%, in line with existing studies. In addition, the application rate of flexible URS-L was higher in patients who developed febrile UTI than those who did not (76.5% vs 42.9%, respectively, p=.009). However, flexible URS-L was not a predictor of febrile UTI in multivariate analysis.

Although the pediatric literature on infectious complications after URS is insufficient, studies in adults can guide. Sohn et al. determined that preoperative bacteriuria, hydronephrosis, and the presence of a catheter (urethral catheter, ureteral stent, or nephrostomy tube) predicted infectious complications after URS (9). Mitsuzaka et al. determined that the presence of preoperative pyuria and pyelonephritis were factors predicting infectious complications after URS (10). In the present study, according to our knowledge, for the first time in the literature, we investigated the factors predicting febrile UTI in pediatric patients who underwent URS-L. We also determined that the history of UTI is the only independent predictor for febrile UTI. The findings of our study are partially consistent with existing studies. Differences may have resulted from factors such as inclusion criteria, patient population, variety of procedures performed, antibiotic prophylaxis policy, and local resistance profile. Additionally, in this study, we determined that postoperative complications except febrile UTI were much more frequent in patients with febrile UTI (23.5% vs 6.7%, p=0.045). We believe this is due to the longer operative time in more complex cases, and therefore the higher likelihood of developing febrile UTI and other complications.

It has been shown that the use of UAS enables repetitive access, decreases intrarenal pressures, and operation time, and improves SFR in the adult population (21). However, there are concerns regarding the use of UAS due to the potential risk of ureteral injury and vesicoureteral reflux in pediatric patients. Although there are studies showing that UAS is safe for use in children, the literature on this subject is insufficient (20, 22, 23). In addition, the advancement of the UAS or flexible ureterorenoscope over the hydrophilic guidewire may be more difficult in pediatric patients with a smaller ureter. In the study of Erkurt et al., 61.5% of the patients could have UAS placed and in 30.8% of their patients, a flexible URS could be advanced over the guidewire. In 7.7% of the patients, the procedure was left for the second session by placing a D-J stent (20). In our study, UAS could be placed in 71.9% of the patients. Compared to the study of Erkurt et al., the reason for our high rate of success may be that our patient population consists of older children, including adolescents (mean age 4.31 vs 10 years).

Postoperative D-J stenting is important in terms of allowing the recovery of local edema and preventing postoperative pain and possible infectious complications secondary to obstruction. However, requiring additional anesthesia limits its use in pediatric patients (14). Studies have shown that the postoperative stenting rate is between 61.7-92.3%. The postoperative stenting rate is higher in patients undergoing RIRS (7, 14, 18, 20). Doğan et al. stated that the rate of complications was higher in patients who underwent postoperative stenting compared to those who did not (14). This may be due to more frequent stenting in complicated cases. Sohn et al. found that postoperative placement of ureteral stent decreased the incidence of infectious complications in adults (9). In our study, the postoperative stenting was 87.5%, and D-J stents were placed routinely in patients using UAS. However, postoperative stenting was not one of the factors affecting febrile UTI positively or negatively in our study.

Our study has some limitations. Its retrospective design is the most important limitation. Another important limitation is that we evaluate stone-free status with US and/or KUB instead of CT to avoid radiation exposure one month after the surgery. Therefore, the actual stone-free rate is likely to be lower than reported. However, we believe this difference is negligible. Also, we cannot comment on the reliability of the use of UAS since patients had no long-term follow-up data. The etiology of patients who had a history of UTI has not been evaluated. The stone analysis results (especially infection stones) of the patients were not recorded. However, we believe that our study will be a guide for prospective multicentric studies to be performed on this subject.

CONCLUSION

In the pediatric patients, infectious complications constitute the majority of complications after URS-L. History of UTI is the only independent factor that predicts postoperative febrile UTI after URS-L. Appropriate antibiotic prophylaxis based on previous antibiotic susceptibility tests and closer follow-up of patients with a history of UTI may be an appropriate approach to prevent postoperative febrile UTI. There is a need for prospective, randomized studies with larger populations to reach more definitive conclusions.

