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European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December. The journal publishes content in English.

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.

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- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

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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

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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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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

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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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Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

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Contents

ORIGINAL ARTICLES

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Original Article

Hyperlipidemia in Patients with Calcific Tendinitis

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ABSTRACT

Objective: Calcific tendinitis is a disease of unclear etiology and is associated with metabolic diseases. Hyperlipidemia, one of the metabolic diseases with systemic effects, may be associated with tendinopathy and tendinitis. In this study, we aimed to evaluate the relationship between hyperlipidemia and hypertriglyceridemia, calcific tendinitis, the location of tendinitis, and the frequency of severely symptomatic tendinitis attacks.

Methods: This retrospective study included a total of 2055 patients diagnosed with calcific tendinitis between August 1, 2019, and August 1, 2021. The patients were evaluated in terms of their hyperlipidemia and hypertriglyceridemia status, statin and/or fibrate use, and the frequency of tendinitis, and the location of attack.

Results: It was observed that 64.4% (n = 230) of the patients had hyperlipidemia and 11.8% (n = 42) had hypertriglyceridemia. It was determined that the most common tendinitis area among 357 patients was the shoulder. There was no statistically significant difference between the frequency of attacks (P = .712), and the location of attack (P = .069) in patients with hyperlipidemia. There was no statistically significant difference between the frequency of attacks (P = .735) and the location of attack (P = .286) in patients with hypertriglyceridemia. However, a statistically significant difference was found between the attack area (P = .032) in patients with triglyceride values higher than the target recommended values.

Conclusion: The frequency of hyperlipidemia is high in patients with calcific tendinitis; it will be useful to evaluate patients with calcific tendinitis in terms of hyperlipidemia.

Keywords: Calcific tendinitis, hyperlipidemia, hypertriglyceridemia, LDL, shoulder

INTRODUCTION

Calcific tendinitis (CT) is a disease characterized by the accumulation of calcium hydroxyapatite deposits in tendons.[1] Calcific tendinitis usually affects individuals between the ages of 30 and 50.[2] Calcific tendinitis is more common in women than men.[3] Although the most common location is shoulder, deposit accumulation may also be observed in many areas such as the gluteus maximus, paravertebral, hip, and foot.[4-6]

The etiology of CT is still not clear yet. Although there are many intrinsic and extrinsic theories regarding its etiology, multiphasic theory is the most widely adopted theory.[7] Studies other than multiphasic theory have shown that CT is associated with many metabolic diseases such as thyroid disorders and diabetes mellitus.[7,8] It has been shown that there is a relationship between hypercholesterolemia, which is one of the systemic metabolic diseases, and tendinopathy. Therefore, the severity of tendinopathies is correlated with the severity of hypercholesterolemia.[9,10] The studies have revealed that hyperlipidemia causes tendinopathy as a result of accumulation of lipid cholesterol and triglyceride deposits on the tendon by increasing the activity of tumor necrosis factor (TNF)-alpha, interleukin (IL)-8, IL-6, increasing the macrophage activity, reducing the amount of type III collagen in the tendon, and causing changes in the tendon structure.[9,11-13]

It is noteworthy that the studies investigating the effect of hypercholesterolemia on CT are limited in our country. In this study, we aimed to evaluate the relationship between hyperlipidemia and hypertriglyceridemia, CT, the location of tendinitis, and the frequency of tendinitis attacks in Turkey.

METHODS

Study Design and Settings

Ethics committee approval for this retrospective study was obtained from the Health Sciences Ethics Committee of Muğla Sıtkı Koçman University (September 10, 2021, 198). Patients diagnosed with CT in the orthopedic outpatient clinic between August 2019 and August 2021 were examined.

Selection of the Participants

Patients between the ages of 18 and 99 who were diagnosed with CT in the orthopedic outpatient clinic between August

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2019 and August 2021 were included in the study. Patients with calcium deposits on x-ray or magnetic resonance imaging were considered to have CT.

Among patients with CT, patients with a lipid profile at the time of diagnosis were included. Patients with lack of information were excluded from the study.

Measurements and Outcomes

Demographic information of patients, chronic diseases (such as diabetes mellitus, hypertension, coronary artery disease, atrial fibrillation, cerebrovascular disease), total cholesterol, Low-density lipoprotein cholesterol (LDL), triglyceride, High-density lipoprotein cholesterol (HDL), white blood cell count, C-reactive protein (CRP), glucose, calcium levels, number of CT attacks in the last 2 years, tendinitis areas, presence of LDL level higher than target value, statin use, presence of TG level higher than target value, and fibrate use were examined. The reference LDL value was accepted as LDL < 116 mg/dL, which is the recommended target value for patients at low cardiovascular risk by the guideline.[14] The triglyceride reference value is <200 mg/dL. The patients were evaluated according to the presence or absence of hyperlipidemia at the time of admission and whether they were above the LDL target value or not, considering their treatment status. Similarly, patients were evaluated according to the presence or absence of hypertriglyceridemia at the time of admission and whether they were above the TG target value or not, considering their treatment status.

Statistical Analysis

Statistical Package for the Social Sciences 25.0 (IBM SPSS Corp., Armonk, NY, USA) program was used for data analysis in the study. Descriptive data on the clinical information and biochemical parameters of the patients were presented as n (%), median (min-max), and mean ± standard deviation. Chi-square test and Fisher's exact test were used to compare patients with hyperlipidemia, patients with LDL higher than target value, patients with hypertriglyceridemia, patients with triglyceride higher than target value, and attack frequency, and attack area. A *P*-value of <.05 was considered statistically significant.

RESULTS

The study included a total of 2055 patients diagnosed with CT in the orthopedic outpatient clinic between August 2019 and August 2021. Of these patients, 357 met the inclusion criteria of the study and 64.4% (n = 230) of the patients were female (Table 1). The mean age was 57.4 ± 11.27 (Table 1). In terms of comorbidities, 55.2% (n = 197) of the patients had hypertension, 23.8% (n = 85) diabetes mellitus, and 15.7% (n = 56) had coronary artery disease (Table 1).

Main Points

- Hyperlipidemia was observed in 64.4% of patients with calcific tendinitis (CT).
- In patients with hyperlipidemia, there was no difference in the location of the tendinitis and the number of attacks.
- Patients with CT should be scanned for hyperlipidemia.

Table 1. Demographic and Clinical Characteristics of Patients

		n	%
Age (years)	57.40 ± 11.27		
Gender	Female	230	64.4
	Male	127	35.6
Diabetes mellitus	No	272	76.2
	Yes	85	23.8
Hypertension	No	160	44.8
	Yes	197	55.2
Coronary artery disease	No	301	84.3
	Yes	56	15.7
Atrial fibrillation	No	340	95.2
	Yes	17	4.8
Cerebrovascular disease	No	350	98.0
	Yes	7	2.0
Number of attacks	1	221	61.9
	>1	136	38.1
Location of tendinitis	Foot or ankle	73	20.4
	Knee or leg	32	9.0
	Hand or wrist	107	30.0
	Forearm or arm	7	2.0
	Pelvis or hip	9	2.5
	Shoulder	116	32.5
	Vertebra	13	3.6
Hyperlipidemia	No	127	35.6
	Yes	230	64.4
Statin use	No	300	84.0
	Yes	57	16.0
LDL value above target value	No	159	44.5
	Yes	198	55.5
Hypertriglyceridemia	No	315	88.2
	Yes	42	11.8
Fibrate use	No	343	96.1
	Yes	14	3.9
Triglyceride value is above target value	No	318	89.1
	Yes	39	10,9

The mean and standard deviation value of age is given LDL, Low-density lipoprotein cholesterol.

While 61.9% (n = 221) of the patients had only 1 attack in 2 years, 136 (38.1%) patients visited the orthopedic outpatient clinic due to more than 1 attack (Table 1). The most common attack location was shoulder (32.5%, n = 116), followed by the hand and wrist (Table 1).

Hyperlipidemia was present in 64.4% (n = 230) of the patients. Only 16% (n = 57) of patients were using statins. LDL values of 55.5% (n = 198) of the patients were higher than the value recommended

Table 2. Biochemical Parameters

	Mean ± SD
Total cholesterol (mg/dL)	210.95 ± 46.54
LDL (mg/dL)	126.48 ± 39.34
Triglyceride (mg/dL)	157.46 ± 89.02
HDL (mg/dL)	53.48 ± 13.87
White blood count	7.34 ± 2.17
CRP (mg/L)	4.35 ± 7.10
Glucose (mg/dL)	114.19 ± 36.44
Calcium (mg/dL)	9.56 ± 0.56

The mean and standard deviation values of biochemical parameters are given.
SD, standard deviation; LDL, Low-density lipoprotein cholesterol; HDL, High-density lipoprotein cholesterol; CRP, C-reactive protein.

by the guideline (Table 1). Hypertriglyceridemia was present in 11.8 (n=42) of patients. The rate of fibrate use was 3.9% (n=14). In 10.9% (n=39) of the patients, triglyceride level was above the target values recommended in the guideline (Table 1). Laboratory results of the patients were as seen in Table-2.

No statistically significant difference was found between the presence of hyperlipidemia, frequency of attacks (P = .712), and location of attacks (P = .069) (Table 3).

There was no statistically significant difference between the presence of hypertriglyceridemia and the frequency (P = .735) and location of attacks (P = .286). There was no statistically significant difference between the triglyceride levels and the frequency of attacks (P = .765). However, there was a statistically significant difference between the location of attacks (P = .032) (Table 4).

DISCUSSION

In our study, 64.4% of the patients had hyperlipidemia and 11.8% had hypertriglyceridemia. It was observed that the most common CT area was the shoulder with a rate of 32.5%. There was no correlation between the patients' hyperlipidemia and the attack area and the frequency of attacks. No correlation was found between the patients having hypertriglyceridemia and

Table 3. Comparison of Attack Frequency and Attack Area, Presence of Hyperlipidemia, and Above of LDL Value Compared to Target Value

		Hyperlipidemia			LDL Value Above Target Value		
		No (n=127)	Yes (n=230)	*P*	No (n=159)	Yes (n=198)	*P*
Number of attacks	1	77 (34.8)	144 (65.2)	.712[a]	98 (44.3)	123 (55.7)	.925[a]
	>1	50 (26.8)	86 (63.2)		61 (44.9)	75 (55.1)	
Location of tendinitis	Foot or ankle	29 (39.7)	44 (60.3)	.069[b]	35 (47.9)	38 (52.1)	.221[b]
	Knee or leg	13 (40.6)	19 (59.4)		17 (53.1)	15 (46.9)	
	Hand or wrist	42 (39.3)	65 (60.7)		49 (45.8)	58 (54.2)	
	Forearm or arm	3 (42.9)	4 (57.1)		4 (57.1)	3 (42.9)	
	Pelvis or hip	2 (22.2)	7 (77.8)		2 (22.2)	7 (77.8)	
	Shoulder	38 (32.8)	78 (67.2)		50 (43.1)	66 (56.9)	
	Vertebra	0 (0.0)	13 (100.0)		2 (15.4)	11 (84.6)	

[a]Pearson chi-square test; [b]Fisher's exact test; P<.05 statistically significant.

Table 4. Comparison of Attack Frequency and Attack Area, Presence of Hypertriglyceridemia, and Above of Triglyceride Value Compared to Target Value.

		Hypertriglyceridemia			Triglyceride Value Above Target Value		
		No (n=315)	Yes (n=42)	*P*	No (n=318)	Yes (n=39)	*P*
Number of attacks	1	194 (87.8)	27 (12.2)	.735[a]	196 (88.7)	25 (11.3)	.765[a]
	>1	121 (89.0)	15 (11.0)		122 (89.7)	14 (10.3)	
Location of tendinitis	Foot or ankle	64 (87.7)	9 (12.3)	.286[b]	65 (89.0)	8 (11.0)	.032[b]
	Knee or leg	31 (96.9)	1 (3.1)		31 (96.9)	1 (3.1)	
	Hand or wrist	96 (89.7)	11 (10.3)		97 (90.7)	10 (9.3)	
	Forearm or arm	5 (71.4)	2 (28.6)		3 (42.9)	4 (57.1)	
	Pelvis or hip	8 (88.9)	1 (11.1)		8 (88.9)	1 (11.1)	
	Shoulder	101 (87.1)	15 (12.9)		103 (88.8)	13 (11.2)	
	Vertebra	10 (76.9)	3 (23.1)		11 (84.6)	2 (15.4)	

[a]Pearson chi-square test; [b]Fisher's exact test; P<.05 statistically significant.

the attack area and the frequency of attacks. There was only an association between the triglyceride value of the patients above the target value and the attack area.

According to the current literature, it is known that CT is frequently seen between the ages of 30 and 50.[2,15] In our study, the mean age of patients diagnosed with CT was found to be 57.40 ± 11.27 years. This may be due to the fact that we included patients with a diagnosis of CT who had a lipid profile at the time of admission and that young patients generally do not have a regular lipid profile, and middle-aged or elderly patients regularly have their lipid profile checked.

Although the etiology of CT is not clear, endocrine disorders such as thyroid disorders and estrogen hormone disorders are thought to play a role in the etiology.[8,16] Since it has been shown that there are estrogen and progesterone receptors in the rotator cuff, rotator cuff injuries are more common, especially in variations related to estrogen-related receptor beta gene.[17,18] This explains why CT is more common in women in the literature. In our study, we observed that females had CT more frequently, which was consistent with the literature.[3]

When we examine the studies on cholesterol and triglyceride levels in tendinopathies, Longo et al.[19] studies showed that there was no significant relationship between rotator cuff tears, cholesterol, and triglyceride levels. In a cohort study in which 498 678 patients were followed for 11 years, hyperlipidemia was found to be a risk factor for rotator cuff diseases.[20] Calcific tendinitis has been observed to be more common in female patients with hyperlipidemia in Taiwanese adults.[15] In our study, hyperlipidemia was also common in CT patients, and it was observed that 64% had hyperlipidemia. This can be considered compatible with studies showing that hyperlipidemia may be a risk factor in tendinitis patients.

Statin use is thought to be protective against rotator cuff diseases due to its anti-inflammatory effects.[20] There are studies showing that the use of statins, particularly simvastatin, reduces the risk of tendinopathy.[21] In our study, it was seen that 16% of the patients were using statins, but since the patients could not reach the target LDL values despite statin use, it was difficult to compare the frequency and location of the attacks.

There are several limitations to our study. Since the study was retrospective, there were only 357 patients meeting the inclusion criteria among 2055 patients with CT. Our mean age was higher than in other studies because young patients did not have routine lipid check. In addition, patients using both statins and fibrates in the study could not be evaluated according to drug use, since they remained above the target values.

CONCLUSION

In patients with CT, hyperlipidemia does not affect the frequency of attacks and the location of tendinitis, but the incidence of hyperlipidemia is high. Therefore, it is useful to examine patients with tendinitis in terms of lipid profile. Similarly, it would be useful to scan patients with hyperlipidemia in terms of CT.

Ethics Committee Approval: Ethical committee approval was received from the Health Sciences Ethics Committee of Muğla Sıtkı Koçman University (Date: September 10, 2021, Decision no: 198).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.P., F.Ö.; Design- E.P., F.Ö.; Supervision – E.P., F.Ö.; Materials – E.P., F.Ö.; Data Collection and/or Processing – E.P., F.Ö.; Analysis and/or Interpretation – E.P., İ.Hİ.; Literature Review – E.P., E.İ.Y., İ.H.İ.; Writing – E.P., F.Ö., İ.H.İ.; Critical Review – E.P., E.İ.Y., İ.H.İ.

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Original Article

Effective Therapeutic Intervention for Left Atrial Appendage Thrombus: Percutaneous Left Atrial Appendage Closure

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ABSTRACT

Objective: The thrombus formation in the left atrial appendage (LAA) can be challenging for operators and increases periprocedural complication risk. However, recent consensus documents discuss that left atrial appendage closure is a potential therapeutic option for malign left atrial appendage. This clinical study aimed to evaluate the procedural safety and early efficacy outcomes of left atrial appendage closure in patients with left atrial appendage thrombus.

Methods: This observational single-center clinical trial included 18 patients with left atrial appendage thrombus. Transesophageal echocardiography was performed before and during the left atrial appendage closure in all patients. All procedures were performed using the Amplatzer Amulet left atrial appendage closure device (Abbott Medical Inc.).

Results: Ten of the patients were male (55.6%). The mean ages were 69.6 ± 7.5 years. CHA_2DS_2-VASc and HAS-BLED scores were calculated at 5 (2-8) and 3 (1-6), respectively. In 4 patients (22.2%), left atrial appendage occlusion was indicated due to malign left atrial appendage. The significant bleeding event under oral anticoagulant treatment was the main indication in 12 patients (66.7%). All patients were referred to Transthoracic Echocardiography (TTE) and transesophageal echocardiography 30 days after the procedure. There were no major or minor adverse clinical events during the first month of follow-up. Also, no patient faced ischemic cerebrovascular events, including transient ischemic attack, hospitalization due to heart failure, or significant bleeding events. Neither device-related thrombus nor peridevice leak was observed in the Transesophageal echocardiography evaluation.

Conclusions: Left atrial appendage closure in patients with left atrial appendage thrombus is a feasible and effective method to reduce thromboembolic risk. It can be performed as an alternative therapy to oral anticoagulants (OACs) in patients with contraindications to OACs or malign left atrial appendage.

Keywords: Anticoagulants, atrial appendage, atrial fibrillation, catheterization closure devices, thrombosis

INTRODUCTION

Left atrial appendage (LAA) closure is a feasible and effective therapy to prevent thromboembolic events in patients with nonvalvular atrial fibrillation (AF). Recent guidelines suggest the LAA closure (LAAC) for AF patients with oral anticoagulant contraindication or high bleeding risk.[1,2] On the other hand, recent trials showed that the outcomes of LAAC are non-inferior to NOACs.[3] Growing procedural experience and device technology improvements expand the LAAC indications, and LAAC can be performed with the indication such as the patient's choice.

The thrombus formation in LAA can be challenging for operators and increases periprocedural complication risk. Even the challenges, case series, and multicenter observational studies showed that LAAC is a safe and effective procedure in patients with LAA thrombus.[4-6] Although LAA thrombus was considered a contraindication for LAAC several years ago, recent consensus documents discuss that LAAC is a potential therapeutic option for malign LAA.[7,8]

This clinical study aimed to evaluate the procedural safety and early efficacy outcomes of LAAC in patients with LAA thrombus.

METHODS

Study Population

In this trial, the patients who were referred to percutaneous LAAC and had LAA thrombus in preprocedural transesophageal echocardiography (TEE) were enrolled. One hundred fifty-eight consecutive patients had undergone percutaneous LAAC in Hacettepe University Department of Cardiology between 2015 and 2022. Thrombus formation in the LAA was observed in 20 patients in preprocedural assessment. In 2 patients, the

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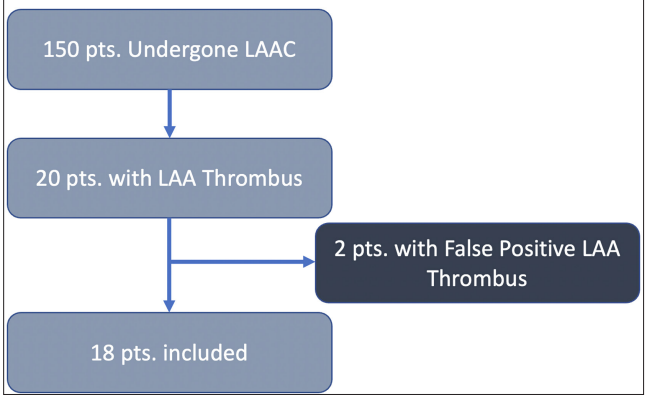
Figure 1. Flowchart of study. LAA, left atrial appendage; LAAC, LAA closure.



intravenous isoproterenol test showed that the thrombus image in preprocedural TEE was false positive for LAA thrombus. This observational single-center clinical trial included the rest of the 18 patients. Patients with overflowing LAA thrombus (type 0), concomitant mechanic heart valve, false-positive LAA thrombus (according to intravenous isoproterenol test), and are younger than 18 years were excluded (Figure 1).

Their baseline characteristics, antithrombotic medication, LAAC indications, and adverse events, including intraprocedural and during follow-up, were recorded. The written informed consent was taken from all patients before the procedure. Hacettepe University Ethics Committee approved the study (May 28, 2019, GO 19/483).

Preprocedural and Intraprocedural Thrombus Evaluation

All patients were examined with TEE cardiography before and during the procedure. After 2021, if any thrombus formation was observed or any suspicion was present in preprocedural TEE, an intravenous isoproterenol test was performed in intraprocedural TEE to confirm the presence of LAA thrombus. The intravenous isoproterenol test was done following the protocol (2 μg/min/kg over 3 min), previously described in the case report by Enomoto et al.[9]

All thrombus formations were defined according to the classification (Type 0 (overflowing), 1 (proximal to distal), and 2 (distal)) that we described in our previous study.[5]

Main Points

- The thrombus formation in left atrial appendage (LAA) is not a contraindication for percutaneous LAA closure.
- Percutaneous LAA closure can be used as a potential therapy for LAA thrombus which is resistant to OACs. Further large-scale trials are needed.
- Left atrial appendage closure in patients with LAA thrombus should be performed carefully to avoid unnecessary manipulations by experienced operators.

Procedure

The procedural technique of LAA occlusion in the patients with LAA thrombus was described in detail in our previous study.[5] All procedures were performed under general anesthesia and with fluoroscopy and TEE guidance. Amplatzer Amulet LAA closure device (Abbott Medical Inc.) was used in all patients. The inferoposterior septum was targeted for transseptal puncture to align the LAA ostium optimally. The manipulations to engage LAA ostium were minimized to avoid interaction with thrombus. Measurements of LAA and decisions on device size were made based on intraprocedural TEE. After optimal engagement to the LAA ostium, the lobe of the device was opened. Then, the disc was opened at the ostium after settlement of correctly placing the lobe in the LAA (Figure 2). The circumflex artery and mitral valve functions were evaluated with 3D-TEE. The device stability was tested before release. Intravenous heparin infusion was continued during the procedure, and dosage was adjusted with activated clotting time monitoring.

Procedural Success

After implantation, all patients were apprised of an effective occlusion (peridevice leak < 3 mm). Successful implantation is defined as the implantation that results in effective occlusion without migration of the device. MACE includes mortality, myocardial infarction, urgent surgery requirement, and clinically significant cerebrovascular ischemic or hemorrhagic events.