Ethics Committee Approval: Ethics committee approval was received for this study from the Bakirkoy Dr. Sadi Konuk Training and Research Hospital Ethical Committee (Decision number: 2021/165, Date: 15.03.2021).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Author Contributors: Concept - I.E., E.S.; Design - I.E., E.S.; Supervision - I.E.; Resources - I.E., E.S.; Materials - I.E., E.S.; Data Collection and/or Processing - I.E., E.S.; Analysis and/or Interpretation - I.E., E.S.; Literature Search - I.E., E.S.; Writing Manuscript - I.E., E.S.; Critical Review - I.E., E.S.

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Original Research

Aggressive Treatment of Refractory Coronary Artery Vasospasm in a Patient with Malignant Ventricular Tachyarrhythmia and Cardiac Arrest

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ABSTRACT

Coronary artery vasospasm (CAVS) is a clinical entity that can cause angina, but also unstable angina pectoris, acute myocardial infarction, fatal arrhythmias, and sudden death. Although it is a condition that is usually controlled with medical treatment, more aggressive treatments may rarely be required. In this case, the patient with a known diagnosis of CAVS had multiple arrests despite optimal medical treatment. We observed that fatal arrhythmias persisted in the Implantable Cardioverter Defibrillator (ICD) records, even though we implanted a stent and gave the patient maximal medical treatment. We performed sympathectomy as a last resort and we did not detect any recurrence in the 6-month follow-up of the patient. ICD implantation and sympathectomy should always be considered in resistant CAVS cases.

Keywords: Vasospastic coronary angina, Sudden cardiac arrest, Sympatectomy

INTRODUCTION

Coronary artery vasospasm (CAVS) is a well-known clinical scenario for development of angina or its equivalents, ischemic electrocardiographic changes, and associated complications (1). Among several proposed mechanisms of CAVS, vascular smooth muscle hyperactivity is considered as the most important factor (2). Clinical presentation varies between asymptomatic status to wide range of conditions including chronic coronary syndrome, acute coronary syndrome [unstable angina, non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI)], and sudden cardiac death (3). Drug-refractory CAVS requiring percutaneous coronary intervention (PCI), implantable cardioverter defibrillator (ICD) implantation, and/or surgical sympathectomy is rarely reported in seperate patients (4). Herein, we presented an interesting patient with a drug-refractory CAVS and multiple admissions to our emergency room due to angina pectoris and subsequent sudden cardiac arrest or ventricular tachyarrhythmia in whom PCI, ICD implantation, and surgical sympathectomy has been performed during follow-up.

Case

A 55-year-old male with hypertension, chronic hepatitis B infection, and smoking history admitted to our emergency room with acute onset chest pain at rest and developed sudden cardiac arrest just after admission. He has undergone coronary angiography and CAVS was demonstrated 3 years ago in our center. His medications included optimal doses of peroral calcium channel antagonist and long-acting nitrate. Spontaneous circulation was achieved after 8 cycles of cardiopulmonary resuscitation (CPR). Post-CPR electrocardiography (ECG) revealed sinus rhythm (heart rate of 72 bpm) and ST-segment elevation at inferior and anterior leads (Figure-1). Bedside echocardiography showed normal left and right ventricular functions, no pericardial effusion and flap appearance at the ascending aorta. He has been immediately transferred to cath lab for coronary angiography which demonstrated diffuse coronary vasospasm in the left anterior descending (LAD), circumflex (Cx), and intermediate arteries (Video 1). CAVS was disappeared after 1000 mcg of intracoronary nitrate (Video 2). There was a non-critical hazzy atherosclerotic plaque at the mid LAD region. The ST-segment

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Figure 1. In the 12-lead ECG of the patient taken after CPR in the emergency room, ST Elevation is seen in all leads.