Postprocedural Follow-Up

The antiplatelet therapy was planned according to individual characteristics, including the indication of LAAC and each patient's thromboembolism and bleeding risk. It was scheduled as dual-antiplatelet therapy (DAPT) or a continuation of anticoagulant therapy.

The patients were examined with TTE and TEE in the first month after the procedure. Any adverse events were recorded, including bleeding complications, thromboembolic events, and heart failure or myocardial infarction hospitalization.

Statistical Analysis

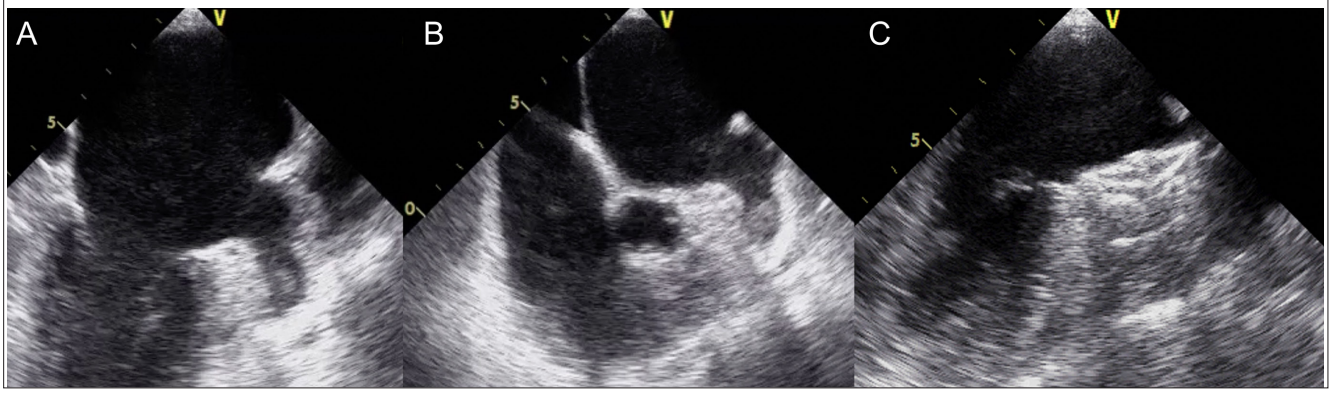
We used Statistical Package for the Social Sciences statistical software, version 20 for statistical analysis. Frequencies and percentages were used to present descriptive categorical variables. Mean values and standard deviation were used to give continuous data with the Gaussian distribution. Quantitative variables with non-Gaussian distribution are expressed with median and range. The distribution of variables was evaluated with the Kolmogorov–Smirnov test.

RESULTS

Baseline Characteristics

Eighteen patients were enrolled in the study. Ten of the patients were male (55.6%). The mean ages were 69.6 ± 7.5 years. Hypertension (17; 94.4%) was the most common comorbidity in the study population. CHA_2DS_2-VASc and HAS-BLED scores were calculated at 5 (2-8) and 3 (1-6), respectively. In 4 patients

Figure 2. (A-B) Thrombus formation in LAA (type 1). (C) Implanted Amulet device. LAA, left atrial appendage.



(22.2%), LAA occlusion was indicated due to malign LAA. The significant bleeding event under oral anticoagulant treatment was the main indication in 12 patients (66.7%). Baseline characteristics are listed in Table 1.

Left Atrial Appendage Thrombus Features

Type 1 and type 2 LAA thrombus were observed in 5 (27.8%) and 13 (72.2%) patients, respectively. Amplatzer Amulet Device was used in all patients. In 6 patients (33.3%) who had performed LAAC after 2021, intravenous isoproterenol was given to confirm LAA thrombus presence and its localization.

Procedural and Follow-up Outcomes

The LAA was occluded successfully in all 18 patients. All patients were discharged after the procedure and applied for 1 month after discharge. The median postprocedural hospitalization duration was 1 day (1-3). No MACE was observed during hospitalization.

Table 1. Baseline Characteristics

Age (years)	69.6 ± 7.5
Male sex	10 (55.6%)
Permanent atrial fibrillation	18 (100%)
Hypertension	14 (77.8%)
Heart failure	7 (39%)
Diabetes mellitus	7 (39%)
Coronary heart disease	12 (66.7%)
Chronic kidney disease	8 (44.4%)
Ischemic stroke	6 (33.3%)
$CHA2DS_2$–VASc score	5 (2–8)
HAS–BLED score	3 (1–6)
LAA closure indication	
• Bleeding	12 (66.7%)
• High bleeding risk	2 (11.1%)
• Malign LAA	4 (22.2%)

LAA, left atrial appendage.

All patients were referred to TTE and TEE 30 days after the procedure. There were no major or minor adverse clinical events during the first month of follow-up. Also, no patient faced ischemic cerebrovascular events, including transient ischemic attack, hospitalization due to heart failure, or significant bleeding events. Neither device-related thrombus nor peridevice leak was observed in the TEE evaluation.

Postprocedural antiplatelet treatment was decided on clopidogrel, DAPT, or oral anticoagulant plus clopidogrel in 4, 10, and 4 patients, respectively. Procedural and follow-up outcomes are stated in Table 2.

DISCUSSION

The main finding of this trial is that percutaneous LAAC can be performed effectively and safely in patients with LAA thrombus. Procedural feasibility is independent of the LAA occlusion indication. Left atrial appendage occlusion could be an alternative and effective treatment for patients with LAA thrombus resistant to effective oral anticoagulation therapy.

The LAA is the primary location in the heart for thrombus formation in non-valvular AF. It is responsible for 90% of the thrombus

Table 2. Outcomes

Procedural outcomes	(n = 18)
General anesthesia	18 (100%)
Amplatzer Amulet device	18 (100%)
Implantation at first attempt	16 (92%)
Procedural success	18 (100%)
Periprocedural bleeding	0 (0%)
Length of stay at hospital (days)	1 (1–3)
One month follow-up outcomes	(n = 18)
Ischemic events	0 (0%)
Bleeding events	0 (0%)
Thrombus on device at 1-month follow-up	0 (0%)
Peridevice leak at 1-month follow-up	0 (0%)

in the left atrium.[10] First-line therapy for preventing thromboembolic events in AF is oral anticoagulants. Non-vitamin K oral anticoagulants are safe and effective in non-valvular AF.[1] However, many non-valvular AF patients have a contraindication for oral anticoagulation or significant bleeding history under oral anticoagulant treatment. In addition, thrombus formation can be observed with TEE in patients who use the effective dosage of oral anticoagulants. The recent guidelines suggest LAA occlusion for AF patients with an oral anticoagulant contraindication or high bleeding risk.[1,2] On the other hand, optimal therapy for LAA thrombus resistant to OACs is not clear. In addition, there is no consensus on managing the patients with high bleeding risk and LAA thrombus. Consequently, we aimed to evaluate the feasibility of LAAC in our study group, which is the patients with LAA thrombus with or without OACs contraindication.

The Munich consensus document on LAAC emphasized that LAA thrombus, resistant to OACs, is one of the indications of LAAC.[7] More recent consensus documents stated that LAAC in patients with malign LAA is considerable.[8] We had published the first case report, which reported LAAC in a patient with high bleeding risk and LAA thrombus.[4] In addition, recent studies showed that LAAC in patients with LAA thrombus is feasible.[11]

Tarantini et al.[11] published the multicenter case series, which included 32 patients with or without high bleeding risk. In this multicenter case series, there were 3 patients with malign LAA. There was no thromboembolic event during the 1-year follow-up after the procedure. Sharma et al.[6] evaluated the patients from Tarantini et al's case series and 26 patients from other case reports. Their findings also supported that LAAC in patients with LAA thrombus is feasible and safe. The findings from our trial were similar to the previous studies and showed that short-term results of LAAC in this patient group are excellent for thromboembolic prevention.

Cerebral protection devices (CPD) are designed to reduce the risk of cardiovascular event (CVE) during cardiovascular procedures, but their role and effect in LAAC are unclear. The case series by Boccuzziet al[12], which included 27 patients, reported that using CPD during LAAC is safe and effective. Limite et al[13] supported these findings with another case series, which enrolled 14 patients. However, they were not designed as controlled studies evaluating CPDs' efficacy in LAAC. In contrast, CPD was used according to the operators' discretion in Marroquin et al's[14] multicenter registry. Although they observed macroscopic embolic material in 19.4% of the cases in which CPD was used, no intraprocedural stroke was observed in patients in which CPD was not used. In our study, CPD was not used in any cases, and we had not attended who had any intraprocedural thromboembolic event.

Bellmann et al[15] defined a fish ball technique to trap thrombus in LAA using an amulet device. Jalal et al[16] reported the thrombus trapping technique in 3 patients. Each technique was similar and performed using an amulet device. The authors of these papers emphasized the importance of minimal manipulation and avoiding interaction with thrombus. We used the same principle, and we believe that this principle should be the cornerstone of the procedure for the operators. Consequently, we preferred to define this technique as a "no-touch technique," previously described in Tarantini et al.'s[11] paper.

Medical treatment for LAA thrombus was compared with LAAC in patients referred to LAAC in Luis Marroquin et al's trial.[14] They performed LAAC on 53 patients, and intensive antiplatelet therapy was decided for 73 patients. Luis Marroquin et al[14] reported that thrombus did not change in 18 of 73 patients and partially resolved in 11 patients. Even though there was no statistical significance, LAAC was feasible with device-related thrombus, and intensified antiplatelet treatment resulted in resolution with higher bleeding events in 60% of the patients. In our study group, we think it is not optimal to intensify the antiplatelet regimen in patients with high bleeding risk. However, LAAC was performed in 4 patients with resistant LAA thrombus after intensifying or changing the anticoagulant regimen. Combining the intensifying antiplatelet treatment and LAAC seems to be the most appropriate therapeutic decision.

Although our study is the clinical trial with the highest volume that enrolled the patient who had undergone LAAC with a single device, it has several significant limitations. First, this study is observational, and the study population is small. Second, the follow-up duration is short. Third, the indications of LAAC are not homogenous in the study group. We think that indication of LAAC may affect the outcomes of LAAC.

Left atrial appendage closure in patients with LAA thrombus is a feasible and effective method to reduce thromboembolic risk. It can be performed as an alternative therapy to OACs in patients with contraindications to OACs or malign LAA.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Hacettepe University (Date: May 28, 2019, Decision no: GO/19483).

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

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Original Article

Relationship Between Coronary Lesion Severity Detected in Fractional Flow Reserve with Monocyte/High-Density Lipoprotein, Neutrophil/Lymphocyte, Lymphocyte/Monocyte, and Platelet/Lymphocyte Ratios: Which Is Most Important?

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ABSTRACT

Objective: In this study, in patients with moderate coronary lesions evaluated in coronary angiography, fractional flow reserve by lesion severity, we aimed to determine the relationship between neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, lymphocyte/monocyte ratio, and monocyte/high-density lipoprotein cholesterol ratio, which has been recently expressed as a predictor of cardiovascular disease risk.

Methods: Stenosis with a fractional flow reserve of <0.80 was considered functionally severe. According to fractional flow reserve lesion severity, a total of 131 patients were analyzed, with fractional flow reserve > 0.8 (group 1) and fractional flow reserve < 0.8 (group 2). Patients with acute coronary syndrome, severe arrhythmia, hemodynamic instability, history of previous revascularization, severe renal and hepatic failure, active infection, malignancy, hematologic disease, familial history of hyperlipidemia, rheumatologic disease, life expectancy <1 year, and age <18 and >90 years were excluded from the study.

Results: There was a statistically significant difference between monocyte/high-density lipoprotein cholesterol ratio, neutrophil /lymphocyte ratio, lymphocyte/monocyte ratio, and platelet/lymphocyte ratio, and fractional flow reserve groups ($P < .001$). Univariate and multivariate regression analyses were applied among the factors affecting the severity of the lesion detected in fractional flow reserve. Monocyte/high-density lipoprotein cholesterol ratio (odds ratio, 1.25; 95% CI, 1.05-1.47; $P = .004$), neutrophil/lymphocyte ratio (odds ratio, 3.15; 95% CI, 1.51-6.57; $P < .001$), hemoglobin A1c (odds ratio, 11.5; 95% CI, 2.76-48.4; $P = .001$), and lymphocyte/monocyte ratio (odds ratio, 0.27; 95% CI, 0.16-0.44; $P = .002$) were found to be independent predictors.

Conclusions: In this study, we would like to emphasize that simple, fast, and low-cost methods such as monocyte/high-densit y lipoprotein cholesterol ratio, neutrophil/lymphocyte ratio, lymphocyte/monocyte ratio, and platelet/lymphocyte ratio can be parameters related to lesion severity detected in fractional flow reserve. These parameters can be widely used as they are easily accessible and repeatable.

Keywords: Fractional flow reserve, high-density lipoprotein, lymphocyte, monocyte, neutrophil, platelet

INTRODUCTION

Coronary artery disease (CAD) is still the disease group most responsible for morbidity and mortality in our age. Coronary angiography (CAG) is one of the main methods used in the diagnosis of coronary artery lesions. However, the qualitative assessment of lesion severity in the coronary arteries by CAG is not always reliable. Anatomical stenosis, which is evaluated as visually severe, may not always be serious in terms of hemodynamics. Evaluation with fractional flow reserve (FFR) is an extremely important method to reveal the severity of the coronary artery lesion, especially when coronary artery stenosis is 40%-70% (i.e., moderate).[1]

The underlying cause of CAD is atherosclerosis. Inflammation is one of the leading steps in the pathogenesis of atherosclerosis. Recently, researches on the connection of inflammatory markers with cardiovascular diseases (CVD) have been the subject of study. In a study involving 105 patients with extracranial carotid artery disease, it was reported that the neutrophil/lymphocyte ratio (NLR) was positively correlated with extracranial carotid

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stenosis and was associated with lesion severity in extracranial carotid artery stenosis.[2] In a study of 963 patients with non-ST elevation myocardial infarction (NSTEMI), high monocyte/lymphocyte ratio (MLR) was stated to be riskier for major cardiac events developing in-hospital than low MLR. It has been reported that MLR is more effective than NLR in reflecting CAD in NSTEMI patients.[3] In a study of 300 patients over 70 years of age, measurements were made using the ankle-brachial index method. High platelet/lymphocyte ratio (PLR), NLR, and monocyte/high-density lipoprotein (HDL) cholesterol ratio (MHR) have been shown to be associated with peripheral arterial disease (PAD). In the study, it was stated that these 3 indices could be simple, easily accessible, and reproducible factors in the diagnosis of PAD.[4] High-density lipoprotein cholesterol protects endothelial tissue from the harmful consequences of low-density lipoprotein (LDL) cholesterol. It also prevents LDL from becoming oxidized. In addition to being an antioxidant, HDL also has antithrombotic and anti-inflammatory effects. Monocytes, on the other hand, are a parameter that plays an active role in the synthesis and distribution of cytokines with proinflammatory and prooxidant properties. In recent years, the proinflammatory effect of monocytes has been described. In addition, HDL cholesterol has been reported to have anti-inflammatory and antioxidant results. The idea has emerged that MHR is a ratio that can determine the level of oxidative stress and inflammation. It has also been associated with CVD development and long-term outcomes.[5]

In this study, with the severity of the lesion in patients who underwent FFR method after CAG, we thought to determine the relationship between NLR, PLR, MHR, and lymphocyte/monocyte ratio (LMR), which have recently been expressed as CVD risk parameters.

METHODS

We designed this study retrospectively. For this purpose, a total of 131 consecutive patients who underwent the elective FFR method were enrolled. All patients participating in our study were given detailed information and a signed consent form was requested. Biochemical, lipid, and hemogram parameters, drugs used, demographic, echocardiographic, and angiographic data of the patients were recorded. Blood tests were taken 24 hours after the patients applied to the health center. Fractional flow reserve measurement results were made at the discretion of the cardiologists. Five thousand units of heparin were given intra-arterially as a bolus. The coronary arteries were then visualized using a guide catheter without side holes. After the calibration was checked, a 0.014-inch guide wire (PrimeWire, Volcano, San Diego, Calif, USA) was placed distal to the stenosis to monitor the pressure level. Before FFR measurements, 200 µg bolus nitroglycerin was administered intracoronally. First of all, the distal intracoronary pressures of the patients were recorded. Hyperemia was triggered by administering gradually increasing doses of intracoronary adenosine until the final value in which the FFR value decreased. Fractional flow reserve value was defined as the result between the pressure measured in the intracoronary distal region and the mean aortic pressure. The highest hyperemia dimension was recorded at that time. An FFR result of <0.80 was considered functionally significant. According to FFR lesion severity, 2 groups were formed; FFR < 0.8 group (84 patients) and FFR > 0.8 group (47 patients).

Inclusion criteria for the study: patients evaluated as stable angina pectoris and undergoing the FFR procedure under elective conditions. Criteria excluded from the scope of the study: acute coronary syndrome (ACS), severe arrhythmia, hemodynamic instability, previous revascularization history, severe renal and hepatic failure, active infection, malignancy, hematologic diseases, familial history of hyperlipidemia, rheumatologic disease, life expectancy <1 year, and age <18 and >90 years.

Statistical Analysis

We obtained the statistical analysis results of the data using the Statistical Package for the Social Sciences software version 25.0 for Windows (IBM SPSS Corp., Armonk, NY, USA). Whether numerical variables were suitable for normal distribution was evaluated by analyzing with Shapiro–Wilk and Kolmogorov–Smirnov tests. The mean and standard deviation values of the numerical variables are given. Independent samples *t*-test was used if normal distribution was achieved to compare the 2 groups in terms of numerical variables. If a normal distribution could not be obtained, it was analyzed using the Mann–Whitney *U*-test. Categorical parameters were shown as number (n) and ratio (%). The correlation between categorical parameters was compared using Pearson's chi-square test and Fisher's exact test. The relationships between NLR, PLR, LMR, and MHR were compared using Spearman's rho analysis. The correlation analysis for NLR, PLR, LMR, and MHR was evaluated using univariate and multivariate regression analyses. Odds ratio and 95% CI values were recorded. In addition, receiver operating characteristic (ROC) analysis was performed for the cut-off value of NLR, PLR, LMR, and MHR ratios. The cut-off value was defined based on the Youden index. Obtaining a $P < .05$ result in all hypotheses was considered statistically significant.

Main Points

- We would like to emphasize that simple, fast, and low-cost methods such as monocyte/high-density lipoprotein cholesterol ratio, neutrophil/lymphocyte ratio (NLR), lymphocyte/monocyte ratio (LMR), and platelet/lymphocyte ratio (PLR) may be parameters related to lesion severity detected in fractional flow reserve (FFR).
- Monocyte/high-density lipoprotein cholesterol ratio predicted the severity of coronary lesion detected in FFR with 80% sensitivity and 75% specificity, NLR with 75% sensitivity and 70% specificity, LMR with 72% sensitivity and 70% specificity, and PLR with 71% sensitivity and 71% specificity.
- Although hemogram parameters play an important role in predicting the severity of the lesion before the FFR procedure, symptoms, other laboratory findings, and non-invasive imaging methods should be carefully examined.

RESULTS

Among the patients included in the study, 2 separate groups were formed as FFR > 0.8 (group I) and FFR < 0.8 (group II).

The mean age of the patients after the analysis was 58.5 (±9.6). Of these patients, 65.6% were male. When the mean age (58.4 (±10.3) vs. 58.5 (±9.3), $P = .941$) and male sex ratio (70.2% vs. 63.1%, $P = .411$) were examined, no statistically significant difference was found between the 2 groups. Of the patients, 94.7% were in the NYHA Class-I category. The most common symptoms detected in the patients were chest pain and shortness of breath (90.8% and 20.6%, respectively). When the 2 groups were compared, no statistically significant difference was found in terms of smoking (36.2% vs. 39.3%, $P = .725$) and alcohol use (4.3% vs. 6.0%, $P = .924$). There was no significant difference in the parameters of hypertension (44.7% vs. 40.5%, $P = .640$), history of CAD (46.8% vs. 54.8%, $P = .382$), and hyperlipidemia (51.1% vs. 52.4%, $P = .885$) (Table 1). Other demographic data and comorbidities between the groups are given in Table 1.

When the biochemical markers are examined, between group I and group II, respectively, HDL (44.30 (±10.68) vs. 38.23 (±9.80), $P = .001$), MPV (8.67 (±0.89) vs. 8.31 (±0.92)), $P = .029$), monocytes (0.73 (±0.19) vs. 0.95 (±0.20)), $P < .001$), lymphocytes (2.83 (±1.0) vs. 2.1 (±0) .51), $P < .001$), HbA1c (5.55 (±0.38 vs. 5.97 (±0.57)), $P < .001$), LMR (4.23) (±2.09) vs. 2.31 (±0.74), $P < .001$), NLR (1.81 (±0.60) vs. 2.50 (±0.95), $P < .001$), PLR (101.65 (±47.39) vs. 136.10 (±49.41), $P < .001$), and MHR (0.017 (±0.0064) vs. 0.026 (±0.0085), $P < .001$), significant difference was detected. The EF values obtained in the groups were compared and no significant difference was found (53.7% (±8.2) and 55.1% (±7.6), $P = .324$) (Table 2). Other hemogram, biochemical, and echocardiographic parameters are summarized in Table 2.

The medical treatments received by the patients are compared in Table 3.

In the applied correlation analysis method, there was a moderate positive correlation between FFR, and NLR and MHR parameters. In addition, it was concluded that there was a moderate negative correlation between FFR and LMR. A weak correlation was found between FFR and PLR (Table 4).