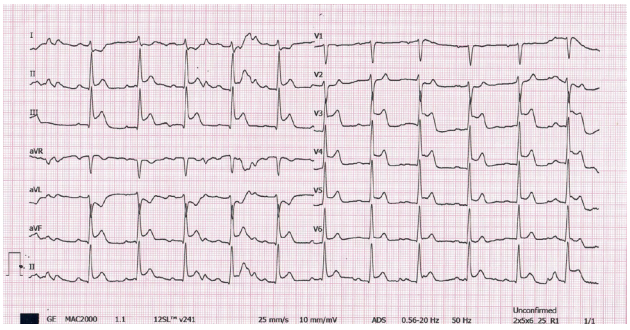
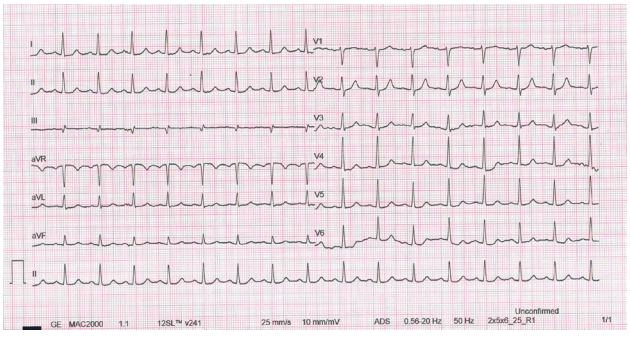


Figure 2. Follow-up ECG of the patient in the coronary intensive care unit after intracoronary nitrate administration during CA, normal sinus rhytm

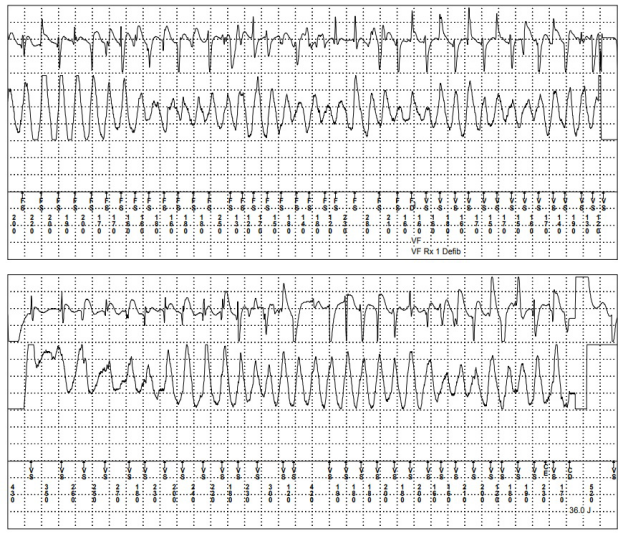


changes in control ECG were also disappeared after coronary angiography (Figure 2). The CAVS was thought as the etiology of chest pain and sudden cardiac arrest. As the patient developed sudden cardiac arrest despite optimal tolerated doses of vasodilators, ICD implantation has been performed as a secondary prophylaxis therapy. He has been discharged with the medications of isosorbide mononitrate, nifedipine, statin and clopidogrel. Two weeks later, the patient re-admitted to the emergency room with chest pain, appropriate ICD shocks, and subsequent cardiac arrest. After 30 minutes of effective CPR, spontaneous circulation was achieved. Intravenous nitrate was initiated immediately. He has been transferred to the cath lab for coronary angiography again. A non-critical hazzy lesion at the mid LAD region became critical after cold-pressor test. Thus, a drug-eluting stent (DES)

Main Points:

- CAVS is not common in clinical practice and is well managed, especially when resistant.
- Malignant ventricular arrhythmias are the most likely cause of refractory CAVS, especially in cardiac arrest. For this reason, ICD implantation is not considered as a priority for patients.
- Sympathectomy should be considered in refractory CAVS cases that persist despite optimal medical therapy.

Figure 3. Documentation of the shock therapy received by the patient for the VF episode upon admission to the emergency department



was implanted to the mid LAD lesion (Video-3). ICD interrogation revealed the development of ventricular fibrillation (VF) which has been converted to sinus rhythm at first 2 attempts and no response to ICD shocks thereafter (Figure-3). 10 days later, he admitted with angina pectoris again and ECG revealed diffuse ST segment depression. During control coronary angiography, there was a diffuse CAVS at the circumflex artery and LAD except stented segment which has been improved with intracoronary nitrate. Thus, sympathetic denervation was planned for the patient. Bilateral thoracic sympathetic chain resection was performed with video-assisted thoracoscopic surgery (VATS) method. The patient was discharged uneventfully. He was clinically stable in the last 6-months follow-up and no episode was detected in the ICD control.

DISCUSSION

The prevalence of CAVS is highest between the ages of 40-70 and tends to decrease after the age of 70, and it has been shown to be more common especially in Japan than in Western countries (5). Although the pathogenesis of CAVS has not been fully elucidated, different pathogenic mechanisms such as vascular smooth muscle cell hyperreactivity, endothelial dysfunction, magnesium deficiency, inflammation, abnormal autonomic nervous system response, genetic factors and oxidative stress are among the suggested causes (4). On the other hand, when we examine the risk factors, smoking, migraine and history of hypertension are known risk factors (6). Calcium channel blockers (CCB), long-acting nitrates (in combination with CCB in resistant angina), statins, Rho-kinase inhibitors and nicorandil are among the recommended treatments for CAVS treatment (4). Apart from this, the use of aspirin is not a recommended treatment in these patients, as it may cause vasospasm by inhibiting the release of vasodilator-acting prostacyclins (7). In our country, nicorandil and Rho-kinase inhibitors can not be prescribed to the patients due to lack of availability.

Our patient had a history of hypertension and smoking, which are risk factors for CAVS. In addition, it may be interesting to think that inflammation also affects CAVS in our patient with chronic hepatitis B infection. The cardiac arrest was probably developed as a result of ventricular malignant arrhythmia despite use of CCB+long-acting nitrate at the maximally tolerated dose. In patients resuscitated due to CAVS for whom medical therapy is ineffective or not tolerated, implantation of an ICD is a recommended approach in the guideline if significant survival beyond 1 year is expected (8). At the same time, when we look at the literature, it has been shown that a substantial number of patients with ICD implanted in patients with resistant CAVS received appropriate treatment for fatal arrhythmias (9). Therefore, we implanted an ICD for secondary protection in our patient. Our patient was also re-admitted to the emergency room after receiving ICD shocks for ventricular tachyarrhythmias during his follow-up. Coronary stent implantation may be considered when a lesion causing organic stenosis is present in CAVS patients (10). Thus, we decided to implant a DES to the mid LAD stenosis which became critical after the cold-pressure test.

Our patient presented to the emergency department again 10 days later with persistent chest pain and ST segment depression on ECG. Control coronary angiography showed extensive CAVS in the circumflex artery and LAD, except for the stented segment, which was resolved with intracoronary nitrate. Thoracic chain resection was performed with VATS method in our patient because of the recurrence of CAVS despite all other therapies. Since 1980s, the sympathectomy has been shown to be successful in cases of resistant vasospastic angina (11). In a study, sympathectomy led to the resolution of all symptoms including pulseless electrical activity and cardiac arrest, and it was shown to prevent recurrence of angina and arrhythmia (12). We discharged the patient uneventfully after all those medical procedures and when the patient came to the follow-up after 6 months, he had no complaints. There was no episode in the ICD interrogation.
In conclusion, if CAVS persists despite maximally tolerated medical treatment, interventional procedures including PCI, ICD implantation in case of cardiac arrest, and sympathectomy should be considered accordingly.

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Author's contributions: BAT: researched the literature, wrote the article, IN: critised and edited the paper

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Review Article

Physical Activity in Neurological Disorders: A Narrative Review

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ABSTRACT

Physical activity levels of people with chronic neurological disorders are lower than those of healthy people. Problems in neurological disorders, including gait abnormalities, muscle weakness/loss of strength, spasticity, tremor, fatigue, balance disorder, and incontinence, results in lower physical activity level. After determining the situations that are contraindicated for physical activity, the patients should be evaluated by physiotherapists, and possible risks that may occur should be determined. Many studies have demonstrated that physical activity significantly reduces mortality and morbidity, increases community participation, and improves health-related quality of life. These benefits show that increased physical activity and exercise should be part of the standard management of neurological disorders. In these patients, physical activity programs should be structured individually by providing appropriate environmental conditions and safety, following the assessment of the functional status and severity of the disease. The duration, intensity, and type of planned physical activity should be adjusted individually; Appropriate rest intervals should be given during the activity, and termination criteria should be determined according to individual tolerance. This literature review aims to provide an up-to-date overview of physical activity recommendations for individuals with chronic neurological disorders.