Table 1. Demographic and Comorbid Characteristic Results

Parameters	Group I (n = 47)	Group II (n = 84)	Total (n = 131)	*P*
Age (years)	58.4 (±10.3)	58.5 (±9.3)	58.5 (±9.6)	.941
Male sex, n (%)	33 (70.2)	53 (63.1)	86 (65.6)	.411
SBP, mmHg	128.7 (±17.8)	127.5 (±16.6)	127.9 (±17.0)	.691
DBP, mmHg	70.7 (±10.5)	70.8 (±10.2)	70.8 (±10.3)	.960
Heart rate, minute	76.4 (±12.3)	73.7 (±12.7)	74.6 (±12.5)	.230
NYHA class I, n (%)	46 (97.9)	78 (92.9)	124 (94.7)	.221
Chest pain, n (%)	41 (87.2)	78 (92.9)	119 (90.8)	.285
Dyspnea, n (%)	10 (21.3)	17 (20.2)	27 (20.6)	.888
Palpitation, n (%)	10 (21.3)	5 (6.0)	15 (11.4)	.008
Tiredness, n (%)	7 (14.9)	6 (7.1)	13 (9.9)	.155
Dizziness, n (%)	3 (6.4)	4 (4.8)	7 (5.3)	.692
Syncope, n (%)	0 (0)	1 (1.2)	1 (0.8)	.453
Smoking, n (%)	17 (36.2)	33 (39.3)	50 (38.2)	.725
Alcohol use, n (%)	2 (4.3)	5 (6.0)	7 (5.3)	.924
Hypertension, n (%)	21 (44.7)	34 (40.5)	55 (42.0)	.640
CAD, n (%)	22 (46.8)	46 (54.8)	68 (51.9)	.382
Hyperlipidemia, n (%)	24 (51.1)	44 (52.4)	68 (51.9)	.885
COPD, n (%)	8 (17.0)	12 (14.3)	20 (15.3)	.676
Thyroid disease, n (%)	4 (8.5)	6 (7.1)	10 (7.6)	.777
Stroke/TIA, n (%)	2 (4.3)	7 (8.3)	9 (6.9)	.376
CKD, n (%)	2 (4.3)	3 (3.6)	5 (3.8)	.592
Peripheral artery disease, n (%)	1 (2.1)	5 (5.9)	6 (4.6)	.315
Pacemaker/ICD/CRT, n (%)	0 (0)	2 (2.4)	2 (1.5)	.286
Malignancy, n (%)	1 (2.1)	1 (1.2)	2 (1.5)	.675
Anemia, n (%)	1 (2.1)	2 (2.4)	3 (2.3)	.926

SBP, systolic blood pressure; DBP, diastolic blood pressure; NYHA, New York Heart Association; CAD, coronary artery disease; COPD, chronic obstructive pulmonary diseases; TIA, transient ischemic attack; CKD, chronic kidney disease; ICD, implantable cardioverter defibrillator; CRT, cardiac resynchronization therapy; Group I, FFR > 0.8; Group II, FFR < 0.8.

Table 2. Hemogram, Biochemical, and Echocardiographic Results

Parameters Mean (±Standard Deviation)	Group I (n=47)	Group II (n=84)	Total (n=131)	*P*
Urea, mg/dL	33.79 (±12.61)	34.07 (±10.17)	33.97 (±11.06)	.893
Creatinine, mg/dL	1.06 (±0.94)	08.8 (±0.20)	0.95 (±0.59)	.111
Uric acid, mg/dL	5.48 (±1.0)	5.23 (±0.91)	5.32 (±0.95)	.150
Total cholesterol, mg/dL	187.40 (±51.27)	198.90 (±44.70)	194.78 (±47.29)	.183
Triglyceride, mg/dL	155.07 (±86.62)	179.09 (±126.06)	170.48 (±113.74)	.248
HDL, mg/dL	44.30 (±10.68)	38.23 (±9.80)	40.38 (±10.09)	**.001**
LDL, mg/dL	112.33 (±49.45)	120.34 (±37.92)	122.13 (±72.31)	.304
Hemoglobin, g/dL	13.51 (±1.24)	13.56 (±1.56)	13.54 (±1.45)	.851
Platelet, $x10^3$/μL	251.79 (±56.29)	274.30 (±71.80)	266.22 (±67.30)	.066
Leukocyte, $x10^3$/μL	8.68 (±2.32)	8.26 (±1.64)	8.41 (±1.92)	.237
MPV, fL	8.67 (±0.89)	8.31 (±0.92)	8.44 (±0.92)	**.029**
Neutrophil, $x10^3$/μL	4.91 (±1.64)	5.09 (±1.62)	5.03 (±1.63)	.541
Monocyte, $x10^3$/μL	0.73 (±0.19)	0.95 (±0.20)	0.87 (±0.19)	**<.001**
Lymphocyte, $x10^3$/μL	2.83 (±1.0)	2.10 (±0.51)	2.34 (±0.67)	**<.001**
Fasting glucose, mg/dL	98.13 (±10.55)	99.30 (±11.25)	98.88 (±10.98)	.557
TSH, μIU/Ml	2.00 (±1.33)	2.14 (±1.48)	2.09 (±1.42)	.593
T4, ng/dL	1.36 (±0.38)	1.47 (±0.50)	1.43 (±0.46)	.200
Ca, mg/dL	9.32 (±0.56)	9.36 (±0.58)	9.35 (±0.57)	.689
Sodium, mmol/L	139.51 (±2.93)	139.45 (±3.04)	139.47 (±13.63)	.915
Potasium, mmol/L	4.46 (±0.47)	4.40 (±0.48)	4.42 (±0.48)	.519
HbA1c, %	5.55 (±0.38)	5.97 (±0.57)	5.82 (±0.55)	**<.001**
LMR	4.23 (±2.09)	2.31 (±0.74)	2,95 (±1,19)	**<.001**
NLR	1.81 (±0.60)	2.50 (±0.95)	2.25 (±0.90)	**<.001**
PLR	101.65 (±47.39)	136.10 (±49.41)	123.74 (±51.27)	**<.001**
MHR	0.017 (±0.0064)	0.026 (±0.0085)	0.023 (±0.0078)	**<.001**
Sinus rhythm, n (%)	47 (100)	78 (92.9)	125 (95.4)	.061
LVEF,%	53.7 (±8.2)	55.1 (±7.6)	54.6 (±7.9)	.324
LVEDD, cm	48.25 (±5.21)	47.06 (±4.83)	47.48 (±4.98)	.189
LVESD, cm	30.47 (±6.30)	28.48 (±5.30)	29.19 (±5.73)	.056
LVDD, n (%)	29 (61.7)	59 (70.2)	88 (67.2)	.318
Moderate-severe MR, n (%)	1 (2.1)	4 (4.8)	5 (3.8)	.450
Moderate-severe MS, n (%)	0 (0)	0 (0)	0 (0)	–
Moderate-severe AR, n (%)	0 (0)	2 (2.4)	2 (1.5)	.286
Moderate-severe AS, n (%)	0 (0)	1 (1.2)	1 (0.8)	.453
Moderate-severe TR, n (%)	2 (4.3)	2 (2.4)	4 (3.1)	.535
Moderate-severe TS, n (%)	0 (0)	0 (0)	0 (0)	–

HDL, high-density lipoprotein; LDL, low-density lipoprotein; MPV, mean platelet volume; TSH, thyroid-stimulating hormone; HbA1C, hemoglobin A1c; LMR, lymphocyte/monocyte ratio; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; MHR, monocyte/HDL ratio; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVDD, left ventricular diastolic dysfunction; MR, mitral regurgitation; MS, mitral stenosis; AR, aortic regurgitation; AS, aortic stenosis; TR, tricuspid regurgitation; TS, tricuspid stenosis; Group I, FFR > 0.8; Group II, FFR < 0.8.

In the univariate and multivariate regression analyses performed among the factors affecting the severity of the lesion detected in FFR, MHR (OR, 1.25; 95% CI, 1.05-1.47, $P = .004$), NLR (OR, 3.15; 95% CI, 1.51-6.57, $P < .001$), HbA1c (OR, 11.5; 95% CI, 2.76-48.4, $P = .001$), and LMR (OR, 0.27; 95% CI, 0.16-0.44, $P = .002$) were found to be independent predictors (Table 5).

Table 3. Results of Drugs Used by Patients

Parameters	Group I (n = 47)	Group II (n = 84)	Total (n = 131)	*P*
Beta-blockers, n (%)	25 (53.2)	52 (61.9)	77 (58.8)	.331
ACE-I, n (%)	10 (21.3)	26 (31.0)	36 (27.5)	.234
Statin, n (%)	28 (59.6)	47 (56.0)	75 (57.3)	.688
Antiaggregant, n (%)	31 (66.0)	58 (69.0)	89 (67.9)	.716
Anticoagulant, n (%)	2 (4.3)	6 (7.1)	8 (6.1)	.508
ARBs, n (%)	9 (19.1)	13 (15.5)	22 (16.8)	.590
Dihydropyridine CCB, n (%)	9 (19.1)	12 (14.3)	21 (16.0)	.467
Loop diuretic, n (%)	3 (6.4)	12 (14.3)	15 (11.4)	.173
Aldosterone antagonist, n (%)	3 (6.4)	8 (9.5)	11 (8.4)	.534
Thiazide diuretic, n (%)	7 (14.9)	21 (25.0)	28 (21.4)	.176
Non-dihydropyridine CCB, n (%)	0 (0)	5 (5.9)	5 (3.8)	.088

ACE-I, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CCB, calcium channel blockers; Group I, FFR > 0.8; Group II, FFR < 0.8.

Table 4. Correlation Analysis to Determine Predictor of FFR Lesion Severity

Parameters	Correlation Analysis	
LMR	*r*	−0.52
	P	<.001
NLR	*r*	0.50
	P	<.001
MHR	*r*	0.58
	P	<.001
PLR	*r*	0.34
	P	<.001

LMR, lymphocyte/monocyte ratio; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; MHR, monocyte/high-density lipoprotein ratio.

ROC analysis was used to evaluate the power of the MHR, NLR, PLR, and LMR parameters to predict the severity of the lesions detected in the FFR. In the results obtained, MHR with 75% specificity and 80% sensitivity (AUC, 0.82; 95% CI, 0.74-0.90; $P < .001$), NLR with 75% sensitivity and 70% specificity (AUC, 0.79; 95% CI, 0.71-0.89; $P < .001$), LMR with 72% sensitivity and 70% specificity (AUC, 0.77; 95% CI, 0.69-0.87, $P < .001$), and PLR with 71% sensitivity and 71% specificity (AUC, 0.74; 95% CI, 0.63-0.78, $P < .001$) predicted lesion severity detected in FFR (Figure 1 and 2).

DISCUSSION

In this study, functionally severe coronary artery lesions evaluated using the FFR method were strongly associated with some inflammatory parameters. Atherosclerosis and CVD are among the most well-known causes of death worldwide.[6] It is known that atherosclerosis develops after inflammatory events and steps. Oxidative stress and inflammation are important steps that play a role in the initiation and progression of atherosclerosis.[7] An increased white blood cell count has been reported to be associated with adverse clinical outcomes in patients with CAD, ACS, percutaneous coronary intervention (PCI), and PAD.[8] Therefore, we aimed to examine these hemogram parameters in our study. The severity of stenosis and unstable plaque in atherosclerotic plaques are also life-threatening. In particular, neutrophils are an important factor in plaque destabilization, determination of reperfusion injury, and remodeling.[9] In a study conducted by Ionita et al.[10] a correlation between the severity of the atherosclerotic lesion in the carotid artery and the basal neutrophil count was found. In his works, neutrophil counts were higher if atherosclerotic plaques prone to dissociation were present with a higher macrophage content, lower collagen content, and smooth muscle cells.[9] In the first place, neutrophils and lymphocytes are involved in the onset of atherosclerosis.[2] Neutrophil; protein hydrolysis can accelerate the development of atherosclerosis through inflammatory and oxidative stress reactions, and lymphopenia has also been thought to be associated with the formation of atherosclerosis.[11] Another accepted process is that the NLR can represent the function of the autonomic nervous system. It has been determined that the distribution of leukocyte-forming subsets in the body is determined by the autonomic nervous system.[2]

Table 5. Univariable and Multivariable Regression Analysis to Determine Predictor of FFR Lesion Severity

Parameters			Model 1		Model 2	
Variables	Univariate, OR (95% CI)	*P* Value	Multivariate, OR (95% CI)	*P* Value	Multivariate, OR (95% CI)	*P* Value
MHR	1.32 (1.10–1.53)	<.001	1.25 (1.05–1.47)	.004		
NLR	3.8 (1.97–7.37)	<.001	3.15 (1.51–6.57)	<.001		
LMR	0.27 (0.16–0.44)	<.001			0.27 (0.16–0.44)	.002
HT	2.0 (0.76–5.42)	.042	1.8 (0.72–4.93)	.20	2.1 (0.78–5.85)	.13
HbA1c	11.5 (3.67–36.5)	.001	11.5 (2.76–48.4)	.001	8.8 (2.15–36.2)	.002
Age	1.00 (0.96–1.03)	.94				
LVEF	1.03 (0.97–1.10)	.29				

LMR, lymphocyte/monocyte ratio; NLR, neutrophil/lymphocyte ratio; HT, hypertension; MHR, monocyte/high-density lipoprotein ratio; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction.

Figure 1. The cut-off value of PLR, MHR, and NLR associated with FFR in the ROC curve analysis. NLR, neutrophil/ lymphocyte ratio; PLR, platelet/ lymphocyte ratio; MHR, monocyte/high-density lipoprotein ratio; FFR, fractional flow reserve; ROC, receiver operating characteristic.

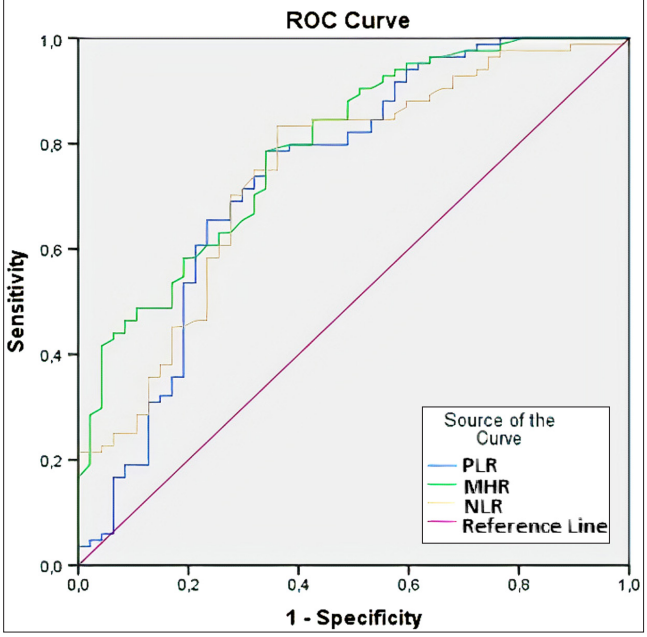
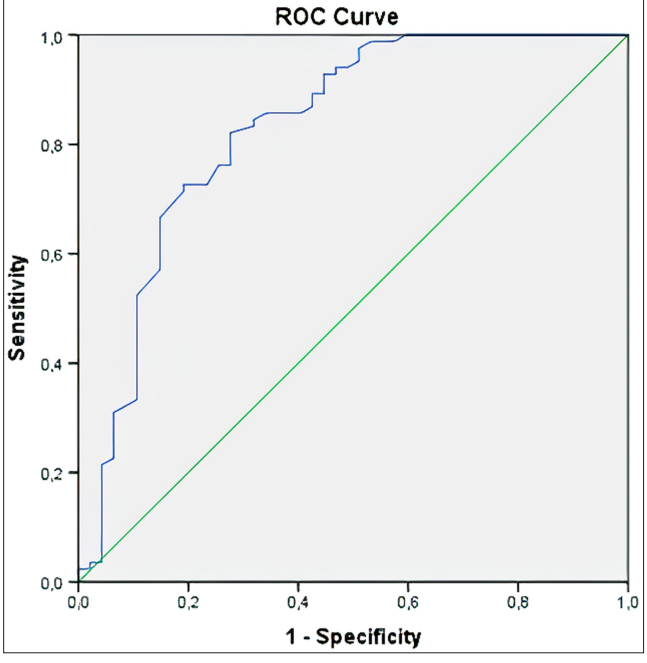


Figure 2. The cut-off value of LMR associated with FFR in the ROC curve analysis. LMR, lymphocyte/monocyte ratio; FFR, fractional flow reserve; ROC, receiver operating characteristic.



It has neutrophil adrenergic receptors. The number and function of neutrophils are determined by the sympathetic nerves. It has lymphocyte cholinergic receptor. Lymphocyte count and function are determined by parasympathetic nerves.[12] Neutr ophil/lymphocyte ratio reflected partial activity of sympatheti c/parasympathetic nerves.[13] A disruption in the autonomic nervous system may play a role in the formation of atherosclerosis.[14] In addition, NLR has been reported to be associated with early diagnosis, follow-up, treatment, and prognosis of patients hospitalized in intensive care units.[15] It has also been associated with systemic inflammatory diseases.[16] In the light of these data reported in the literature, it is consistent with the results obtained in our study. In addition, we found that NLR could be an independent predictor in patients in whom we detected severe lesions with the FFR method. In another study, a PLR ratio of >144 was found to be associated with high mortality in patients who underwent PCI after myocardial infarction (MI).[17] In addition, recent studies have reported that there is a positive correlation between the severity of PAD and NLR and PLR values, and these values may be poor prognostic markers.[18] Lymphocytes and monocytes are defense system parameters associated with the initiation and progression of the atherosclerotic process. It has been reported that low lymphocyte count and high monocyte count may have predictive and prognostic value in conditions such as stable CAD, MI, and heart failure.[19] It has been suggested that an increase in lymphocyte apoptosis and therefore a decrease in lymphocyte count has a negative effect on tissue healing and remodeling after infarction.[20] It has also been reported that deterioration in coronary microcirculation is closely associated with an increased incidence of MI and an increased risk of mortality.[21] In a few studies, it has been stated that LMR is an effective parameter that determines the systemic inflammatory response. In addition, it has been reported to be closely related to patient prognosis in many clinical conditions, including malignancies, PAH, and CAD severity.[22] In another study, LMR level before bare metal stent implantation was found to be independently associated with restenosis in patients with stable angina pectoris.[23] In our study, when we compared the PLR and LMR values, we observed that there was a statistically significant difference between the groups. Platelet/lymphocyte ratio showed poor correlation in predicting FFR lesion severity. We observed a moderate negative correlation between FFR lesion severity and LMR. In addition, we identified LMR as an independent predictor of FFR lesion severity. This result obtained in our study supports the previously reported results on this subject. Like neutrophils, monocytes play an important role in the formation of oxidative stress, inflammation, and atherosclerosis. The interaction of activated monocytes with the damaged endothelial structure leads to excessive secretion of proinflammatory cytokines.[24] Monocytes then phagocytose the oxidized LDL cholesterol molecules and differentiate into damaging macrophage cells that form foam cells. On the other hand, HDL cholesterol reduces macrophage accumulation and ensures the removal of oxidized cholesterol from the arterial wall structure. In addition to its antioxidative and anti-inflammatory properties, HDL increases the release of nitric oxide synthase in endothelial tissues and supports vasorelaxation.[25] Monocyte/high-density lipoprotein ratio is a marker that can reflect atherosclerosis severity and inflammation status. In a study by Korkmaz et al.[26] they found a correlation between atherosclerotic lesion severity and MHR after FFR. Also here,

there was no significant difference between the level of lesion severity detected in FFR and NLR, PLR, and LMR.[26] In the emergence of these findings, we think that the demographic data of the patients examined in the study, the distribution of risk factors, the inclusion criteria of the patients, and the number of patients included in the study may be effective. Cetin et al[27] reported that MHR is a predictor of stent thrombosis, severity of CAD, and CVD in long-term follow-up in patients with ACS. We can say that this finding is compatible with the results of our study. In addition, in our previous study, we found a significant correlation between the HbA1c value and the severity of the lesion detected by the FFR method.[28] In a meta-analysis examining 5 studies and a total of 1366 (606 FFR patients and 760 CAG patients) patients, MI was shown to be significantly lower in patients after FFR-guided interventional procedures compared to the CAG group. With this study, it was concluded that the FFR-based management of patients can significantly reduce the incidence of MI as it will improve the quality of life of patients, reduce the rate of rehospitalization, and reduce medical costs.[28,29] For this reason, we preferred to examine the severity of atherosclerotic lesion with the FFR method instead of CAG findings to determine more meaningful results in our study.

Study Limitations

Our study has strengths as well as some limitations. The study was retrospective. The number of patients studied was relatively small. Prospective studies with larger numbers of patients are needed to generalize the results. Many other important inflammatory parameters, such as highly sensitive C-reactive protein, were not used in the design of this study (it is unlikely to include all inflammatory parameters and perform a comprehensive analysis). The parameters we considered in the study were based on only 1 MHR, NLR, LMR, and PLR value. In other words, we did not examine THE changes in these inflammatory parameters that may develop over time.

CONCLUSION

In this study, we would like to emphasize that simple, fast, and low-cost methods such as MHR, NLR, LMR, and PLR may be parameters related to lesion severity detected in FFR. These parameters are easily accessible, reproducible, and widely used. Therefore, these parameters may be an alternative option in cases where it is difficult to apply invasive methods due to patient preference or other reasons.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of İzmir Bakırçay University (Date: April 4, 2021, Decision no: 264).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – T.G., M.K.; Design – T.G., M.K; Supervision – T.G., M.K.; Funding – No funding; Materials – T.G., M.K.; Data Collection and/or Processing – T.G., M.K.; Analysis and/or Interpretation – T.G., M.K.; Literature Review – T.G., M.K.; Writing – T.G.; Critical Review – T.G., M.K.

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Original Article

Assessment of Compatibility Between Cardiologists and Radiologists for Interpreting and Reporting Carotid Duplex Ultrasound Images

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ABSTRACT

Objective: This study aimed to evaluate the degree of agreement between cardiologists and radiologists for interpreting and reporting carotid duplex ultrasound images.

Methods: This prospective observational study was conducted in a cardiology outpatient clinic. For the sample size calculation, according to the kappa (2 raters) hypothesis testing method, assuming minimum acceptable kappa of 0.6, expected kappa of 0.8, a significance level of 0.05, power of 90%, and considering the expected dropout rate of 10% in the study, 116 patients were enrolled in the study. Demographic findings, personal histories, and laboratory test results were recorded. Carotid artery duplex ultrasonography was performed simultaneously and recorded by cardiologists and radiologists.

Results: This study included 116 patients who were treated in cardiology outpatient clinics for ischemic stroke, trans-ischemic attack, amaurosis fugax, dizziness, and severe headache complaints. While 50.9% of them are female, 49.1% were male. The age range of patients included in the study was a minimum of 32 years and a maximum of 71 years. Their mean age and deviation were 58.6 ± 10.1. Examination of their distribution according to chronic disease states revealed that 44.8% had hypertension, 58.6% were smokers, 36.2% had diabetes mellitus, 22.4% had dyslipidemia, 13.8% had ischemic heart disease, 29.3% had chronic obstructive pulmonary disease, and 16.4% had congestive heart failure. According to the criteria for carotid stenosis measurement of ≤50% and >50%, a significant and almost perfect agreement was found between the measurements by cardiologists and radiologists (Cohen's kappa coefficient $\kappa = 0.811$; $P < .0001$).

Conclusion: Diagnostic compatibility with radiologists was found to be near-perfect for carotid ultrasound evaluation.

Keywords: Carotid ultrasound, dizziness, handheld ultrasound, POCUS, stroke

INTRODUCTION

Carotid artery disease is observed in less than 3% of the general population.[1] It has a wide range of clinical presentations. It is considered one of the causes of the transient ischemic attack, ischemic stroke, sudden vision loss, dizziness, and severe headaches. It is common in patients with coronary and peripheral artery diseases.[2]

Cerebrovascular events due to carotid artery diseases are common in developed countries.[3] In clinical practice, imaging obtained using carotid duplex ultrasound (USG) is important in both the management of patients with acute stroke and the risk assessment of coronary artery disease or stroke.[4]

Treatment approaches are generally determined by cardiologists based on carotid ultrasonography results. The increase in the number of patients, together with the increasing elderly population, has further increased the workload of radiologists and sonographers.

The carotid arteries can be evaluated if the vascular imaging application is selected in echocardiography devices and a linear probe is used. If short-term training is provided to cardiologists who know the features of ultrasound devices, carotid ultrasound can be performed simultaneously by cardiologists along with echocardiography. Thus, patients are evaluated in a short time.

Our study aimed to determine whether the reports of carotid duplex USG performed and interpreted by cardiologists who have attended an accredited USG course are compatible with reports interpreted by radiologists.

METHODS

Study Design and Settings

This prospective observational study was conducted in 2022. The Ethics Commission of Gazi Yaşargil Training and Research Hospital authorized the study and waived the need for informed consent (Date: March 11, 2022, Decision no: 2022-45). This study was conducted as per the Declaration of Helsinki (2013).

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Selection of Participants

Patients over the age of 18 who provided written informed consent and were admitted to the cardiology outpatient clinics with ischemic stroke, trans-ischemic attack, amaurosis fugax, dizziness, and severe headache were included in the study. First, unstable patients were excluded. Patients with neck tumors, masses, diffuse goiters, recent neck trauma, or neck surgery were excluded from the study.

Study Protocol

This study included only patients who met the inclusion criteria. Demographic findings, history, and laboratory test results were recorded. Carotid duplex USG imaging was interpreted by cardiologists with at least 5 years of experience, attended an accredited ultrasound course, and achieved success. Before the study, the cardiologists received 2 hours of didactic and 2 hours of practical training. In a preliminary study, accurate measurements were obtained at least 30 times. Ultrasound images were transferred to a computer and interpreted by an experienced and independent radiologist. Figure 1 shows the flow diagram of the patients enrolled in the study. Images with poor quality were excluded from further analyses.

Imaging

The carotid artery imaging procedure was performed according to the recommendations of the American Society of Echocardiography and the European Society of Radiology.[5] The carotid arteries were evaluated in the transverse and sagittal planes (Figure 2). Intima-media thickness and arterial flow velocity were measured from the transverse plane following the recommendations of the American Society of Echocardiography.[6] The examinations were performed with a linear probe (L 12-3) using a Philips brand ultrasonography device (Model HD7 XE).

Pre-Study Power Analysis

For the sample size calculation, according to kappa (2 raters) hypothesis testing method, assuming minimum acceptable kappa of 0.6, expected kappa of 0.8, a significance level of 0.05, power of 90%, and considering the expected dropout rate of 10% in the study, 116 patients were enrolled in the study.

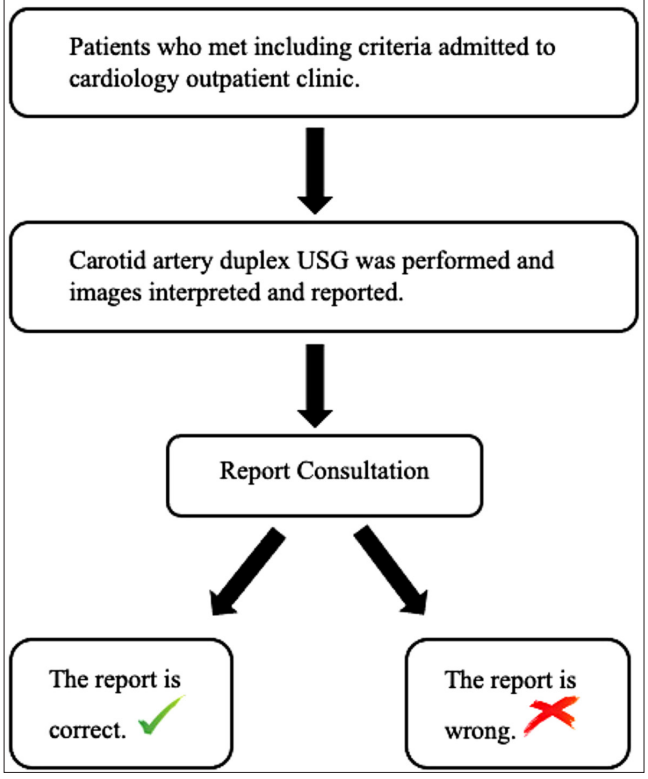
Statistical Analysis

The Statistical Package for the Social Sciences program was used to conduct all analyses (version 24.0, Chicago, Ill, USA). The mean and standard deviation and the minimum and maximum values of the features were used to establish categorical variables, such as frequency and percentage values. The compatibility of different doctors' nominal-level measurement results was evaluated using Cohen's kappa. The statistical significance level was set at $P < .05$.

Main Points

- Cardiologists can perform carotid duplex ultrasounds as part of the exam and speculate about the severity of coronary artery disease.
- Cardiologists can easily detect non-stroke subclinical carotid artery stenosis using carotid duplex ultrasound.
- Interventional cardiologists who perform carotid artery stenting can follow up the patients before and after the procedure with carotid ultrasound.

Figure 1. Study flow diagram.



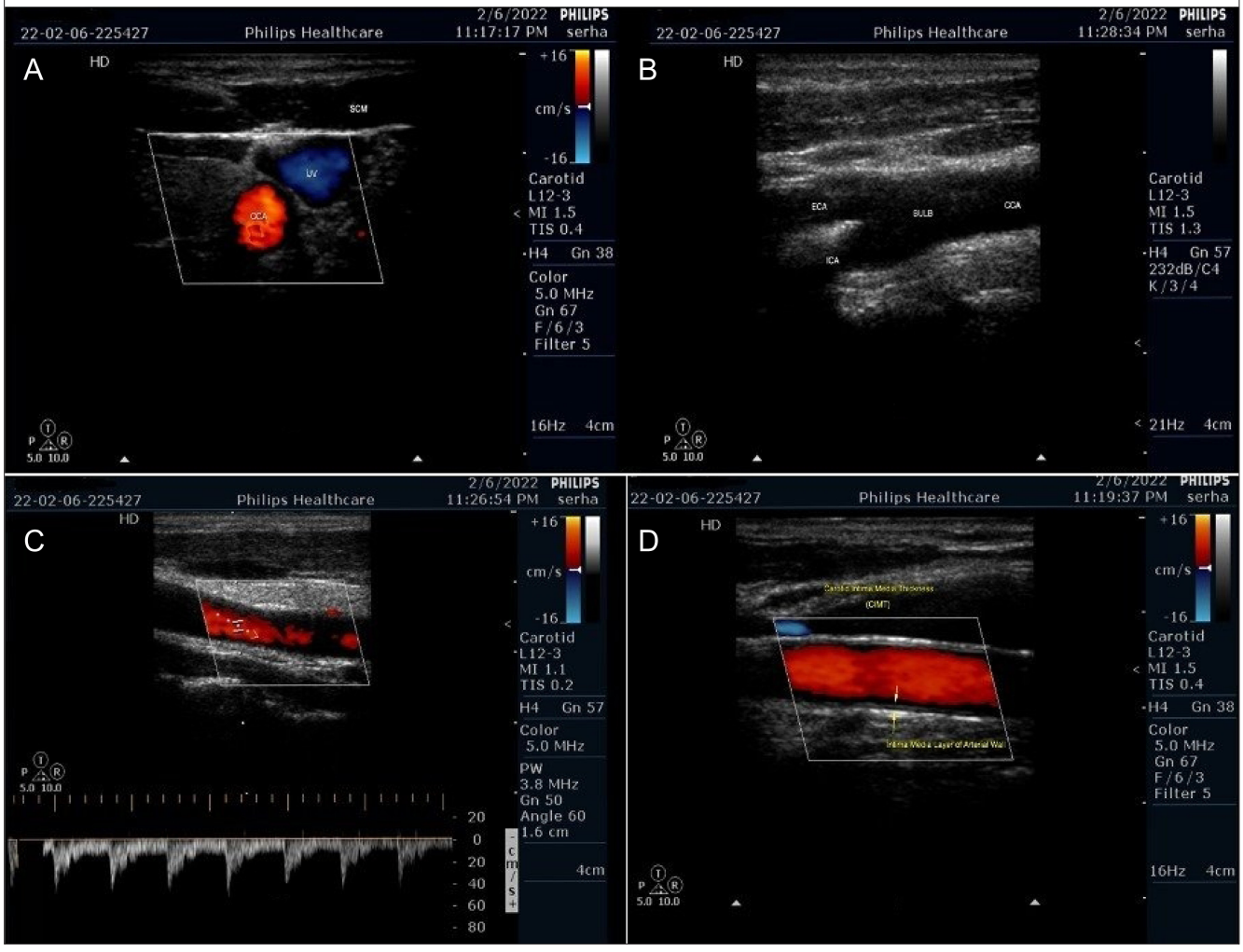
RESULTS

The socio-demographic and clinical history of the patients are shown in Table 1. While 50.8% of them were female, 49.2% were male. The age range of the patients included in the study was a minimum of 32 years and a maximum of 71. Their mean age and deviation were 58.6 ± 10.1. When their distribution according to chronic disease states was examined, 44.8% had hypertension (HT), 58.6% were smokers, 36.2% had diabetes mellitus (DM), 22.4% had dyslipidemia, 13.8% had ischemic heart disease (IHD), 29.3% had chronic obstructive pulmonary disease (COPD), and 16.4% had congestive heart failure (CHF) (Table 1). The mean and min-max measurements of vital signs and laboratory parameters of the patients are shown in Table 2. Table 3 shows physician compliance statistics between radiologists and cardiologists according to carotid artery stenosis measurements. As seen in Table 3, according to the criteria for carotid stenosis measurement of ≤50% and >50%, a significant and almost perfect agreement was found between the measurements of cardiologists and radiologists (Cohen's kappa coefficient (κ) = 0.811; $P < .0001$) (Table 3).

DISCUSSION

This study suggests that cardiologists who attended accredited carotid USG training can successfully interpret and report carotid artery USG images with short-term training (κ = 0.811; $P < .001$).

Figure 2. (A) Transverse Doppler image of common carotid artery (CCA) and internal jugular vein (İJV); (B) sagittal B-mode view of CCA, BULB, internal carotid artery (ICA), and external carotid artery (ECA); (C) normal carotid artery duplex; (D) image of carotid intima-media thickness (CIMT).



Carotid atherosclerosis is observed in 25% of men and women worldwide. While it is 1% between the ages of 50 and 60, this rate can reach 80% between the ages of 70 and 90. A recent study observed a higher rate of carotid atherosclerosis in women in Chinese society.[7,8] Smoking, high LDL levels, obesity, hypertension, diabetes, COPD, advanced age, and family history were identified as risk factors.[9-11] In our study, the average age of the patients was 58.6 ± 10.1 years, and the incidence in females was 50.8%. Smoking was observed in 58.6%, dyslipidemia in 22.4%, overweight in 19%, hypertension in 44.8%, diabetes in 36.2%, COPD in 29.3%, CHF in 16.4%, and CVD in 20.7% rates. Mean SBP was measured at 137.0 ± 6.75 mmHg, DBP was 87.9 ± 2.91 mmHG, heart rate was 91.3 ± 20.82 beats/min, and glucose was 158.2 ± 74.37 mg/dL. Other laboratory findings were normal.

Coronary heart disease, ischemic stroke, and peripheral vascular disease are caused by atherosclerosis. Cardiovascular diseases are closely related to each other.[12] Echocardiography is usually performed in patients who visit a cardiology outpatient clinic. A certain majority of the patients had HT, diabetes, and ischemic stroke. Carotid duplex ultrasound is sometimes required for optimal treatment of these patients. Atherosclerotic plaques in the carotid artery cause a significant part of ischemic strokes and are easily assessed using duplex ultrasonography.[13] Assessment of arterial stiffness and atherosclerotic load in the carotid arteries can provide crucial prognostic information regarding the risk of future cardiovascular events.[14] Carotis intima-media thickness (CIMT) is a biomarker used in the diagnosis of atherosclerosis.[15] It is an independent risk factor for stroke and myocardial infarction.[16,17] Therefore, it has been the focus of attention of both cardiologists and neurologists. Cardiologists with the skill of using an ultrasound device can determine the severity of cardiovascular disease by calculating the CIMT or by directly assessing the volume and morphology of the plaque.

Continuous advances in ultrasound technology have led to an era of widespread access to these devices. Point-of-care

Table 1. Socio–Demographical and Disease History Distribution

N = 116		$\bar{x} \pm SD$	Min–Max
Age		58.6 ± 10.1	32–71
N = 116		**n**	**%**
Gender	Female	59	50.9
	Male	57	49.1
Smoker	No	48	41.4
	Yes	68	58.6
HT	No	64	55.2
	Yes	52	44.8
DM	No	74	63.8
	Yes	42	36.2
IHD	No	100	86.2
	Yes	16	13.8
CVD	No	92	79.3
	Yes	24	20.7
COPD	No	82	70.7
	Yes	34	29.3
CHF	No	97	83.6
	Yes	19	16.4
DL	No	90	77.6
	Yes	26	22.4
BMI	Underweight	7	6
	Normal weight	87	75
	Overweight	22	19

Values are reported as n (%) for categorical variables. HT, hypertension; DM, diabetes mellitus; CVD, cerebrovascular disease; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; CHF, congestive heart failure; BMI, body mass index; SD, standard deviation.

ultrasound and handheld ultrasound devices with dual probes, which are considered stethoscopes of the 21st century, have been adopted by many experts for diagnosis and treatment. It has been applied in areas such as the gastrointestinal, musculoskeletal, obstetric, respiratory, vascular, and cardiovascular systems.[18] Its use in intensive care units and emergency departments is becoming increasingly common.[19,20] Oluku et al.[21] achieved excellent results in bone fracture diagnostics. In comparison with radiologists, diagnostic compatibility with high sensitivity and specificity was observed. Ultrasound training has been integrated into the education of emergency physicians. Thus, the need for radiologists has gradually decreased.[22] Simultaneously, the workload of the radiologists was alleviated by decreasing the ultrasound orders.[23]

The performance of carotid ultrasonography and interpretation of the exam are delayed because of the heavy workload of the sonographers and radiologists. Cardiologists can evaluate the carotid arteries simultaneously with echocardiography instead of waiting for USG reports from radiologists to organize optimal

Table 2. Distribution of Vital Findings and Laboratory Measurements

N = 116	$\bar{x} \pm SD$	Min–Max
Systolic blood pressure (mmHg)	137.0 ± 6.75	130–159
Diastolic blood pressure (mmHg)	87.9 ± 2.91	85–98
Pulse (beat/min)	91.3 ± 20.82	60–170
Glucose (mg/dL)	158.2 ± 74.37	72–421
Creatinine (mg/dL)	1.04 ± 0.54	0.41–3.75
Na (mmol/L)	139.3 ± 3.32	128–147
K (mmol/L)	4.0 ± 0.46	3.0–5.19
HBG (g/dL)	13.4 ± 1.86	9.0–17.1
HCT (%)	40.5 ± 4.93	27.1– 50.2
AST (U/L)	28.2 ± 17.51	10–99
ALT (U/L)	18.6 ± 10.42	4–56
WBC ($10^3/mm^3$)	11.5 ± 3.15	6.09–18.34
PLT ($10^3/mm^3$)	268.8 ± 70.45	67–633

Values are reported as mean ± SD for continuous variables. Na, sodium; K, potassium; HBG, hemoglobin; HTC, hematocrit; ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cells; PLT, platelets; INR, international normalized ratio; SD, standard deviation.

treatment. Thus, they can notice subclinical carotid artery stenosis without developing a stroke or having an idea of the severity of the concomitant coronary artery disease.[24,25] In addition, interventional cardiologists who perform carotid artery stenting will more easily perform pre- and post-procedure follow-ups of patients with carotid artery USG without the need for radiologists.[26,27]

Performing carotid ultrasonography and echocardiography will increase time and labor intensity. The lack of reimbursement for carotid ultrasonography may not convince cardiologists. Carotid artery duplex USG by cardiologists will bring a big change in favor of patients if suitable conditions are provided.[28] Stroke and coronary artery events can be reduced through early diagnosis and treatment.

The prominent limitation of this study was its single-center prospective nature with a small patient cohort. During the procedure, calcification obscuring a large-vessel segment in some patients requires another imaging modality. Carotid USG could

Table 3. Evaluation of Inter–Physician Compliance

N = 116		Cardiology		Cohen's Kappa	*P*
Carotis Artery Stenosis		**≤50%**	**>50%**		
Radiology	**≤50%**	78	6	***0.811****	***<.001****
	>50%	3	29		
	Total	81	35		

*Cohen's kappa coefficient is accepted at the $\kappa > 0.6$.
**$P < .05$.

not be performed in patients suspected or diagnosed with COVID-19 during the COVID-19 pandemic.

CONCLUSION

We found almost perfect compatibility between cardiologists and radiologists in interpreting and reporting of carotid duplex USG findings. We believe that this will be beneficial in decision-making for patients who present to the cardiology outpatient clinic with complaints of sudden numbness or weakness of the unilateral arm and leg, sudden loss of vision, dizziness, and severe headache. Cardiologists should have vascular ultrasound training as part of their education.

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Original Article

Comparison of the Efficacy of *Lactobacillus rhamnosus* GG and Lactulose Treatments in Minimal Hepatic Encephalopathy

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ABSTRACT

Objective: Minimal hepatic encephalopathy is a condition characterized by decreased perception and consequently deterioration in quality of life, and there is still limited data on its treatment. The aim of this study is to compare the efficacy of lactulose and *Lactobacillus rhamnosus* GG treatments by critical flicker frequency test in minimal hepatic encephalopathy patients.

Methods: Patients with a critical flicker frequency test result of <39 Hz were considered to have minimal hepatic encephalopathy. Eighty-four minimal hepatic encephalopathy patients were divided into 3 groups as lactulose, *Lactobacillus rhamnosus* GG, and control group. Critical flicker frequency control was performed 4 weeks after treatment. Critical flicker frequency values before and after treatment were compared according to the treatment groups and evaluated.

Results: Minimal hepatic encephalopathy was detected in 84 (54.5%) of 154 cirrhosis patients. Of the patients with minimal hepatic encephalopathy, 31 (36.9%) received lactulose, 31 (36.9%) *Lactobacillus rhamnosus* GG treatment, and 22 (26.2%) did not receive any treatment. In patients with minimal hepatic encephalopathy compared to those without minimal hepatic encephalopathy, there were statistically significant differences in terms of age ($P = .003$), body mass index (BMI) ($P = .019$), albumin ($P < .001$), sodium ($P = .010$), model for end-stage liver diseases score ($P < .001$), and Child Pugh Classification (CHILD) score ($P < .001$).There was no significant difference between cirrhosis etiology and treatment response ($P = .535$). Statistically significant increase was found in critical flicker frequency values in the lactulose ($P = .011$) and *Lactobacillus rhamnosus* GG ($P = .007$) groups after treatment. No statistically significant difference was found in the placebo group ($P = .804$). There was no statistically significant difference between lactulose and *Lactobacillus rhamnosus* GG ($P = .576$).

Conclusion: In the treatment of minimal hepatic encephalopathy, *Lactobacillus rhamnosus* GG treatment is as effective like lactulose treatment and can be used safely.

Keywords: Critical flicker frequency, lactobacillus rhamnosus, lactulose, minimal hepatic encephalopathy

INTRODUCTION

Hepatic encephalopathy (HE) is a brain dysfunction that can be associated with hepatic failure, cirrhosis, or portosystemic shunts, can range from subclinical disease to coma, and is accompanied by neurological or psychiatric abnormalities.[1] In minimal hepatic encephalopathy (MHE), no mental or neurological disorder is detected during clinical examination. Minimal hepatic encephalopathy is the early stage of covert hepatic encephalopathy (CHE), which can be diagnosed by neurophysiological and psychometric tests.[2] There is no obvious impairment of cognitive functions in MHE. However, there is a significant decrease in the quality of life in these patients due to decreased visual perception, impaired ability to drive, and difficulty in performing tests that require psychomotor speed and attention.[3]

Minimal hepatic encephalopathy is diagnosed by neurophysiological and psychometric tests. Psychometric tests can be affected by factors like age and education level and standardization. In addition, these tests take a long time to perform and, if repeated, may give erroneous results because patients learn the tests and memorize them. Test results may be influenced by these disadvantageous situations.[4] Other tests used in the diagnosis of MHE are Inhibitory Control Test, Cognitive Drug Research Test, Scan Test, STROOP App Test, and the critical flicker frequency (CFF) test.[5] The level of education, age of the patient, the fact that the test is not affected by frequent repetitions, and its prediction of overt hepatic encephalopathy (OHE) make the CFF test superior to the others. In addition, improvement with treatment observed in the test results is one of the advantages of the CFF test.[6] Once CHE develops in patients with cirrhosis,

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50% of the patients develop OHE, an indication that the prognosis will be poorer within three years. After the development of OHE, neuropsychiatric disorders may become persistent and unresponsive to medical therapy. In addition, it can cause major problems which can require liver transplantation. As a result, treatment of CHE is important.[7]

There is still no consensus on which treatment scheme is the best once CHE is detected. Probiotics, non-absorbable disaccharides, rifaximin, and L-ornithine L-aspartate (LOLA) are currently the most studied and recommended therapeutic agents in the treatment of CHE.[8] The common goal of treatments is to reduce the formation and absorption of ammonia and other toxins in the intestine. Lactulose has properties such as acidifying the feces, increasing beneficial organisms in the intestine, and shortening the colonic transit time, in addition to its laxative effect.[9] The effect of dysbiosis on the development of HE is currently well known, and there are many studies showing that the use of probiotics reduces episodes of HE.[10] Rifaximin is a gastrointe stinal-specific antibiotic, and when used together with lactulose has been shown to improve cognitive functions and to decrease ammonia levels.[11]

Although there are studies on the use of lactulose and probiotics in the treatment of MHE, the number of studies evaluating the comparison of these treatments is limited. Many different probiotics have been used in the treatment of MHE, and there are no studies on *Lactobacillus rhamnosus* GG (LbGG). The first aim of our study was to determine the prevalence of MHE in patients with cirrhosis. It was also aimed to compare the efficacy of lactulose or probiotic (LbGG) treatments on MHE in patients followed up for cirrhosis and diagnosed with MHE by the CFF test.