Keywords: Exercise, Neurologic disorders, Physical activity

INTRODUCTION

People with neurological disorders (stroke, multiple sclerosis (MS), Parkinson's disease, spinal cord injury (SCI), Alzheimer's disease, etc.) have no obstacles to mechanically performing fundamental body movements such as walking, speaking, and grasping. However, they have difficulty performing these movements due to the damage of the disease to the nervous system. [1] In addition, neurological disorders such as gait abnormalities and muscle weakness/loss of strength, spasticity, tremor, fatigue, coordination/balance disorder, and incontinence in individuals with chronic neurological disorders also seriously affect physical activity (PA). [2,3,4] Decreased PA in people with various neurological disorders may cause a loss of independence during the disease. Also, this situation initiates a cycle that will accelerate the worsening and progression of disability independently of progressive disease processes, resulting in a deterioration of health-related quality of life.[3]

Numerous types of research have indicated the significant benefits of exercise and PA in reducing mortality and morbidity, increasing social participation, and improving health-related quality of life, emphasizing that increasing exercise and PA should be a part of the standard management of neurological disorders. [1,3,5] However, people with neurological disorders may not be able to participate sufficiently in leisure physical activities and behave more inactively during work and home activities. The most important reasons for avoiding PA in people with neurological problems are shown as health concerns or limitations, symptom severity, pre-existing comorbid conditions, hospitalizations, learning or cognitive problems, societal and environmental factors, insufficient time, risk of injury, lack of self-efficacy, insufficient energy and motivation, and high prevalence of fatigue. [6,7] There is an important relationship between PA and physical fitness, which affects health outcomes. [8] A summary of this relationship is presented in Figure 1.

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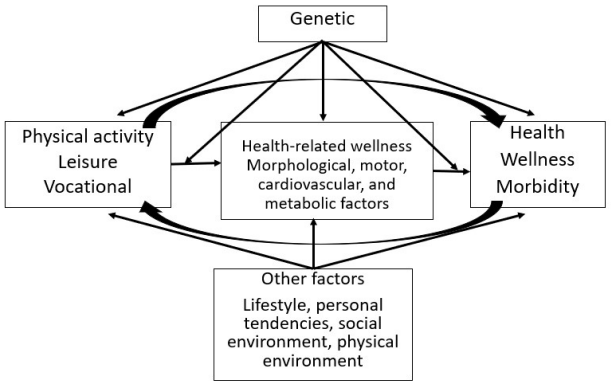
Figure 1. The relationship between physical health and physical fitness and effective factors



The Importance of Physical Activity in Neurological Disorders

Regular PA, which has an important role in preventing the progression of neurological disorders, has been shown to have positive effects on metabolic disorders which are risk factors for neural system involvement. Regular PA increases the resistance of cells, tissues, and organs to oxidative stress and regulates energy metabolism. It also provides vascularization and neurotrophin synthesis. All of the listed effects are important inducers of muscle growth. [9] It is known that the neurotrophin brain-derived neurotrophic factor (BDNF) is responsible for regulating the beneficial effects of PA on cognitive functions. [10] It has been demonstrated that moderate and vigorous PA significantly increases BDNF levels. BDNF exists in two forms in the hippocampus region and has a very important role in neuroplasticity, neurogenesis, and neuron preservation. It has a very important place in the recovery of motor movements after neurodegenerative diseases. [11] Significant evidence from neuroimaging studies in humans indicates that PA promotes structural and functional adaptations in motor and cognitive pathways by improving neuroplasticity (the brain's capacity to change and adapt), both in old age and following brain lesions. [12]

Main Points:

- Measuring physical activity (PA), and especially mobility, lets clinicians figure out a patient's functional capability and determine treatment or prognosis. Therefore, PA evaluation is one of the important sub-parameters in planning the rehabilitation program.
- Before starting any PA program, patients with neurological disorders should be evaluated by a specialist physiotherapist and risk screening should be performed.
- Regular PA can help improve cognitive function by stimulating the brain, it improves cognitive capacity with the vessel and neuron formation in certain regions of the brain. Thus the risk of developing neurological disorders such as Parkinson's and Alzheimer's could be decreased with regular PA.
- Regular PA and exercise (aerobic and strength training) have been shown to be beneficial at all stages of recovery and help the management of risk factors in neurological disorders.
- For people with neurological disorders, 150 minutes of moderate-intensity exercise for 10 minutes or longer is recommended as aerobic exercise. Progressive muscle-strengthening activities that involve major muscle groups of the body are to be 2 or more days per week.