METHODS

Seven hundred eighty-four patients diagnosed with liver cirrhosis and admitted to the hepatology outpatient clinic of Gaziantep University Medical Faculty Hospital were evaluated retrospectively. The patients were diagnosed with cirrhosis by evaluating together with anamnesis, physical examination, laboratory findings, imaging methods, and/or liver biopsy. Since 51 patients died due to various reasons, the files of the remaining 723 patients were evaluated in detail. The exclusion criteria were history of OHE, alcohol use in the last 3 months, receiving treatment for HE, visual and/or hearing impairment, hepatocellular cancer (HCC) or other malignancy, active gastrointestinal bleeding, electrolyte imbalance, alcohol-induced liver cirrhosis, presence of a psychiatric or neurological disease, use of an anti-psychotic or sedative, etc., presence of overt porto-systemic shunt, previous shunt surgery, use of antibiotics, lactulose or LOLA in the last 6 weeks, presence of active infection, and not giving written consent for inclusion in the study. A total of 154 patients who met the inclusion criteria, aged above 18 years, and did not have findings suggestive of OHE (euphoria, depression, sleep disorders, impaired handwriting, changes in mental functions, memory disorders, mild disorientation, and coordination disorders) were included in the study.

Main Points

- It may be recommended that patients with advanced Child-Pugh stage and high model for end-stage liver diseases scores should be approached more carefully in terms of minimal hepatic encephalopathy (MHE) screening.
- The etiology of cirrhosis did not play an important role in the development of MHE.
- In our study, the treatment efficacy for MHE was found to be higher in the groups given lactulose and *Lactobacillus rhamnosus* GG compared to the placebo group. However, no superiority was found between the 2 treatment groups.

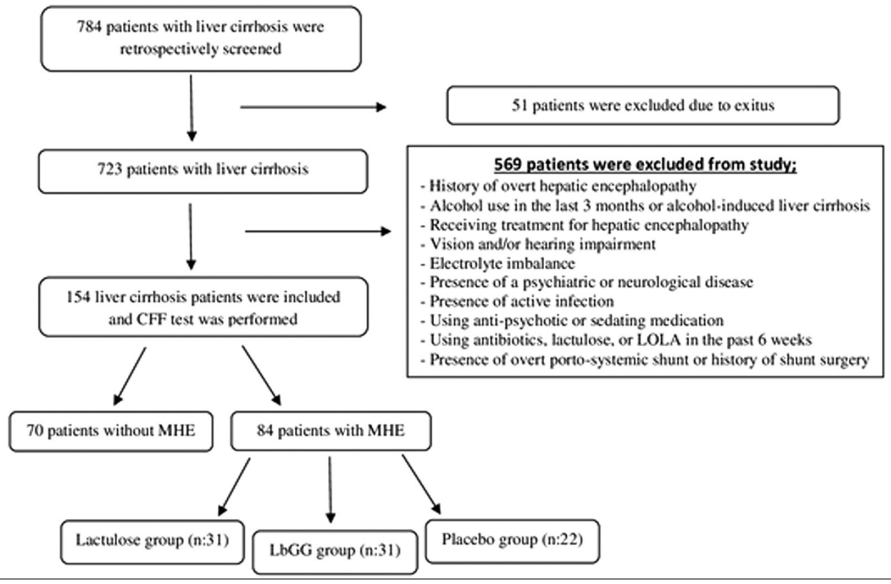
Medical history of patients was obtained, and complete blood count and biochemical tests were requested for all patients. The etiology of cirrhosis (viral, cryptogenic, autoimmune, metabolic, etc.) and complications of cirrhosis were recorded from patient files. Child-Pugh and model for end-stage liver diseases (MELD) scores were calculated. Based on the presence of MHE, patients were compared according to their demographic characteristics, laboratory parameters, BMI, Child-Pugh, and MELD scores. The patients were classified as compensated and decompensated cirrhosis according to the history of ascites, jaundice, and varicose bleeding other than HE. The BMI of the patients was calculated as kg/m^2. The literacy status of the patients was recorded.

All patients underwent the CFF test. Eighty-four patients with MHE based on the CFF test were divided into 3 groups. The first group (n = 31) was given lactulose (duphalac 3.335 mg/5 mL) oral solution 1-2 times a day to provide soft stools. The second group (n = 31) was given probiotic tablets containing 6 billion LbGG (kaleidone 60 mg capsule) twice a day. No treatment was given to the third group (n = 22) (Figure 1). The patients underwent the CFF test at the time of their first tests and 30 days after treatment. Patients with follow-up CFF values of >39 Hz after the MHE treatment were considered to have benefited from the treatment. The relationship between treatment benefit and the treatment groups, laboratory parameters, demographic characteristics, cirrhosis etiology, and cirrhosis complications were then compared. Ethics committee approval was obtained from the Ethics Committee of Gaziantep University (Date: July 24, 2017, Decision no: 2017/280). Oral and written explanations were given to the patients included in the study and their consent was obtained.

Minimal Hepatic Encephalopathy Evaluation

The presence of MHE was evaluated with the CFF test. This is a test that measures the highest range of flickering of light from a light source perceivable by the patient. The test was performed using a HEPAtonorm analyzer (R&R Medi-Business Freiburg GmbH, Freiburg, Germany). Each patient was taken to a comfortable, quiet test room away from external stimuli. Each patient was told how to perform the test. To perform this test, a device was attached to the patient's head, and after a steady red light arrived in the patient's eyes, the patient was asked to observe and follow this red light and press the button in his/her hand when he/she noticed that the light was flickering. After this test was repeated several times by the patient, recording was started.

Figure 1. Flow chart of the study. LOLA, L-ornithine L-aspartate; MHE, minimal hepatic encephalopathy; CFF, critical flicker frequency; LbGG, *Lactobacillus rhamnosus* GG.



The test was performed on the patients 9 times and recorded. The mean and standard deviation of these 9 tests were then determined. Patients with a CFF test result of <39 Hz were considered to have MHE.

Diet and Drug Habits

Patients with ascites were recommended to take a salt-poor diet (<2 g sodium) throughout the study. Protein intake was not restricted and all patients were encouraged to take a diet containing 1-1.5 g/kg/day of protein. Decision to give treatment to patients whose CFF results were not normal was made from the files. Treatments received by the patients were recorded. It was confirmed that none of the patients was treated with rifaximin, LOLA, lactulose, or LbGG. The treatment of patients who were taking diuretics, spironolactone for ascites, or beta-blocker therapy for varicose bleeding prophylaxis was not discontinued.

Statistical Analysis

Data evaluations were made using software package programs Statistical Package for the Social Sciences 22 (SPSS Inc., Chicago, IL, ABD). Data were expressed as mean ± SD. The Student's *t* test, chi square, dependent groups *t*-test, Mann-Whitney *U* test, and Kruskal–Wallis analysis were used together with descriptive statistics. The statistical significance value was considered as $P < .05$.

RESULTS

The medical records of 784 patients with cirrhosis were evaluated. Seventy-nine (51.3%) of the 154 patients with cirrhosis included in the study based on the exclusion criteria were male. The mean age was 55.1 ± 13.4 years. No statistically significant difference was found between the mean ages by gender ($P = .087$). The mean BMI of the patients was 27.8 ± 4.4 kg/m^2, while the mean BMI of female patients was found to be statistically significantly higher than of the men (29.1 ± 4.8 kg/m^2 vs. 26.5 ± 3.6 kg/m^2, respectively, $P < .001$). The mean value for the CFF test applied to the patients was 40.0 ± 5.9 (min = 26.9, max = 56.3). Patients with a CFF test result of <39 Hz were considered to have MHE. Minimal hepatic encephalopathy was detected in 84 (54.5%) of the patients.

Comparison of the demographic characteristics, laboratory parameters, MELD, and CHILD scores of the patients according to the presence of MHE demonstrated that there was a significant difference between the 2 groups in terms of age ($P = .003$), BMI ($P = .019$), albumin ($P < .001$), sodium ($P = .010$), MELD scoe ($P < .001$), and CFF values ($P < .001$) (Table 1). Of the patients without MHE, 48 (68.5%) were Child A, 21 (30%) were Child B, and 1 (1.5%) was Child C. Of the patients with MHE, 24 (28.5%) were Child A, 52 (61.9%) were Child B, and 8 (9.6%) were Child C. The rate of developing MHE was observed to be statistically significantly higher with increased Child-Pugh scores ($P < .001$) (Table 1).

With respect to the etiology of cirrhosis, 13 (15.4%) of the patients with MHE were reported to be cryptogenic, 13 (15.4%) had nonalcoholic steatohepatitis, 25 (29.7%) had Hepatitis C virus (HCV), 19 (22.6%) had Hepatitis B virus (HBV), 3 (3.6%) had Budd-Chiari syndrome, 2 (2.4%) had Wilson's disease, 3 (3.6%) had celiac disease, 3 (3.6%) had portal vein thrombosis, 2 (2.4%) had primary biliary cirrhosis while 1 (1.2%) had autoimmune hepatitis. There was no statistically significant relationship in terms of development of MHE in the patients according to the etiology of cirrhosis ($P = .573$) (Table 2). Of the patients without MHE, 58 (82.8%) had compensated cirrhosis, while 12 (12.8%) had decompensated cirrhosis. Of the patients without MHE, 41 (48.8%) had compensated cirrhosis, while 43 (51.2%) had decompensated cirrhosis. When compared according to the type of cirrhosis, the rate of MHE was found to be statistically significantly higher in patients

Table 1. Comparison of Demographic Characteristics and Laboratory Parameters of Patients According to the Presence of Minimal Hepatic Encephalopathy

Parameters	Non-MHE	MHE	*P*
Age	51.5 ± 13.7	58.0 ± 12.5	.003
BMI (kg/m²)	28.5 ± 4.6	26.9 ± 3.9	.019
WBC (µL)	4502 ± 1714	4763 ± 2194	.419
Hb (g/dL)	12.6 ± 2.1	12.2 ± 2.2	.189
PLT (µL)	96 940 ± 62 435	99 310 ± 51 930	.798
AST (U/L)	46.3 ± 27.2	43.8 ± 23.4	.532
ALT (U/L)	33.5 ± 26.8	29.3 ± 17.6	.247
INR	1.5 ± 0.2	1.6 ± 0.3	.132
Kreatinin (mg/dL)	0.7 ± 0.3	0.8 ± 0.2	.100
Albumin (g/dL)	3.8 ± 0.4	3.0 ± 0.4	.001
Total bilirubin (mg/dL)	1.7 ± 1.7	1.8 ± 1.5	.692
Sodium (mmol/L)	138.3 ± 2.6	133.4 ± 3.5	.010
MELD	13.9 ± 3.1	16.6 ± 3.8	.001
CFF	45,3 ± 4,1	35.7 ± 2.9	.001
Child A	48 (66.7%)	24 (33.3%)	.001
Child B	21 (28.8%)	52 (71.2%)	.001
Child C	1 (11.1%)	8 (89.9%)	.001

MHE, minimal hepatic encephalopathy; CFF, critical flicker frequency; INR, International normalized ratio; ALT, alanine aminotransferase; AST, aspartat aminotransferase; HBG, hemoglobine; PLT, platelet.

with decompensated cirrhosis than in patients with compensated cirrhosis (78.2% vs. 41.4%, respectively, $P < .001$).

Patients with MHE were divided into 3 groups as lactulose (n = 31, 36.9%), LbGG (n = 31, 36.9%) and placebo (n = 22, 26.2%) patient groups. There was no significant difference between the treatments received and the treatment response according to the etiology of cirrhosis ($P = .535$). When the CFF values before and after the treatment were compared according to the groups, there was a statistically significant increase in the CFF values in the lactulose ($P = .011$) and LbGG ($P = .007$) groups after the treatment. No statistically significant difference was found in the placebo group ($P = .804$) (Table 3). Minimal hepatic encephalopathy was found to be resolved (CFF > 39 Hz) in 24 (77.4%) of the 31 patients with MHE who were treated with lactulose and in 22 (70.9%) of the 31 patients who were treated with LbGG. However, no statistically significant difference was found between the 2 groups ($P = .576$).

Table 2. The Relationship Between the Development of Minimal Hepatic Encephalopathy According to the Etiology of Cirrhosis

	Non-MHE		MHE		
Etiology of Cirrhosis	(n)	(%)	(n)	(%)	*P*
Cryptogenic	11	45.8	13	54.2	.573
NASH	13	50	13	50	
Hepatitis C	14	35.9	25	64.1	
Hepatitis B	18	48.6	19	51.4	
Budd-Chiari syndrome	4	57.1	3	42.9	
Wilson disease	5	71.4	2	28.6	
Celiac disease	2	40.0	3	60.0	
Portal vein thrombosis	1	25.0	3	75.0	
Primary biliary cirrhosis	1	33.3	2	66.7	
Autoimmune hepatitis	1	50.0	1	50.0	

MHE, minimal hepatic encephalopathy; NASH, nonalcoholic steatohepatitis.

There was no statistically significant difference between the improvement of MHE after treatment and age, BMI, laboratory parameters, and MELD scores of the patients ($P > .05$) (Table 4). No statistically significant difference was also found when the relationship between treatment benefit status and the Child-Pugh classification and cirrhosis status (compensated-decompensated) was examined ($P = .138$, $P = .175$, respectively).

DISCUSSION

Contrary to OHE, MHE is a condition which is rarely recognized because there are no clinically detectable symptoms of mental and neurological dysfunction.[12] Minimal hepatic encephalopathy can significantly affect the daily life of patients by impairing many factors such as learning and driving skills, job performance, and cognitive function. Detection and treatment of MHE as early as possible is very important for improving the outcomes of patients with cirrhotic.[13,14]

Cognitive deficits in patients with MHE are hard to detect during routine physical or neurological assessment. Neuropsychological and/or neurophysiological tests should be performed to detect such deficits. Neurophysiological tests are electroencephalogram, evoked potentials, and CFF. Neuropsychological tests include number combination test, finger connection test, and line and circle drawing tests. Imaging methods used to diagnose MHE are computed tomography, magnetic resonance imaging, and magnetic resonance spectroscopy.[15]

For the diagnosis of MHE, a test which can allow detection of neuropsychiatric disorders shows similar results when repeated and does not give different results according to the person performing the test should be used.[16] The first study conducted by Kircheis et al[17] suggested that retinal gliopathy occurring in cirrhosis may reflect subclinical hepatic encephalopathy and cerebral gliopathy. Another study suggested that one of the most sensitive methods in the diagnosis of MHE was the CFF test, and when the threshold value was taken as <39 Hz, the sensitivity was 96% and the specificity was 77%.[18] In the study conducted by Romero-Gómez et al., when the significant threshold value for the diagnosis of MHE was taken as <38 Hz, the sensitivity was found as 72.4% and the specificity as 77.2%, and this was considered the best value.[19] We used the CFF test because education level, patient age, and frequent repetitions do not affect the test; it is a non-invasive, easily applicable method, and improvement can be shown with follow-up tests after treatment.

Table 3. Comparison of CFF Values Before and After Treatment According to Treatment Groups

	CFF Values Before Treatment			CFF Values After Treatment			
Treatment Groups	**Avarage ± SD**	**Min**	**Max**	**Avarage ± SD**	**Min**	**Max**	***P***
Lactulose	34.9 ± 3.1	26.9	37.8	40.9 ± 3.9	30.9	45.1	.011
Probiotic (LbGG)	35.3 ± 3.1	27.6	37.3	41.7 ± 5.4	28.3	52.8	.007
Placebo	37.2 ± 1.5	33.5	37.8	37.1 ± 3.3	30.6	43.5	.804

CFF, critical flicker frequency; LbGG, *Lactobacillus rhamnosus* GG.

The prevalence of MHE may vary between 23% and 56% in different studies. In the study conducted by Kircheis et al.[17] the rate of MHE was found to be 27%. In this study, it was suggested that, the fact that 65% of the patients in the study were in the Child A group may have caused this difference. In 2 different studies, the prevalence of MHE was found to be 53% and 60%, while the mean ages of the patients were 41 and 39.[18,20] In another study, it was shown that there is a correlation between CFF test values and age in both the healthy group and the cirrhosis patient group. Dhiman et al[21,22] showed that CFF values decreased with age and that age-adjusted values of the CFF test may be necessary. The prevalence of MHE was 54.5% in our study, although these studies show parallelism with our study. The mean age was 55.1, and a significant correlation was found between age and MHE. However, no correlation was found between gender and education level.

One of the factors affecting MHE is the stage of cirrhosis. In the study conducted by Romero-Gómez et al.[19] a weak correlation was found between CFF test results and Child-Pugh staging; however, no correlation was found with the MELD score.[19] Two different studies supported the relationship between MHE and Child-Pugh.[23] In our study, a statistically significant relationship was found between MELD score, Child-Pugh stage, and MHE, supporting the studies mentioned. In line with this information, it can be suggested that the more advanced and complicated the cirrhosis was, the higher the MHE detection rate. However, indicators that predict the progression of cirrhosis may be markers for the development of MHE. In this patient group, screening for MHE should be performed even if there is no OHE.

Factors contributing to the development of MHE are similar to those for OHE, and these are hyperammonemia, sarcopenia, excessive bacterial growth, dysbiosis, and increase in inflammatory cytokines and hyponatremia.[20] Recently, it has been reported that intestinal microbiota is impaired in patients with cirrhosis and this dysbiosis plays a substantial role in the formation of ammonia.[21] Probiotics reduce inflammation and oxidative stress in hepatocytes. In addition, it also reduces intestinal permeability and absorption of ammonia by regulating impaired microbiota in the intestines by colonic acidification. It also plays an important role in the regulation of immune response.[22]

There are many studies evaluating the efficacy of probiotic treatment in cirrhotic patients with MHE. In a meta-analysis including 14 different randomized controlled trials in which a total of 1132 patients were evaluated, many

Table 4. Comparison of Age, BMI, Laboratory Values, and MELD Score According to Benefit from Treatment

	Benefit from Treatment			No Benefit from Treatment			
	Avarage ± SD	**Min**	**Max**	**Avarage ± SD**	**Min**	**Max**	***P***
Age	54.1 ± 14.6	20	72	60.3 ± 11.6	24	83	.126
BMI	27.2 ± 4.5	20.8	35.1	29.4 ± 4.9	20.2	41.6	.121
WBC	4592 ± 2114	2430	10 390	4920 ± 2456	1560	14 550	.495
HGB	11.8 ± 1.7	8.8	14.4	12.1 ± 2.3	8.8	18.0	.895
PLT	89 611 ± 40 733	52 000	2 27 000	1 05 886 ± 58 508	32 000	38 5000	.147
AST	45.3 ± 24.4	22	125	40.5 ± 18.5	14	107	.514
ALT	27.9 ± 15.5	10	76	26.0 ± 13.1	5	76	.792
INR	1.6 ± 0.6	1.2	3.9	1.6 ± 0.3	1.2	2.7	.407
Kreatinin	0.7 ± 0.2	0.4	1.2	0.9 ± 0.3	0.5	1.8	.149
Albumin	3.0 ± 0.3	2.6	3.6	3.0 ± 0.4	1.2	3.8	.851
Bilirubin	1.7 ± 1.0	0.6	4.7	1.7 ± 1.1	0.3	5.7	.901
Sodium	134.2 ± 4.4	125	142	134.2 ± 3.6	125	144	.895
MELD	16.2 ± 4.0	11	26	17.1 ± 4.2	10	28	.427

MELD, model for end–stage liver diseases; BMI, body mass index; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartat aminotransferase; HBG, hemoglobine; PLT, platelet.

different probiotics (*Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, *Bacterium lacticum*, *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus acidophilus*) were investigated and it was found that probiotics were found to be effective in the treatment of MHE. Most of these studies evaluated MHE using the number connection test (NCT). In all studies, probiotics were shown to be superior in preventing progression to OHE and improving MHE compared to the placebo or no treatment group. In studies comparing probiotics with lactulose, better results were obtained in the NCT test in the lactulose treatment group compared to the probiotic treatment group.[23,24] In another study evaluating the efficacy of probiotics in the treatment of MHE, it was found that *Clostridium butyricum* and *Bifidobacterium infantis* were effective in improving MHE in patients with cirrhosis due to HBV.[25] In the prospective study conducted by Goyal et al.[26] after 3 months of rifaximin and lactulose treatment of patients with MHE, a significant improvement was found in MHE. Relapse was observed in 50% of the patients six months after discontinuation of treatment.[26]

In our study, the treatment efficacy for MHE was found to be higher in the groups given lactulose and LbGG compared to the placebo group. There was a statistically significant increase in the CFF test values in both groups. But there is no statistical difference between LbGG and lactulose. The reason why there was no difference between probiotics and lactulose may be due to the necessity of receiving treatment for more than 1 month for LbGG colonization in the intestinal flora. The intestinal colonization rate must increase in order for LbGG to be effective, although the effect of lactulose occurs immediately through known mechanisms. No significant parameters were found when factors that could predict benefit from treatment in the treatment groups were analyzed statistically. By increasing the number of patients, clearer conclusions can be drawn about potential factors which can predict treatment success. The fact that only CFF was used for the diagnosis of MHE in our study, the absence of any neuropsychiatric test used, and the shorter treatment period compared to other studies can be considered as limitations of our study.

In this light, it is known that MHE can progress to OHE, increases mortality, morbidity and health expenditures, and lead to a decrease in the driving performance, quality of life, and work performance of patients. Based on the results of our study, it can be suggested that patients with advanced Child-Pugh stage and high MELD scores should be approached more carefully in terms of MHE screening.

CONCLUSION

In the treatment of MHE, LbGG treatment is as effective as lactulose treatment and can be used safely. Also that it is beneficial for patients, especially those who use vehicles-construction machines and have occupations defined as blue-collar jobs, to protect both the patient and the people they serve.

Ethics Committee Approval: Ethics committee approval was received from the Ethics Committee of Gaziantep University (Date: July 24, 2017, Decision no: 2017/280).

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

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Original Article

Is Gastric Residual Volume Measurement Really Necessary to Achieve Targeted Calories?

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ABSTRACT

Objective: Malnutrition, energy protein, and inadequate or excessive intake of other nutrients have measurable effects on tissues, body function, and clinical outcomes of patients. We aimed to determine the time to reach target calories, nutritional failures, and complications during feeding in measured and unmeasured gastric residual volume patients under ventilation in the intensive care unit.