Recent studies in the literature have discovered a strong link between PA and preventing or delaying neurodegenerative symptoms. These studies have demonstrated the role of PA in preventing or delaying the deterioration of motor abilities and mental capacity in patients suffering from many neuro-diseases such as MS and Alzheimer's. [13] There are shown that PA that offers brain protective effects can be applied to provide endogenous neuroprotection in patients with stroke and act as prototypical preconditioning stimuli that are safe treatment alternatives in other studies. [14,15] Treadmill training, which is among the types of PA, has become a special focus in trying to understand the effects of PA on neurological disorders in the literature. There is evidence to recommend that prolonged treadmill training for a total of 4 or more weeks, usually involving 3 to 5 sessions per week (30–40 min), leads to motor development and regulation of neurotrophic factor expressions associated with a rise in cell number and the process of generating functional neurons (neurogenesis). Aerobic exercise causes suppression of programmed cell death, a rise in anti-inflammatory cytokine levels, and increases vascularity. [16–18]

Physical Activity Evaluation in Neurological Disorders

Measuring PA, and especially mobility, lets clinicians figure out a patient's functional capability and determine treatment or prognosis. In the literature, questionnaires, scales, activity diaries, and wearable monitors are used in the evaluation of PA in neurological patients.

3.1. Questionnaire and Scales for Assessment of Physical Activity:

Questionnaires and scales are frequently used in PA assessments in people with neurological disorders. Among these questionnaires, the most commonly used are the Physical Activity and Disability Questionnaire-Revised and the Godin Leisure-Time Exercise Questionnaire. [19,20]

The Physical Activity and Disability Questionnaire-Revised (PADS-R): It is a questionnaire that evaluates PA conceptually and psychometrically in people with neurological disorders. [19]

Godin Leisure-Time Exercise Questionnaire: The questionnaire developed by Godin is aimed at investigating the general activity habits of the subjects rather than questioning them for a certain period. These PA habits try to be defined with a few short questions. The questions are aimed at determining the weekly frequency of activities that get the heart rate up (vigorous exercises), non-strenuous (moderate-intensity exercises), and activities that require the least effort (light exercises). The questions in the other part are about the frequency of activities that are long enough to result in sweating. [20]

Table 1. Physical activity/Exercise type, frequency, duration, and intensity for patients with neurological disorders

Physical Activity/Exercise Type	Frequency	Duration	Intensity
Aerobic Exercises: - Major muscle activities (e.g. walking, cycling ergometry, treadmill, arm ergometry), functional activities	3-4 days/week	20-60 min. / session (or multiple 10-minute sessions); 5-10 minute warm-up and cool-down activities	40-70% of VO2 reserve or heart rate reserve; 55-80% of maximum Heart Rate
Muscle Strength/Endurance Exercises: - Resistance training of upper-lower extremities and trunk using free weights, elastic bands, spring coils - Circuit training - Functional mobility	2-3 days/week	1-3 sets of 8-10 exercises involving major muscle groups; 10-15 reps.	50-80% of maximum 1 repetition; Resistance is gradually increased over time as endurance allows
Flexibility Exercises: - Stretching exercises (trunk, upper and lower extremities)	2-3 days/week	Static stretches: hold for 10-30 seconds; Before or after aerobic or strength training	Within the normal range of motion
Neuromuscular Exercises: - Balance and coordination activities, tai chi, yoga - Recreational activities - Active play video games and interactive computer games	2-3 days/week	Use as a complement to aerobics, strength/endurance training, and stretching activities	Balance exercises should be of high intensity (progressively challenging)

Activity Logs

An activity log is a document that describes how, where and when time is spent. One of the most effective ways to identify where time is wasted is to keep an activity diary. Thus, after analyzing the activity logs of people with neurological disorders, they can be directed to do more effective PA by using time more efficiently. [21]

3.3. Wearable monitors

Pedometers, accelerometers, multisensor systems, and smartphone accelerometer are used as wearable monitors to measure PA in people with neurological disorders. [21-23] Pedometers have the lowest evidence value for use as a tool for exercise prescribing and assessing PA levels in individuals. [22] Accelerometers detect acceleration in one, two, or three directions. These devices allow the determination of the amount and intensity of motion. [22] Multisensor systems combine with other sensors such as accelerometers that measure data such as heart rate, galvanic skin response, or temperature, providing more data based on PA estimates. [21,22] A review study, shows that among the wearable monitors, biaxial or triaxial accelerometer type tools worn on the ankle provide the most proper measurement for the group with neurological disorders. [2] Smartphone accelerometer provides better mobility and disability predictions for both healthy and mobility impaired. Because there is no need for an additional device, the smartphone accelerometer is a more useful, accessible, and accurate device for measuring real-life mobilization for healthy individuals and individuals with chronic disorders. [23]

4. Physical Activity Recommendations in Neurological Disorders

When the literature is examined, flexibility, aerobic, muscle strength/endurance, and neuromuscular exercises are listed among the PA recommendations for people with neurological disorders. Aerobic exercises include large muscle activities and functional activities. Muscle strength/endurance exercises comprise trunk using free weights, resistance training of upper-lower extremities, functional mobility, and circuit training. The neuromuscular exercises include balance/coordination exercises, tai chi, pilates, yoga, recreational activities, the active play of video games, and interactive computer games. [24,25] The frequency, duration, and intensities of these PA/exercise types are shown in Table 1 below.

4.1. Stroke and Physical Activity

Stroke is a clinical picture that can range from loss of motor control and sensory function, balance problems, speech, and cognitive function loss, and visual disturbances to coma, which occurs when cerebral blood flow suddenly decreases or stops. [26]

Physical activity in stroke lowers blood pressure, body weight, and pulse rate. It raises HDL cholesterol. It lowers LDL cholesterol and improves glucose tolerance by reducing insulin sensitivity. While PA reduces the risk of stroke, it accelerates functional recovery after stroke. [27]

One of the reasons for the decrease in PA in individuals with stroke is the inability to understand its importance, and the other is the

factors that individuals perceive as barriers to PA participation. [28] In addition to psychological and social factors (self-efficacy, beliefs about PA, and social support) physical and environmental factors (lack of professional support and follow-up, transportation difficulties, lack of control, and negative effects) have been reported to be perceived as barriers to PA participation in stroke individuals. Along with all these factors, the individual's functional and motor involvement also affects PA participation but this does not fully explain it. The level of improvement perceived by the individual also plays an important role in adaptation to social life and participation in PA. [28]

Physical activities including walking, treadmill, stationary bicycle, and bicycle ergometer can be selected as aerobic exercise types in stroke patients. According to the physical fitness level of the patient, it is recommended that each session should be 20-60 minutes (maybe in 10-minute sets during the day) at 50-80% of the max. heart rate 3-7 days a week. Programs should include warm-up and cool-down periods, and vital signs should be monitored during activities. [29–33]

4.2. Multiple Sclerosis (MS) and Physical Activity

The most common autoimmune disease of the central nervous system is multiple sclerosis. [34] MS which is a progressive disease reveals a wide variety of symptoms that vary from person to person. Physical activity is recommended because it can control the symptoms and improve muscle function, aerobic fitness, mobility, and quality of life. Problems such as pain, fatigue, heat sensitivity, and lack of knowledge about PA cause a decrease in PA levels of individuals with MS in the post-diagnosis period, and only less than 20% of patients can reach the recommended PA level. [35] Also MS patients often choose not to do PA to avoid fatigue and body temperature rise. [34]

Participation in PA is one of the most debated topics in the MS literature, and there is a lot of evidence showing the positive effects of structured PA studies including exercise on balance, fatigue, depression, aerobic capacity, muscle strength, and quality of life in individuals with MS. [35,36] The general recommendation is to perform 30 minutes of moderate-intensity aerobic activity 2 times a week and strengthening exercises involving major muscle groups 2 times a week, depending on the condition of the disease. [37] Gradual exercise is recommended to cope with fatigue, and over time the fatigue experienced may decrease. A warm shower after exercise, a cooling suit, air conditioning, or a sunscreen hat can help with heat intolerance. [37]

A study in individuals with MS showed that self-efficacy is not directly related to PA, but indirectly to goals, barriers, and outcome expectations, providing additional support to social cognitive theory. This study suggests that components of social cognitive theory should address PA involvement and intervention. [38]