Methods: The study was completed with 62 patients under mechanical ventilatory support in the intensive care unit. According to the consultation order, patients were divided into 2 groups. Gastric residual volume was measured in the control group (gastric residual volume, n = 31) and not in the other (non-gastric residual volume, n = 31). Nutrition nurses continuously monitored all enteral-fed patients, and the results were recorded.

Results: The feeding pause of the gastric residual volume group was longer than that of the non-gastric residual volume group ($P < .001$). The time to reach target calories was higher in the gastric residual volume group than in the non-gastric residual volume group ($P = .010$). The rate of vomiting as a complication was 9.7% (3 patients) in the gastric residual volume group and 6.5% in the non-gastric residual volume group, although the difference was not significant ($P = .641$). The observation rate of abdominal distension was 6.5% (2 patients) in the gastric residual volume group and the non-gastric residual volume group ($P = .999$). The positive end-expiratory pressure (PEEP) values were higher in patients who vomited, but the difference was not significant ($P = .203$). In patients with abdominal distension, PEEP values were higher than in patients without distension, but the difference was not significant ($P = .282$).

Conclusion: In conclusion, gastric residual volume measurement in patients with mechanical ventilatory support prolonged nutritional breaks and extended the time required to reach target calories compared with patients without gastric residual volume measurement.

Keywords: Enteral nutrition, gastric residual volume, intensive care unit, malnutrition, target calorie

INTRODUCTION

Over the past 30 years, with a better understanding of the molecular and biological effects of nutrition, more emphasis has been placed on nutrition, which has positively impacted the treatment of critical care patients.[1] Nutritional homeostasis refers to all metabolic regulatory mechanisms that aim to maintain the physiological functions, energy, and other nutrient stores in a constant state.[2] Nutritional support is an important component of the treatment strategy for intensive care patients.

In intensive care units (ICU), most patients do not achieve targeted caloric and protein intake, although various nutritional supplements are available today. This leads to malnutrition. Malnutrition, energy protein, and inadequate or excessive intake of other nutrients have measurable effects on tissues, body structure, body function, and clinical outcomes of patients receiving treatment. It is a broad term that encompasses protein-energy malnutrition and nutrient deficiencies such as micronutrients. It increases hospital-acquired infections, hospitalizations, and intensive care prolongs and leads to complications.[3] A compilation assessing malnutrition rates in patients presenting to the ICU found that malnutrition rates ranged from 37.8% to 78.1% in heterogeneous ICU patients.[4] Uncontrolled factors are related to the nutrition of ICU patients. Although several measures have been proposed to support the nutritional status of these patients, unfortunately, there are currently no standard guidelines. Nutritional Risk Screening (NRS 2002) is one of the most established screening tools for inpatient medical care.[5] Nutritional Risk Screening assesses the patient's nutritional status (weight loss, body mass index, based on general condition or dietary intake) and severity of illness (stress metabolism associated with severity of illness) and is associated with a higher risk of adverse outcomes. Each area is evaluated from 0 to 3, with patients receiving an additional point if they are 70 or older.[6] Heyland et al[7] to determine the causes of malnutrition, found that 52% of patients could not tolerate enteral nutrition. One of

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the common causes of this tolerance failure was gastric residual volume (GRV).[7] The GRV is the amount of undigested nutrients remaining in the stomach after enteral feeding. It is composed primarily of undigested food formula and gastric fluid. In enteral nutrition patients, GRV measuring is frequently used to determine nutritional tolerance. Gastric aspiration contents can be measured with a 50 mL injector or by draining into a bag using gravity.[8] Adam and Baston[9] found that only 76% of the targeted calories could be administered to ICU patients. In another study by McClave et al[10] 44 patients could be fed enteral nutrition and received only 78.1% of the planned calories. It was found that only 14% of the patients reached their daily target calories in the first 72 hours. Gastric residual volume was the leading cause of this deficit in the same study.[10]

Increased GRV carries the risk of gastroesophageal reflux and aspiration.[11] It delays gastric emptying and increases the risk that the patient's tolerance to food will decrease, leading to interruption of food intake.[12] This risk could lead to long-term access to target calories and malnutrition. Our study aimed to observe the time to reach target calories, nutritional failures, and complications during feeding in measured and unmeasured GRV patients receiving enteral nutrition under ventilation in the ICU.

METHODS

The study was performed at the Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital Anesthesiology and Reanimation intensive care unit. Approval was obtained from the Erzincan Binali Yıldırım University ethics committee (Date: April 26, 2021, Number: 06/22). The full study protocol was registered in the Clinical Trials Database (NCT05238051). Nutritional procedures were explained to the legal heirs of all patients hospitalized in the ICU, and their informed consent was obtained. Patients who received tracheal intubation with mechanical ventilatory support between May 2021 and January 2022 were included in the study. Patients with a history of gastrointestinal bleeding, parenteral nutrition support, hospital stay of fewer than 2 days, and those under 18 years of age were excluded from the study. Our nutrition team screened patients treated in the ICU with the malnutrition screening tool and assigned patients with a score of 2 or higher to the nutrition department. Patients in whom enteral nutrition was not contraindicated were divided into 2 groups according to the consultation order. All patients were placed with a 110 cm polyurethane 14F nasogastric tube and their location was confirmed. The patients' target calories were calculated using the Schofield equation because the hospital did not have an indirect calorimetry device. Feeding pumps were used in the study, and patients were fed continuously. All patients received a head elevation of 30˚ during feeding. Ready-to-eat foods found in the hospital pharmacy and approved by the ICU physician were used. The calculated target calorie amount was administered via a nasogastric tube using a continuous infusion method. Nutrition nurses continuously monitored all enteral-fed patients, and the results were recorded.

Main Points

- Malnutrition is more common than anticipated, but there are problems with its detection.
- Nutritional procedures in intensive care may be a cause of malnutrition that is difficult to detect.
- This study opened a new window to the discussion of gastric residual volume measurement in the routine nutritional procedures.

A total of 72 patients were enrolled in the study. Gastric residual volume was measured in one group and not in the other. In the GRV group, feeding was initiated at an infusion rate of 20 mL/h. The GRV was measured every 4 hours. When it was less than 200 mL, the infusion rate was increased by 20 mL/h. The infusion rate, which was increased every 4 hours according to the GRV, was continued constantly when the target calorie was reached. It was kept constant when the GRV was above 200 mL and then the feeding rate was reduced to half when the GRV volume was above 400 mL. In 4 patients, enteral nutrition was discontinued due to melena and excluded from the study. In 6 patients with persistently high GRV values, enteral nutrition was discontinued and parenteral nutrition was initiated. The study was completed with 62 patients; GRV (n = 31) and non-GRV (n = 31).

In patients without measuring GRV, the feeding rate was increased by 20 mL/h every 4 hours. The infusion rate was kept constant when the target calories were reached. All patients were observed for vomiting, diarrhea, recovery, and constipation for 10 days. When complication was present, the infusion rate was reduced by 20 mL/h. In patients who experienced vomiting and flatulence, the intensivist initiated treatment of complications. Enteral nutrition was discontinued if the complication persisted despite the reduced dose, and parenteral nutrition was started. The dietitian recorded all the patients' daily data.

Malnutrition Screening Tool

We used NRS 2002 screening tool to determine malnutrition. Weight loss and food consumption are measured, and scores are tallied. Patients with a score of 0 or 1 are deemed not at risk, whereas those with a score of 2 or more are considered at risk.

Scofield Equation

Target caloric intake was measured using the Scofield equation, which is a simple, practical, widely used, and more accurate method of predicting resting energy expenditure. Energy intake corrected for stress factors or metabolic values was held constant for all patients throughout the study.[13]

Statistical Method

All statistical analyses were performed using IBM Statistical Package for Social Sciences software package program version 22 (IBM SPSS Corp. Released 2013, Armonk, NY, USA). Categorical variables were summarized as number and percentage, and continuous variables were summarized as average and standard deviation or median. We used chi-square test for analyzing categorical variables, the Kolmogorov-Smirnov test to analyze the compatibility of variables with the normal distribution, Student's *t* test for group comparisons of data with normal distribution, and Mann–Whitney *U* test in cases where there was no assumption of

Table 1. Patients Characteristics

Gastric Residual Volume		Age	Corr. Weight	Target Calories	Break Time (Hours)	Reach Time (Hours)
Group non-GRV	Mean	72.81	64.48	1449.74	4.35	46.10
	Standard deviation	13.370	11.524	208.852	1.199	10.137
	Median	74.00	65.00	1430.00	4.00	44.00
	Minimum	26	41	1100	3	32
	Maximum	89	85	1800	6	72
Group GRV	Mean	67.71	66.81	1542.10	7.74	52.45
	Standard deviation	16.485	8.822	176.426	2.449	13.125
	Median	71.00	65.00	1500.00	7.00	48.00
	Minimum	26	47	1297	3	24
	Maximum	89	83	2000	16	96

The mean age of the GRV group was 67.71 years and that of the non-GRV group was 72.81 years. The adjusted weight was 66.81 for the GRV group and 64.48 for the non-GRV group.
GRV, gastric residual volume.

normality. In all statistical tests, $P < .05$ was deemed statistically significant.

RESULTS

The gender distribution of male and female patients included in the study was equal. The mean age of the GRV group was 67.71 years and that of the non-GRV group was 72.81 years. The adjusted weight was 66.81 for the GRV group and 64.48 for the non-GRV group. Age and adjusted weight did not differ in a statistically significant way (Table 1). The feeding pause of the GRV group was longer than that of the non-GRV group ($P < .001$) (Figure 1). The time to reach target calories was higher in the GRV group than in the non-GRV group ($P = .010$) (Figure 2).

The rate of vomiting as a complication was 9.7% (3) in the GRV group and 6.5% (2) in the non-GRV group and there was no significant difference. The observation rate of abdominal distension as a complication was 6.5% (2 patients) in the GRV group and the non-GRV group.

Although there was no significant difference, positive end-expiratory pressure (PEEP) values were higher in vomited

Figure 1. The feeding pause of the GRV group was longer than that of the non-GRV group ($P < .001$). GRV, gastric residual volume.

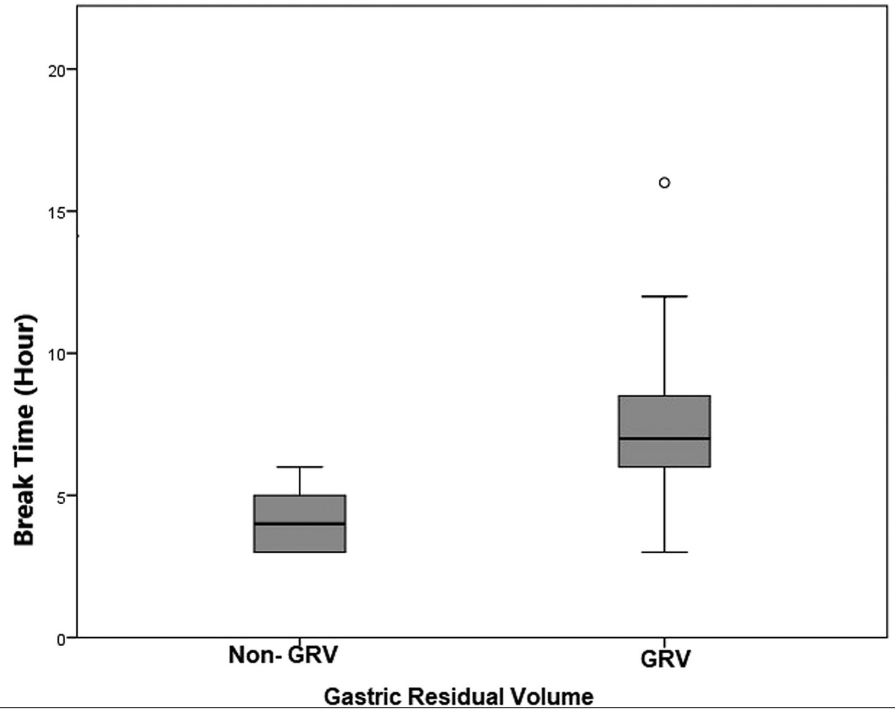
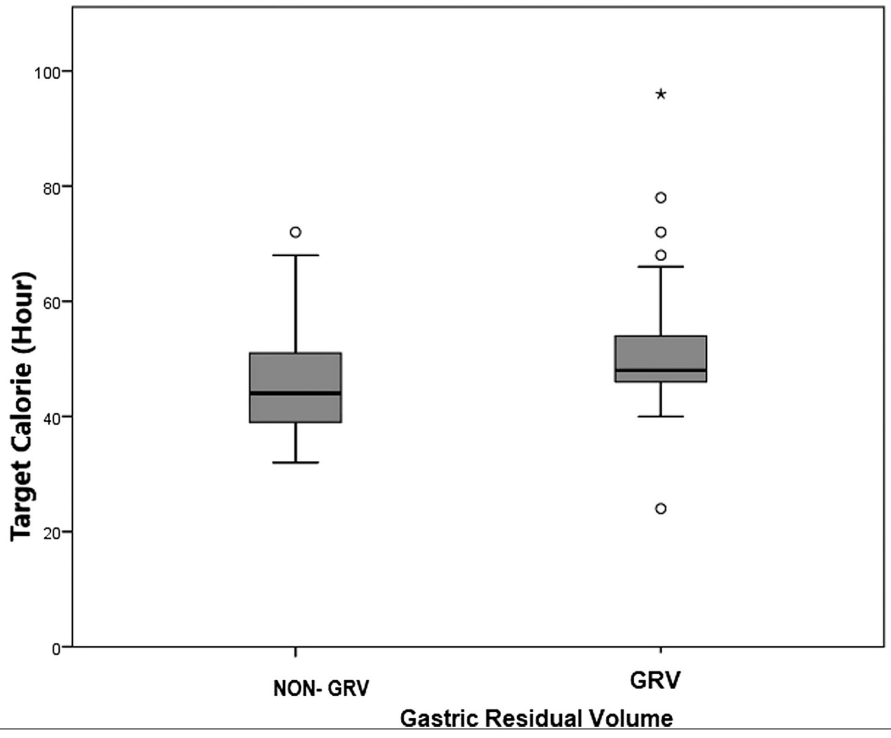


Figure 2. The time to reach target calories was higher in the GRV group than in the non-GRV group ($P = .010$). GRV, gastric residual volume.



patients. In patients with abdominal distension, PEEP values were higher than in patients without distension, but the difference was not statistically significant (Table 2).

DISCUSSION

Critically ill patients are exposed to many adverse conditions and diseases that lead to intensive care. In these patients, malnutrition can quickly occur, negatively affecting the recovery of the underlying diseases.[2] Measurement of GRV is a common method for assessing nutritional tolerance in ICU patients. However, various factors, including patient posture, feeding tube placement, feeding tube inner diameter, syringe size, and measuring method, might impact the amount of GRV.[14] One study found that GRV was approximately 2-fold higher on average compared to patients with large feeding tubes and narrow feeding tubes.[15] Our study used a 14F, 110-cm-long, PVC-coated polyurethane feeding tube. The patient's head position was kept elevated by 30˚ during feeding. No problems occurred with the probe.

Table 2. Comparison of PEEP on Enteral Nutrition Complications

Vomiting		PEEP	Distension		PEEP
None	Mean	5.93	None	Mean	6.00
	Standard deviation	1.613		Standard deviation	1.707
	Median	5.00		Median	5.00
	Minimum	4		Minimum	4
	Maximum	12		Maximum	12
Yes	Mean	7.20	Yes	Mean	6.50
	Standard deviation	2.168		Standard deviation	1.291
	Median	8.00		Median	6.50
	Minimum	5		Minimum	5
	Maximum	10		Maximum	8

PEEP values were higher in vomited patients. In patients with abdominal distension, PEEP values were higher than in patients without distension, but the difference was not statistically significant.
GRV, gastric residual volume.

There are no universally accepted GRV values in the dietary guidelines. The acceptable GRV value stated by The American Parenteral and Enteral Nutrition Society[16] was 500 mL and was 250 to 500 mL by the Canadian Clinical Practice Guidelines.[17] Clinical practices and procedures related to high GRV levels also vary. In a study of 2298 critical care nurses, 36.5% of nurses accepted a high amount of GRV that required interruption of enteral feeding as 250 mL and 25% as 500 mL.[18] Another study reported that reflux and aspiration could occur even with deficient GRV levels of 150 mL.[19] In our study, the volume of GRV in the measured group below 200 mL was accepted as usual and continued by increasing the dose, a fixed dose between 200 and 400 mL was maintained, and the dose above 400 mL was of halved. Food intolerance is described as vomiting, abdominal distension, diarrhea, and elevated GRV levels.[20] Although it is hypothesized that high GRV levels lead to increased food intolerance, many studies have provided conflicting results. There

is no consistent association between a GRV level and gastric intolerance; it can develop even in patients with a low GRV level. Patients with a high GRV are considerably more likely to vomit, according to Mentec et al.[21]

Abdominal distension is a common but late sign of nonocclusive intestinal necrosis associated with early enteral feeding.[22] In contrast, Montejo et al[20] found in their work that there was widespread intolerance of food in the 500-mL GRV group compared with 200 mL. There was no difference in complications, including vomiting and flatulence, between patients with and without GRV measurement in our study. Akinci et al[23] found that GRV did not increase up to PEEP =13, but gastric pH decreased at values above 13. There was no significant difference in PEEP values in our study in patients who had vomiting and abdominal distension. There is no standard for measuring the residual gastric volume. Several studies have reported that these measurements are unnecessary.[24] Wiese et al did not measure GRV and performed dose titration. As a result, they found no difference in the target calorie lead time and complication rates of the patients.[25] Some patients had taken prokinetic agents during the diet phase in their studies. Some patients began taking prokinetic when vomiting was observed. We did not administer prophylactic prokinetic agents to any patient during the study. In our study, patients with GRV measurement reached target calories longer than those without measurement and took longer breaks.

In their meta-analysis, Wang et al[26] came to similar conclusions as our work. They discovered that not monitoring GRV did not affect the incidence of feeding intolerance, ventilator-associated pneumonia, or death. There was also no change in the duration of mechanical ventilation or length of stay in the ICU. Failure to monitor GRV was associated with a significant increase in vomiting.[26] In our study, vomiting was not different between groups. Bouwet et al found that GRV measurement with ultrasound was more reliable than measurement with gavage in their studies comparing monitoring with gastric ultrasound. Their results suggest that gastric ultrasound is a feasible and promising tool for monitoring gastric volume in clinical practice.[27] In their studies of patients fed via a nasogastric tube in an intensive care unit, Kaçmaz et al[28] found that measuring GRV volume is unnecessary to determine gastrointestinal motility function and reduce complication rates. When compared with GRV measurement in a methanol study that included 5 studies with 998 patients, it was found that the absence of GRV monitoring decreased the rate of food intolerance in critical patients and did not increase ventilator-related pneumonia or mortality rates. These results supported our work.[28]

Our study has several limitations. It was conducted at a single center, and blinding was not permitted due to the critical care environment. There is the possibility that other changes in medical or nursing care were made in the study that could lead to a different assessment of enteral nutrition (EN) competence and affect patient outcomes.

CONCLUSION

As a result of this study, we found that GRV measurement in patients with mechanical ventilatory support prolonged nutritional breaks and extended the time required to reach target calories compared with patients without GRV measurement. In addition, we found that complications such as gastroesophageal reflux (GER), abdominal distension, and vomiting did not increase when GRV was not measured. We believe that GRV measurement, which may lead to malnutrition, should be reviewed.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Erzincan Binali Yıldırım University (Date: April 26, 2021, Decision no: 06/22).

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

Peer-review: Externally peer-reviewed.

Declaration of Interests: The author has no conflict of interest to declare.

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Original Article

New Age Borders Obtained from Spot Photoscreener by Using Multivariate Cluster Analysis

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ABSTRACT

Objective: The aim of this study is to analyze all the variables obtained from photoscreener using hierarchical cluster analysis to create more homogeneous age groups for more reliable and consistent measurement by photoscreener.

Methods: The variables obtained from photoscreener examination of consecutive children who attended the ophthalmology department were evaluated. Medical records of the children were evaluated to ontain data including refractive parameters, deviation angle, pupil diameter variables and the calculated spherical equivalent, the cylindrical power vector J0, J45 values. These variables were analyzed by the multivariate cluster analysis.

Results: Based on a dendrogram, 4 main clusters of similar quality variables were created. The calculated spherical equivalent decreased gradually from cluster I to IV, from 0.745 D to –0.235 D. The average pupil size in the Ist cluster was 5.06 mm, while in the IVth cluster, it was 6.38 mm. The proposed new age borders are distinct and statistically significant ($P < .001$). The ultimate proposed new age borders were found as 1-20, 21-64, 65-101, and 102-120 months, respectively.

Conclusions: We proposed new age borders for the evaluation of refraction and pupil size of children which create new groups with a statistically different and homogeneous distribution. The proposed new age borders in this research would provide more reliable and consistent measurement results for clinical diagnosis.

Keywords: Age border, classification, cluster analysis, dendrogram, photoscreener

INTRODUCTION

Refractive errors are still a well-known public eye health problem frequently diagnosed in daily practice. Refractive error detection is critical for the prevention of amblyopia, especially in the pediatric age group. Various refractive error measurement techniques, such as retinoscopy, autorefraction, and photorefraction, have developed from the past to the present.[1]

Photorefraction was first described by Howland in 1974. The MTI photoscreener (Medical Technology and Innovations Inc, Lancaster, PA) was the first device introduced in 1995.[2] Due to short measurement time, ease of use, and portability, photoscreeners have been commonly used in refraction scans and measurements. They identify refractive errors by analyzing reflected (red) reflex images of the pupils using the infrared camera as a working principle. With these devices, which can take binocular measurements from a distance of about 1 m, the heads of pediatric patients do not need to be fixed and are believed to be able to eliminate accommodation due to short measurement times. For these reasons, photoscreening may be a good option for young children and groups of patients who cannot cooperate.[3]

Studies have shown that photoscreeners have acceptable sensitivity and specificity to detect refractive errors and risk factors for amblyopia.[4] Many different devices have been developed and presented along the way from past to present by different companies for clinical use. These devices can measure refraction errors as well as the deviation angle, the pupil size, and the interpupillary distance.

It is well known that many photoscreening devices which are currently used clinically assess the parameters obtained by age-based measurements. As a result of this assessment, it provides a report to the clinician that includes information that measurements are within normal limits or a full eye examination is required. These devices analyze the measurement results based on the age criteria defined in the software installed on the device.[5] Although there is no particular standardization of age classification, each company uses its own distinct age classification system in the photoscreening device software.