4.3. Parkinson and Physical Activity

Parkinson's disease develops due to the loss of neurons in the substantia nigra from the basal ganglia, the globus pallidus, and the subthalamic nucleus, and the deterioration of the connection between these structures and the motor cortex. [39] Parkinson's patients have deficiencies at the level of body functions and structures, including tremor, rigidity, bradykinesia, difficulty getting out of bed, and loss of postural control, leading to activity limitations (dressing, getting up from a chair, and walking). These limitations lead to fear of falling, reduced participation in PA, and reduced quality of life. [40]

Various forms of exercise or physiotherapy can maintain and improve mobility by improving daily functioning and decreasing the risk of falls and related injuries. Therefore, early post-diagnosis interventions to promote PA should focus on strength and balance to prevent future falls. A study comparing PA levels in older adults with individuals with Parkinson's found that individuals with Parkinson's are about one-third less active than older adults. [41]

A cross-sectional study of 260 Parkinson's patients showed that participants with high self-efficacy were more likely to exercise regularly than those with low self-efficacy. [42]

4.4. Spinal Cord Injury and Physical Activity

A spinal cord injury overthrows the connections between the brain and the region distal to the injury. This will proportionally affect the individual's ability to be physically active, depending on the level of SCI. A high level of spinal cord injury with complete tetraplegia can make the patient completely dependent on caregivers. So basic PA of these people may be limited to passive movements and stretching made to reduce complications.[43]

However, an exercise program that addresses how aerobic fitness, muscle strength, coordination, and balance can be improved for lower or incomplete SCI should be created individually. This process should be planned, directed, and managed by expert physiotherapists. The patient and caregivers should be encouraged to continue the physiotherapy program for a long time. [44]

Increased levels of PA in people with SCI significantly decreased fatigue, anxiety, depression, and lower exercise self-efficacy compared to non-disabled controls. Because PA can raise the quality of life for people with SCI, poor exercise self-efficacy is considered a modifiable factor of PA behavior change, especially in this population. Therefore, it is crucial to improve strategies with the capacity to high exercise self-efficacy to increase PA participation and achieve improvements in quality of life. [44-46]

4.5. Alzheimer's and Physical Activity

Alzheimer's is a chronic progressive disease in which cognitive dysfunction, psychiatric symptoms and behavioral disorders, and difficulties in performing activities of daily living are experienced. [47] In studies investigating the effectiveness of PA in patients with cognitive disabilities, it has been stated that individuals with mild and moderate cognitive problems have improvements in their general functional levels similar to healthy individuals. [48] According to a review describing the relationship between improvement in cognitive functions and exercise; there is evidence that PA and exercise have positive effects on cognitive effects, executive functions, memory, attention, and com-

munication in Alzheimer's patients. [49] However, despite the lack of consensus on applied exercise programs, numerous studies have shown that endurance, balance, and strength exercises can be beneficial. [49,50]

CONCLUSION

When creating PA programs for people with neurological disorders, it is possible to refer to clinical practice by including the points to be considered. This review examines the importance of PA levels, PA assessments, and exercise recommendations in different neurological disorder groups (Stroke, Multiple Sclerosis (MS), Parkinson's disease, Spinal Cord Injury (SCI), and Alzheimer's disease. Multicomponent aerobic training involving strength, and balance exercises induce crucial beneficial effects on health. Therefore, considering the neurodegenerative feature of neurological disorder, we believe that PA is very important to avoid the progression of both cognitive and motor symptoms of the disease. Physical activity programs should be included in the treatment program of the neurological disorders group.

Comprehensive studies are needed to establish standard protocols and to understand the mechanisms by which PA produces positive effects. However, PA can be overlooked by most people, including those with neurological disorders.

Within the scope of our review, we think that it will raise awareness about increasing PA by emphasizing the importance of PA in the group with neurological disorders. Our review also could be an up-to-date guide that can be used to increase PA in the group with neurological disorders.

For this reason, it is recommended to do regular PA as a preventive measure without having a neurological disorders. In addition, many beneficial effects of regular PA are found in individuals with neurological disorders diagnoses. We suggest further studies that show the positive effects of PA on body systems and encourage doing it by separating people with neurological disorders into specific groups.

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