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Table 1. Descriptive Statistics for Continuous Variables of Children

	Minimum	Maximum	Mean	Standard Deviation
Spherical value (D)	−5.5	7.000	1.013	1.369
Astigmatism (D)	−3.000	0.000	−0.984	0.715
Spherical equivalent from Spot Vision	−5.500	6.250	0.528	1.308
Calculated spherical equivalent (D)	−5.625	6.500	0.521	1.299
Vector presentation of cylindrical power J0 (D)	−0.887	1.499	0.364	0.425
Vector presentation of cylindrical power J45 (D)	−1.042	1.149	0.003	0.237
Pupil diameter (mm)	3.90	8.80	5.674	1.0000
Angle of vertical deviation (°)	0.0	5.0	1.193	1.068
Angle of horizontal deviation (°)	0.0	5.0	1.329	1.166

D, diopter; (°), degree; mm, milimeter.

The aim of this study is to analyze all the variables obtained from photoscreener in order to create more homogeneous age groups for more reliable and consistent measurement by photoscreener. In this analysis, hierarchical k-means cluster analysis of multivariate statistical methods that considers the variation in all variables as a whole was used to obtain more sensitive and more powerful methods instead of univariate statistical methods.

METHODS

Patient Data Collection and Exclusion Criteria

In this retrospective study, children attending the ophthalmology department either for vision screening or routine ophthalmic examination within the age group of 1 to 120 months were recruited in this study from March 2018 to December 2018. The data were extracted from medical records of children undergoing vision screening with photoscreener. The data included age, gender, and measurements obtained by the photoscreener (Welch Allyn Spot Vision Screener Skaneateles Falls, NY software: 3.0.05.00) including the pupil diameter, cylindrical (C), spherical (S) and spherical equivalent (SE) values, cylindrical axis (α), and angle of ocular deviation with direction. Spherical equivalents, vector presentation of cylindrical power referred as J0 and J45 were calculated by the following formulas: $SE = C/2$, $J0 = (-C/2)\cos(2\alpha)$, $J45 = (-C/2)\sin(2\alpha)$, respectively. Myopia was defined as $SE \leq -0.5$ diopter (D) and hypermetropia ≥ 0.50 D. Emmetropia was defined as SE between >-0.5 D and $<+0.50$ D. The same medical technician performed all the refraction measurements. It was ensured that the room was in dim light during the measurement and the device was at the same level as the patients' eyes from a distance of 1 m. All children underwent complete ophthalmological and orthoptic evaluations. Strabismus cases (>5 degree/10Δ diopter prism), media opacity, retinal disease, nystagmus, previous ocular surgery, and history of eye-head trauma were excluded from the study. Additionally, cases with refractive errors outside the limits of the recommendation by the manufacturer guidelines (spherical value interval +7.5/−7.5 diopter (D), cylindrical value interval +3/−3 D) were not included in the study.

Main Points

- Photoscreeners have acceptable sensitivity and specificity to detect refractive errors and risk factors for amblyopia.
- There is no particular standardization of age classification, and each company uses its own distinct age classification system in the photoscreening device software.
- Hierarchical cluster analysis of multivariate statistical methods is useful for many applications in terms of classification.
- New age borders were proposed with a large data set with high clinical evidence by using a hierarchical cluster method of multivariate statistics, which is an advanced analysis to obtain valid and reliable results.
- The proposed new age borders would provide more reliable and consistent measurement results for clinical diagnosis.

Statistical Analysis

The data obtained were evaluated with hierarchical cluster analysis of multivariate statistical methods, which is useful for many applications in terms of classification. Using this method, the relationships among variables and clustering trends were determined by dendrogram, allowing us to obtain greater detail to the clinical interpretation. The data were analyzed using "R programming, version 3.6.2 (2019-12-12)—CRAN" adapted by statistical experts as a standard software package for data analysis.

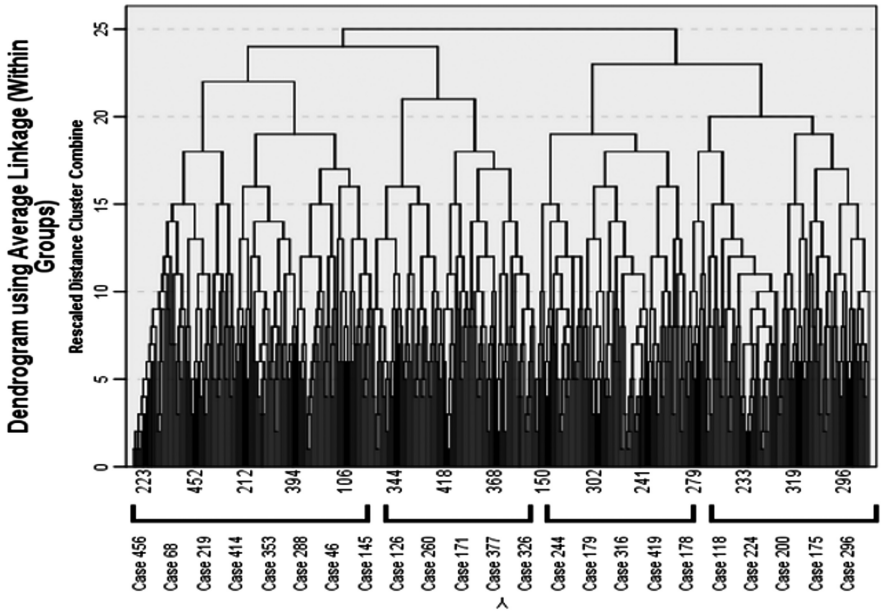
Power Analysis

Based on literature information, photoscreener has 87% accuracy in refraction in children.[6] In our study, it was calculated that when this rate was accepted as 0.05% higher, than 92% and according to 85% power, the sample size was calculated as 406 patients by using R programming. The sample size included in the current study was 458.

Ethical Statement

The study was approved by the Ethics Committee of Health Sciences University, Istanbul Training and Research Hospital on January 18, 2019, with the number 1641. The study was carried out in line with the principles of the World Medical Association Helsinki Declaration.

Figure 1. The dendrogram for the measurements of the children by using "hierarchical cluster method."



RESULTS

Nine hundred sixteen eyes of 458 children (222 boys, 236 girls; average age, 43.37 ± 34.91 months; range, 1-120 months) met the inclusion criteria and were included in this study. Descriptive statistics (minimum, maximum, mean, and standard deviation) for continuous variables of patients are presented in Table 1. The distribution of refractive error according to the calculated SE in 916 eyes was as follows: hypermetropia, 436 eyes; myopia, 124 eyes; and emmetropia, 356 eyes.

The clusters and subclusters formed by the related variables were shown by dendrogram using cluster analysis (Figure 1). The dendrogram allowed us to see how clusters were combined and at the same time determined the appropriate number of clusters formed by the variables. Based on the dendrogram, 4 main clusters of similar quality variables were created. Based on the results of the dendrogram, the "hierarchical clustering method" demonstrated that children form 4 very smooth and different clusters according to their ages (months) in Table 2 and Figure 2. Descriptive statistical values (number of individuals, mean and standard deviation values) and analysis results related to the distribution of variables in 4 different sets are presented in Table 3. From cluster I to cluster IV, the calculated SE decreased gradually from 0.745 D to −0.235 D. The average pupil size in the Ist cluster was 5.06 mm, while in the IVth cluster, it was 6.38 mm.

Table 2. Ultimate Border Values Obtained for the Ages (in Months) of the Children in 4 Different Clusters to be Considered in Clinical Diagnosis

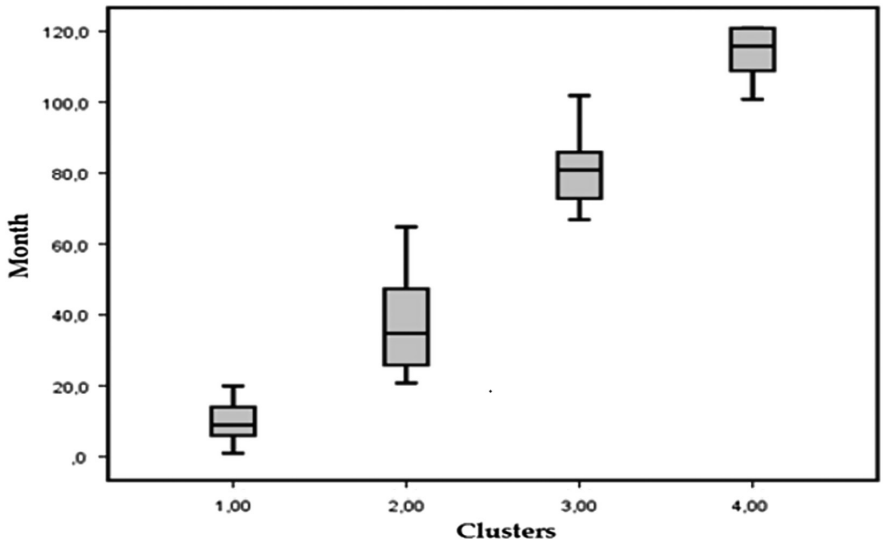
	Month	
Main Clusters	**Minimum**	**Maximum**
I	1	20
II	21	64
III	65	101
IV	102	120

DISCUSSION

The most common cause of visual impairment affecting all age groups is refractive errors.[6] Thus, early detection of refractive errors and risk factors of amblyopia in children may lead to better and more stable final visual results, with shorter treatment times and more rapid improvement in visual acuity.[7,8]

In the pediatric age group, photoscreeners are now commonly used to evaluate refractive parameters and perform community vision screening programs. Photoscreeners allow a large number of children to be screened in a short period of time in a wide geographic area and provide time advantage over conventional methods such as cycloplegic retinoscopy.[9] In addition, some studies reported that these devices are effective and reliable in detecting refractive erors.[4,6] Panda et al.[6] reported that the difference between the measurements of photoscreener and cycloplegic retinoscopy was -0.3 D and stressed that this difference in measurement was not clinically significant. Therefore, the frequency of clinical use of photoscreener devices is increasing day by day. The fact that each producer company uses a different age classification is the most important proof that no consensus exists in this regard. To the best of our knowledge, there is no specific study done so far to classify the age borders according to the variables used by photoscreener. As a result, the age limits used in photoscreeners in clinical use are not based on evidence, especially in the pediatric age group. In order to obtain more reliable and consistent photoscreener measurements, we believe

Figure 2. The distribution of the minimum and maximum values obtained for the age (in months) of the children in 4 different clusters to be considered in clinical diagnosis.



that it is necessary to establish homogeneous age groups. Therefore, using the hierarchical cluster method of multivariate statistics and advanced analysis, we proposed 4 age ranges for children aged 120 months and below.

In this study, 4 clusters were determined according to variables that including refractive error, pupil size, angle of ocular deviation, and direction obtained from children aged between 1 and 120 months. The distribution of age ranges in these 4 clusters is 1-20, 21-64, 65-101, and 102-120 months, respectively (Table 2). In the cluster I consisting of children under 20 months, SE and J45 values were the highest, while pupil diameter was the lowest. From cluster I to cluster IV, there was a gradual decrease in SE and J45, as well as an increase in pupil diameter. Cluster IV, which included children aged 102-120 months, had the lowest SE and J45 as well as the highest pupil diameter. Ocular structures are constantly changing in childhood, so it is important to determine the characteristics of this change by assessing the visual system and refractive parameters that are still developing according to the age of children. It is a well-known fact that refraction changes with age and hyperopia are the predominant refractive status in early childhood.[10] Myopic shifts become more evident as the age progresses.[11] Consistent with other studies, our study showed that SE was gradually decreasing among these 4 groups. The SE values obtained from the device and calculated were found to be the same (0.74 D) in cluster I that included children aged 1-20 months. In cluster IV, children aged 102-120 months, these values were-0.24 and-0.23, respectively, and a shift in myopia was observed.

Studies show that the prevalence and amount of astigmatism decrease as the child grows and the greatest change occurs between the ages of 2 and 4 years.[12,13] Dobson et al.[12] found the highest prevalence of astigmatism in infants and toddlers in their

Table 3. Descriptive Statistical Values and Analysis Results of the Variables Measured in 4 Different Clusters Obtained by the "Hierarchical Clustering Method"*

KMC		A	S	SE	CSE	J0	J45	VD	HD	PD
I	$\bar{x}$	9.820	1.345	0.745	0.745	0.445	0.025	1.390	1.570	5.065
	SD	5.140	1.325	1.270	1.280	0.470	0.285	1.185	1.245	0.715
II	$\bar{x}$	36.29	1.060	0.610	0.585	0.345	−0.005	1.110	1.205	5.750
	SD	11.97	1.240	1.195	1.170	0.410	0.210	1.030	1.105	0.915
III	$\bar{x}$	80.38	0.880	0.435	0.425	0.345	−0.030	1.195	1.410	6.195
	SD	9.550	1.470	1.445	1.435	0.380	0.240	1.025	1.480	1.040
IV	$\bar{x}$	112.6	0.090	0.240	−0.235	0.215	0.005	0.960	1.060	6.380
	SD	7.490	1.395	1.360	1.345	0.345	0.165	0.875	1.070	0.985

*The mean values of the variables obtained in 4 different clusters were found to be significantly different with the ANOVA test (P <.001).
n, Number of individuals; $\bar{x}$, mean value, SD, standard deviation; KMC, k–mean cluster no, A, age (month); S, spherical value, SE, spherical equivalent from Spot Vision; CSE, calculated spherical equivalent; J0 and J45, vector presentation of cylindrical power; VD, vertical deviation; HD, horizontal deviation; PD, pupil diameter.

study between the ages of 0 and 9.5 years and reported that astigmatism disappeared at school age. Our research has demonstrated that the importance of astigmatism decreases with growing age. The J0 value of astigmatism had the highest value (0.44 D) in cluster I, and it decreased with age. It was found 0.34 D in the IVth astigmatism was measured as close to zero and did not change significantly with age.[14] According to our findings, the importance of astigmatism decreases with age. Photoscreener devices provide information about the pupil size, angle of ocular deviation and direction as well as refractive error.[15,16] Silbert et al[17] in their study with photoscreener showed that pupil size increased with age in children between ages 0 and 16 years. However, they did not report any specific pupil size values for these age groups. In our study, the average pupil size was found as 5.06 mm in the Ist cluster, and it significantly increased with age and reached 6.38 mm in the IVth cluster. In addition, pupil diameter in children shows similar characteristics in 4 different groups according to age.

The spot vision photoscreener performs all measurements based on age groups in the software of the device and the corresponding age range must be selected prior to patient screening. For children under 10 years of age, the age groups defined in the software device are 6-12 months, 12-36 months, 3-6 years, and 6-20 years, respectively (Figure 3). However, no research or paper can be found demonstrating the criteria by which these age limits have been defined. In addition, when the age limits of 1-20, 21-64, 65-101, and 102-120 months determined in our study are compared with the age limits in the software of the device, it is observed that there are significant differences between the age limits. When these findings are evaluated, the recommended age limits can be used to create a more homogeneous age group.

One of the limitations of this study is that the results are based on data related to a single population. Different results may be obtained in different populations due to differences in ethnicity. In addition, we did not include ocular biometric parameters associated with refraction such as axial length, corneal radius, anterior chamber depth, and personal characteristics such as body weight and height. Further studies in different ethnic groups and that contain more parameters will provide additional information.

Figure 3. Spot vision photoscreener age classification.

CONCLUSION

In this current study, new age borders were proposed with a large data set with high clinical evidence by using a hierarchical cluster method of multivariate statistics, which is an advanced analysis to obtain valid and reliable results. As a result, new age borders for the evaluation of refraction and pupil size of children which create new groups with a statistically different and homogeneous distribution are proposed. The proposed new age borders in this research would provide more reliable and consistent measurement results for clinical diagnosis.

Ethics Committee Approval: Ethics committee approval was received from the Ethics Committee of Health Sciences University, Istanbul Training and Research Hospital (Date: January 18, 2019, Decision no: 1641).

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Original Article

Acute Renal Infarction: A Single-Center Experience

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ABSTRACT

Objective: The aim of this study is to evaluate the clinical characteristics and short- and midterm renal functions in patients with acute renal infarction.

Methods: The medical records of the patients who were diagnosed with acute renal infarction by computed tomography in our clinic between 2012 and 2019 were retrospectively reviewed. Twenty-four patients who had follow-up data for at least 1 year were included in the study. Clinical, radiological, and laboratory findings of the patients at the time of admission and the results of serum creatinine level and glomerular filtration rate at first month and first year were recorded.

Results: The mean age of the patients was 49.5 ± 20.7 years. In half of the cases, cardiac origin diseases were the underlying risk factor of acute renal infarction. Flank/abdominal pain was the most common presenting symptom. At admission, mean white blood cell count, serum lactate dehydrogenase, serum creatinine, and glomerular filtration rate values were 12 507 ± 6367/μL, 437.4 ± 261 U/L, 1.4 ± 1.9 mg/dL, and 85.3 ± 47.7 mL/min/1.73 m^2, respectively. Chronic kidney disease developed in 7 patients.

Conclusions: Acute renal infarction should be taken into consideration in patients with flank or abdominal pain and increased serum lactate dehydrogenase level. In addition, patients with acute renal infarction are at risk of developing chronic kidney disease.

Keywords: Glomerular filtration rate, infarction, kidney, kidney function tests

INTRODUCTION

Acute renal infarction (ARI) is a condition that results from acute disruption of blood flow in the ipsilateral main renal artery or segmental branches. Cardioembolic diseases, injury of the renal artery, and hypercoagulation disorders are the most common etiologic factors of ARI.[1] Acute renal infarction is an uncommon condition which has an estimated incidence rate of 0.004%-0.007% among emergency department admissions.[2,3] Most patients with ARI presented with non-specific symptoms including flank pain, abdominal pain, nausea, and vomiting that mimic more common conditions such as urinary tract stone disease and acute abdominal diseases. Both rarity and presentation with non-specific symptoms often lead to delay in diagnosis that increase the risk of impairment in renal functions.[4,5] Today, contrast-enhanced computed tomography (CT) is the standard radiological tool for the diagnosis of ARI.[2]

In the current study, we aimed to share our experience of patients with ARI and to describe the characteristics, etiological factors, and short- and midterm renal functions of the patients with ARI.

METHODS

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Ankara City Hospital (Date: August 25, 2021, Ethics Committee Ruling number: E1-21-1953). Written informed consent was obtained from all the patients. Following ethical committee approval, we retrospectively reviewed the medical records of the patients that were evaluated by our department between 2012 and 2019 for ARI diagnosed by contrast-enhanced CT. Our review revealed that 31 patients with ARI were evaluated by our department. Patients with missing data at presentation (n = 2), lack of follow-up data (n = 4), and unavailable CT images (n = 1) were excluded. Following application of the exclusion criteria, 24 patients who were followed up for at least 1 year and the patients with available CT images at admission and laboratory data were included.

Demographic, clinical, radiological, and laboratory findings of the patients at the time of admission and the results of the kidney function tests including serum creatinine level and estimated

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glomerular filtration rate (eGFR) at the time of presentation, first month, and first year were recorded.

Infarcts were classified based on CT as focal (single wedge-shaped lesion), multifocal (more than 1 lesion), and global (uniformly >50% of the renal tissue was involved).[6] In addition, the volume of infarction/kidney volume was calculated for each patient based on CT findings. Estimated glomerular filtration rate was calculated by using the modification in diet and renal disease (MDRD) formula.[7] Chronic kidney disease (CKD) was defined as eGFR < 60 mL/min/1.73 m^2 over 3 months using the MDRD equation.[8]

To evaluate the infarct lesions and kidney function, Tc-99m dimercaptosuccinic acid (DMSA) scintigraphy was performed 3 months after admission. At the time of hospitalization, all patients were assessed by the cardiology department to start or regulate the anticoagulant/antiplatelet therapy.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) v. 25 for Windows (SPSS Inc. Chicago, Ill, USA). Continuous variables were presented as mean ± standard deviation (SD) with or without min–max, and dichotomous values were expressed as number and percentage. The Friedman test was used to compare the eGFR values at admission, first month, and first year. A value of $P < .05$ was considered to be statistically significant.

RESULTS

The mean age of the patients was 49.5 ± 20.7 (18-88) years. Of the 24 patients, 41.7% (n = 10) were female and 58.3% (n = 14) were male. In half of the cases, ARI is of cardiac origin including atrial fibrillation, valvular heart disease, or cardiac thrombus. However, 6 of them had not been diagnosed with any cardiac origin comorbidity until ARI occurred. No predisposing factors were found in 7 patients and classified as idiopathic. In 4 patients, focal ARI occurred following blunt trauma due to motor vehicle accident, and extravasation or perinephric hematoma was not detected in the CT scan. Hypertension was the most common comorbidity (29.2%). At admission, 10 patients were under anticoagulant/antiplatelet therapy. The other drugs that the patients had been using were antihypertensive drugs (n = 7), oral antidiabetics (n = 6), beta-adrenergic blockers (n = 3), antihyperlipidemic drugs (n = 3), non-steroidal anti-inflammatory drugs (n = 2), and proton pump inhibitors (n = 3). Characteristics of the patients are summarized in Table 1.

Seventeen patients had flank and/or abdominal pain at the time of admission. Based on CT, detection rates of focal, multifocal, and global renal infarct were 50%, 20.8%, and 29.2%, respectively. In 2 patients with global infarct (1 had solitary kidney), endovascular procedures were suggested; however, the patients refused the intervention due to possible complications. Therefore, all patients were managed conservatively and received therapeutic doses of low-molecular-weight heparin (LMWH). After initial treatment with LMWH, warfarin was started in 2 patients and acetylsalicylic acid in 4 patients for maintenance therapy. Among the 7 patients with global infarct, 2 had impaired contribution to total renal function (18% and 23%). Five patients had non-functioning kidney findings at DMSA scintigraphy, 3 of whom underwent laparoscopic simple nephrectomy while 2 denied to undergo nephrectomy. Renal scintigraphy showed that 14 patients had

Main Points

- Acute renal infarction (ARI) is a rare condition and patients with ARI are admitted to the hospital with non-specific complaints including flank or abdominal pain, nausea, and/or vomiting.
- The most common predisposing factor of ARI is cardiac origin diseases, namely atrial fibrillation, valvular heart disease, and cardiac thrombus.
- Contrast-enhanced computed tomography is the standard imaging tool for the diagnosis of ARI.
- Acute renal infarction is associated with impaired kidney function and may lead to chronic kidney disease.

Table 1. Characteristics of the Patients with ARI

Age (years)	49.5 ± 20.7 (18–88)
Sex, n (%)	
Male	14 (58.3)
Female	10 (41.7)
Body mass index, (kg/m^2)	26 ± 3.3 (20.9–33.1)
Smoking, n (%)	
Active smoker	13 (54.2)
Ex-smoker	4 (16.7)
Never smoked	7 (29.2)
Suspected cause of ARI, n (%)	
Cardiac	12 (50)
Renal injury/trauma	4 (16.7)
Idiopathic	7 (29.2)
Hypercoagulable status	1 (4.2)
Comorbidities, n (%)	
Hypertension	7 (29.2)
Diabetes mellitus	6 (25)
Cardiac	6 (25)
Other	5 (20.8)
None	7 (29.2)
Under anticoagulant/antiplatelet therapy, n (%)	10 (41.7)
Acetylsalicylic acid	6 (25)
Clopidogrel	2 (8.3)
Warfarin	2 (8.3)

ARI, acute renal infarction.
Data are presented as mean ± standard deviation (min–max) or number (%).

Table 2. Clinical, Radiological, and Laboratory Findings at the Time of Presentation

Clinical presentation, n (%)	
Flank/abdominal pain	17 (70.8)
Nausea/vomiting	3 (12.5)
Fever	1 (4.2)
Other	3 (12.5)
Side of ARI on CT, n (%)	
Right	10 (41.7)
Left	10 (41.7)
Bilateral	4 (16.7)
Configuration of infarct on CT, n (%)	
Focal	12 (50)
Multifocal	5 (20.8)
Global	7 (29.2)
Infarction volume/kidney volume (%)	53.9 ± 31.8 (6.1–100)
Laboratory data (reference range)	
WBC (4500–11 000 /μL)	12 507 ± 6367 (5140–29 050) /μL
Serum LDH (0–248 U/L)	437.4 ± 261 (181–1210) U/L
Serum AST (0–50 U/L)	56.2 ± 61.6 (12–257) U/L
Serum ALT (0–50 U/L)	46.3 ± 41.2 (11–183) U/L

ARI, acute renal infarction; CT, computed tomography; WBC, white blood cell; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
Data are presented as number (%) or mean ± standard deviation (min–-max).

reduced tracer uptake at the site infarction while 3 patients with focal ARI had normal findings.

The mean white blood cell count and serum lactate dehydrogenase (LDH) value of the patients were 12507 ± 6367 /μL and 437.4 ± 261 U/L, respectively. The mean systolic blood pressure of the patients at admission was 147.3 ± 17.3 mmHg, and diastolic blood pressure was 94.2 ± 19.2 mmHg. The clinical, radiological, and laboratory findings of the patients at the time of admission are shown in Table 2.

The mean serum creatinine and eGFR values at admission were 1.4 ± 1.9 mg/dL and 85.3 ± 47.7 mL/min/1.73 m^2, respectively. At the time of clinical presentation, 8 patients had higher serum creatinine levels and 7 patients had <60 mL/min/1.73 m^2 eGFR value. In a 54-year-old male patient who had congenital solitary right kidney, global right ARI occurred. This patient required acute hemodialysis and was included in the chronic hemodialysis program. The kidney functions of the patients at admission, first month and first year are shown in Table 3.

DISCUSSION

Acute renal infarction mostly affects middle-aged population with a mean age of 60 years[9-11]; in our series, the mean age of the patients was 49.5 years. We believe that 4 patients who had renal trauma were younger (21-41 years); therefore, it might decrease the mean age of our population. Patients with ARI frequently have cardiac diseases including atrial fibrillation and ischemic or valvular heart diseases that increase the risk of thromboembolism.[2] Similar to the previous studies,[2,10,12,13] cardiac diseases were the most common suspected cause of ARI in our study. Renal trauma and renal vascular injury were the other causes of ARI. Kagaya et al.[14] reported that renal trauma was the underlying cause of ARI in 8% of the patients in their study. in another study that included 89 patients, renal vascular injury was the accused cause of ARI in 14.6% of the patients.[10] In our study, 4 of 24 patients had ARI due to renal injury/trauma. However, in some cases, predisposing factor of ARI is unknown. Faucon et al.[15] reported that the mechanism of ARI was idiopathic in 3.8% of 186 patients. However, another study reported that 47% of the patients had idiopathic ARI.[13] In our study, the rate of idiopathic ARI was 29.2%.

Most of the patients with ARI present with abdominal or flank pain. Ongun et al. reported that 56.5% of the patients presented with abdominal pain and 43.4% of the patients presented with flank pain.[9] In another study, it was reported that 72% of the patients presented with flank pain.[11] In our study, 70.8% of the patients had flank or abdominal pain at the time of admission. However, these signs are not specific to ARI, and the diagnosis of ARI needs to be proved by imaging tools. Although earlier studies reported that angiography and isotope scans were the main diagnostic techniques, contrast-enhanced CT has been the gold standard diagnostic tool for ARI with increased use since the 1990s.[12] According to the CT configuration, ARI is classified as focal, multifocal, or global. In a study by Suzer et al. CT features of ARI were evaluated in 37 patients. The authors reported that focal, multifocal, and global infarcts were observed in 23 (62.2%), 5 (13.5%), and 9 (24.3%) patients, respectively.[6] In our series, focal infarcts were the most common type of ARI with 50% of incidence.

Table 3. Summary of Renal Functions During 1–Year Follow–Up

Variables	At Presentation Time	At First Month	At First Year	*P*
Serum creatinine (mg/dL)	1.4 ± 1.9	1.4 ± 1.2	1.5 ± 1.5	.54
eGFR (mL/min)	85.3 ± 47.7	74.6 ± 46.8	82.9 ± 48.2	.54
Patients with increased serum creatinine (>1.2 mg/dL), n (%)	8/24 (33.3)	11/24 (45.8)	8/24 (33.3)	
Patients with decreased eGFR (<60 mL/min), n (%)	7/24 (29.2)	11/24 (45.8)	7/24 (29.2)	

eGFR: estimated glomerular filtration rate.
Data are presented as mean ± standard deviation or number (%).

Serum LDH is a marker of cell necrosis,[4] and elevated LDH level is the most common laboratory finding in patients with ARI.[12] Several studies showed that elevated LDH level was observed over 70% of the patients.[3,4,9,11,12] Our results were comparable with the literature, and elevated LDH level was recorded in 17 of 24 patients (70.8%) at presentation.

The treatment options for ARI are medical treatment including anticoagulants, antiplatelets, and thrombolytics, endovascular procedures, and open surgery. However, the optimal treatment for ARI is not clear due to the lack of comparative studies among these treatment modalities.[16,17] Previous studies reported that vast majority of the patients with ARI were treated with anticoagulant/antiplatelet drugs.[5,10,11,18] In a study by Fontán et al. only 4 patients underwent fibrinolysis with urokinase. The authors reported that 3 of them were successfully treated while 1 of them suffered upper gastrointestinal bleeding due to the treatment.[18] Yang et al. reported that 2 patients were given thrombolytic treatment for uncontrolled abdominal pain.[10] Endovascular radiologic procedures are the other and uncommonly used treatment options for ARI. Mesiano et al. reported that none of their patients underwent endovascular procedures.[11] A recent study reported that percutaneous angioplasty was performed in only 7% of the patients.[19] In our study, all patients were initially treated with LMWH, and long-term oral anticoagulant/antiplatelet therapy was started in 6 patients.

Acute renal infarction is a rare entity[2,3]; however, it is a clinically important condition as it may cause impairment in renal function. In a study by Huang et al. the mean serum creatinine level of 20 patients with ARI at presentation was 1.3 ± 0.3 mg/dL. The authors reported that during hospitalization 4 patients had mildly elevated serum creatinine levels (>1.5 mg/dL), and in 3 patients, no improvement was observed during more than 1-year follow-up.[3] In another study, it was found that 40.4% of the patients had impaired kidney function at admission.[4] In 2014, Bae et al. retrospectively reviewed 100 patients with ARI. The authors of the study stated that 30 patients had acute kidney injury and 7 of them progressed to CKD.[5] Ongun et al.[9] reported that 5 of 23 patients had impaired renal function at 1 month and 1-year follow-up. In another study with 89 patients with ARI, impaired kidney function rate was found as 27.4%.[10] A multicenter study by Eren et al. included 121 patients with ARI. In this study, the mean serum creatinine and eGFR values at the time of admission were 1.5 ± 0.1 mg/dL and 68 ± 3 mL/min, respectively. The authors of the study stated that CKD development rate was 28.9% during an average follow-up of 14 months and 4 patients required chronic dialysis.[13] According to our results, the mean serum creatinine value was 1.4 ± 1.9 mg/dL, and at 1-year follow-up, CKD developed in 7 patients (29.2%) which was similar to previous studies. In addition, our results suggested that mean serum creatinine and eGFR levels at presentation were not statistically different compared to the levels at first month and first year.

In the current study, we aimed to present our experience with ARI and to evaluate the short- and midterm kidney functions in patients with ARI. However, our study has some important limitations. First, we used the database and medical records of the patients; therefore, there are missing patients and clinical data including proteinuria and hematuria. Second, renal function values of the patients before ARI were unavailable.

CONCLUSION

ARI is a rare condition and presents with non-specific symptoms; however, it is associated with deteriorated renal function in short- and midterm follow-up periods. Therefore, ARI should be taken into consideration in patients with flank or abdominal pain and increased serum LDH level. In addition, according to our results, serum creatinine and eGFR levels at presentation may be suggestive for midterm renal functions.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Ankara City Hospital (Date: August 25, 2021, Ethics Committee Ruling number: E1-21-1953).

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

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Original Article

Scoliosis After Liver Transplantation in Pediatric Patients

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ABSTRACT

Objective: Little is known about the development of scoliosis after pediatric liver transplantation. In this study, we aimed to evaluate the frequency of scoliosis and its relationship with potential risk factors in children after liver transplantation.

Methods: Pediatric liver transplantations (under of age 18) performed between January 2009 and December 2017 at Malatya İnönü University Institute of Liver Transplantation were scanned retrospectively. In the spinal axis, >10° lateral deviations were accepted as scoliosis. The curve patterns were classified according to the Lenke classification.

Results: Among 287 pediatric liver transplantationss, 17 of them were scoliosis (6%). Nine patients were females and eight were males. The median Cobb angle was 12° at the time of diagnosis and then 17° at the last follow-up. According to the Lenke classification, 11 patients had type 5 curve pattern. During the follow-up period (ranging from 1 to 11 years), scoliosis progression was slow and no patient requiring surgical treatment was detected.

Conclusion: We found that the prevalence of scoliosis increased after pediatric liver transplantation, but we do not have any definite information about the cause. Comparable new studies with more patients are needed to make a definitive conclusion in this regard.

Keywords: Liver transplantation, musculoskeletal system, pediatric, scoliosis

INTRODUCTION

Liver transplantation (LT) is the only curative treatment for acute liver failure, and end-stage liver disease.[1] However, in pediatric patients, mortality and morbidity rates after LT are higher than those in adults.[1,2] The surgical techniques and immunosuppressive medication developed in recent years provide satisfactory results. Increasing the quality of life of transplant patients and avoiding the side effects of immunosuppressive drugs have become important issues.[3,4]

During childhood, growth and development are affected by many factors. Rapid development makes muscle and bone tissue more sensitive to external effects. In pediatric cases where the patient's life expectancy is considered, the effects of an organ transplant and immunosuppressive drugs on the musculoskeletal system should be understood and closely monitored.[5] A decrease in bone mineral density may occur due to medical treatment, immobilization, and metabolic bone disease after LT. Additionally, musculoskeletal system pathologies including developmental retardation, osteoporosis, atraumatic fractures, and spinal deformity may occur.[6,7]

Scoliosis is the lateral curvature of the spine in the coronal plane.[8] It is divided into 2 types: structural and non-structural scoliosis. Idiopathic scoliosis is structural scoliosis and is the most common type. Other causes of structural scoliosis are neuromuscular, congenital, trauma, infection, and tumors. Non-structural scoliosis includes scoliosis caused by posture, nerve root irritation, inflammation (such as appendicitis), and lower limb asymmetry.[9,10] There is a strong relationship between congenital heart disease, thoracotomy/sternotomy, and scoliosis. In most cases, scoliosis can be seen in the vertebral column without morphological abnormalities.[11] However, only few studies have been conducted on the development of scoliosis after pediatric LT and the factors that may affect it. Sharing the experiences of high-volume centers will contribute to the literature. In this study, we present the relationship between probable prognostic factors by retrospectively scanning the frequency of scoliosis after pediatric LT.

METHODS

The Ethics Committee of İnönü University approved this retrospective study (Approval No: 2017/8-14). Two hundred eighty-seven patients (under of age 18) who underwent LT at

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Malatya İnönü University Liver Transplant Institute between January 2009 and December 2017 were included in this study. Age, gender, height, weight, pediatric end-stage liver disease (PELD) score (<12 years), model for end-stage liver disease (MELD) score (≥12 years), Child–Pugh score, primary liver disease, transplantation type, medical treatment information, and radiological images of the patients were scanned using the hospital automation system and archived. The inclusion criteria were radiography images for spinal evaluation and at least 1-year follow-up after LT. The exclusion criteria were spinal axis deformity in vertebral column before LT and having comorbid musculoskeletal disease. Routine triple drug immunosuppression with tacrolimus, mycophenolate mofetil, and corticosteroids was applied to pediatric liver transplant recipients in our center. In cases where calcineurin inhibitory toxicity was suspected or antiproliferative effect such as malignancy was desired, everolimus treatment was initiated by stopping or decreasing tacrolimus treatment in the first postoperative month. Vitamin D and calcium supplements were given.

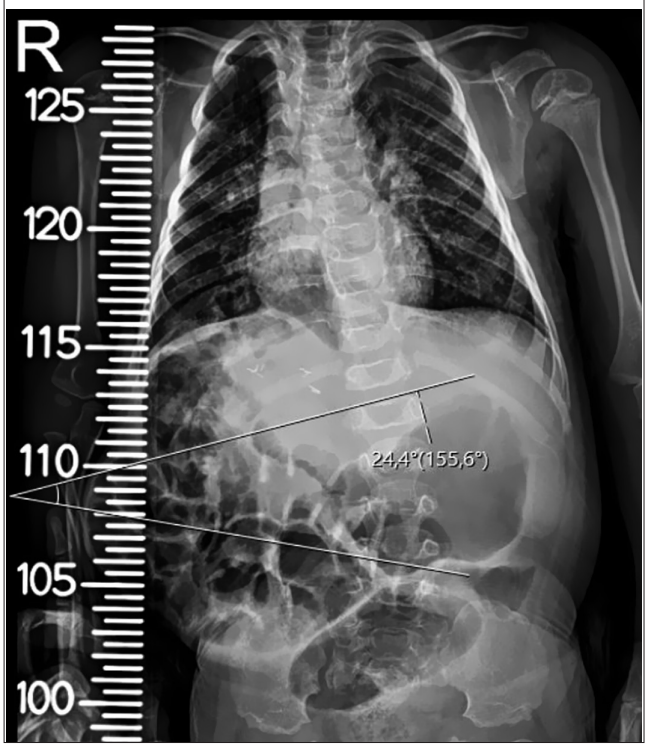
The Cobb method was used to diagnose scoliosis in this study. First, the upper and lower end vertebrae of the curvature were detected. Then, perpendicular lines were drawn to the upper end plate of the upper end vertebra and the lower end plate of the lower end vertebra. The angle formed between these lines was the Cobb angle (Figure 1). In the spinal axis, >10° lateral deviations were accepted as scoliosis.[12] The curve patterns were classified according to the Lenke classification. The main thoracic represents type 1, double thoracic type 2, main thoracic and lumbar curves type 3, triple major (proximal, main thoracic, and lumbar) type 4, thoracolumbar or lumbar type 5, and thoracolumbar/lumbar and main thoracic type 6 curves.[13] Measurements were made via radiograph and computed tomography (CT) by 2 pediatric radiologists with 5 and 10 years of experience, respectively.

Descriptive statistics were calculated and presented as number, degree and percentage. The continuous data were expressed as median (range).

RESULTS

Scoliosis was detected in 17 (6%) of the 287 patients included in the study. Nine patients were females and 8 were males. The diagnosis time for scoliosis was 0.6 years at the earliest and 1.8 years at the latest. The patients were followed up for 1 to 11.2 years after transplantation. The median Cobb angle was 12° at the time of diagnosis and then 17° at the last follow-up. In 13 patients, the angle ranged from 10° to 19°. In 4 patients, the angle was measured between 20° and 30°. According to the Lenke classification, 11 patients had type 5, 4 patients had type 1, and 2 patients had type 3 curve patterns. Liver transplantation indication was end-stage liver disease in 12 patients. Four of them had Wilson disease and 3 had neonatal cholestasis. In 5 patients, LT indication was fulminant hepatitis. Of the LTs performed, 13 were living donor liver transplantation and 4 were deceased donor liver transplantation. The median age was 4.4 years when LT was performed and 47% of the patients (8/17) were in the 0-3 age range. The median PELD score of 12 patients under the age of 12 was 31. The median MELD score of the other 5 patients was 20. The median Child–Pugh score was 10 (n = 17). When scoliosis was detected, 9 patients had incisional hernias (Figure 2). Routine immunosuppression medications were prescribed in 14 patients after LT. In 3 patients, tacrolimus was stopped and everolimus treatment was started. The patients' demographic and scoliosis characteristics are summarized in Table 1.

Figure 1. In a standing posterior–anterior radiograph, Cobb angle was measured at 24° in a 9-year-old male patient.



Main Points

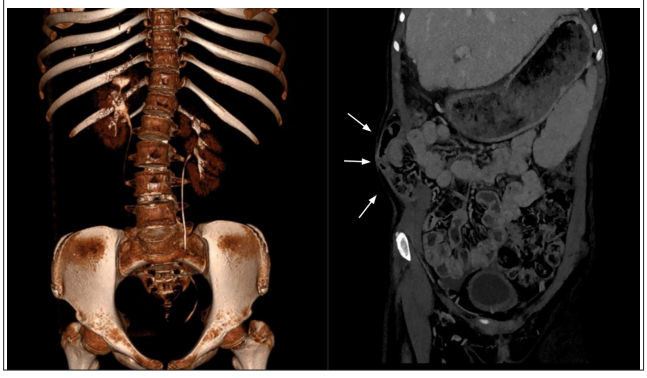
- Liver transplantation (LT) is the only curative treatment for acute liver failure, and end-stage liver disease.
- Musculoskeletal pathologies such as growth retardation, osteoporosis, atraumatic fractures, and spinal deformity may occur after LT.
- It should be kept in mind that scoliosis may develop in pediatric patients after LT, and the patient should be evaluated in this respect.

DISCUSSION

In our study, we found that the frequency of scoliosis increased after pediatric LT. Early age and growth-developmental retardation were noteworthy among possible risk factors. However, scoliosis progression was slow in the follow-up, and no patient requiring surgical treatment was detected.

Scoliosis prevalence in children is 1-3% worldwide.[9] In a study conducted in Turkey, this rate was reported as 2.3%.[14] Structural

Figure 2. A 10-year-old male patient had left-facing scoliosis at the lumbar level on volume-rendered CT image, and on coronal plane CT image, incisional hernia (white arrows) was observed. CT, computed tomography.



scoliosis is more common than non-structural scoliosis. It occurs most often in the adolescent period, and the vast majority of cases are idiopathic. Non-structural scoliosis is rarely seen.[15] The rib cage plays an important role in keeping the spine upright and balanced.[16] Large surgical procedures in this area can lead to unstructured scoliosis. Some studies report the development of scoliosis in children who underwent a thoracotomy/sternotomy.[11]

Little is known about the development of scoliosis after pediatric LT. One study demonstrated that the prevalence of scoliosis increased after a solid organ (heart, liver, kidney) transplantation. The study found that scoliosis after LT did not reach serious degrees. The results of the present study were similar. However, we do not have certain information about why scoliosis increased after pediatric LT. Factors leading to this condition may be impaired liver function, immunosuppressive therapy, or growth hormone therapy (GHT). Additionally, incisional hernia and pain can lead to posture disorder, causing scoliosis. Rapid growth and development during childhood makes bone tissue vulnerable.[17] Therefore, even the smallest risk factors can lead to pathological consequences.

Pre-LT, fat-soluble vitamin levels are generally low in children with end-stage liver disease. This is often caused by intestinal absorption disorders due to bile deficiency. Also, the activation of vitamin D in the liver is impaired. For these reasons, musculoskeletal system problems such as growth-development retardation, bone quality deterioration, and sarcopenia can be seen. The intrinsic support structures of the spine are the vertebrae, discs, and intraspinal-erector spinal muscles. Impairment in liver function negatively affects these spinal support structures. The therapeutic effect of LT can usually take 2 to 3 years.[7,18,19] All these problems may lead to an increase in the frequency of scoliosis after LT. Additionally, scoliosis was detected in all patients within the first 2 years after LT. This demonstrates that scoliosis develops before the therapeutic effect of LT is complete. Children receive long-term immunosuppressive therapy after LT.

Table 1. Patients' Demographic and Scoliosis Characteristics

Variables (n=17)	Number/Degree (Percentile/Range)
Age (at transplantation), median, year	4.4 (0.8–15.5)
0–4 years	9 (54%)
5–12 years	4 (23%)
13–18 years	4 (23%)
Gender	
Female	9 (53%)
Male	8 (47%)
Height, median, cm	88 (58–163)
Weight, median, kg	12 (5–60)
Height percentiles < 5th	10 (59%)
Weight percentiles < 5th	9 (53%)
PELD score, median (n=12)	31 (13–44)
MELD score, median (n=5)	20 (10–21)
Child–Pugh score, median	10 (7–13)
Primary liver disease:	
Fulminant hepatitis	5 (29%)
Wilson's disease	4 (23%)
Neonatal cholestasis	3 (18%)
Biliary atresia	2 (12%)
Cryptogenic hepatitis	2 (12%)
Autoimmune hepatitis	1 (6%)
Type of transplant:	
LDLT	13 (77%)
DDLT	4 (23%)
Cobb angle (last), median	17° (12°–29°)
10°–19°	13 (77%)
≥20°	4 (23%)
Lenke type	
Type 5	11 (65%)
Type 1	4 (23%)
Type 3	2 (12%)
Time to diagnosis after LT, median, year	1 (0.6–1.8)
Follow-up after LT, median, year	5 (1.0–11.2)

PELD, pediatric end-stage liver disease; MELD, model for end-stage liver disease; LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation; LT, liver transplantation.

Immunosuppressive agents, especially glucocorticoids, have many side effects on the skeletal system that negatively affect quality of life, including osteoporosis and fractures.[6] Glucocorticoids affect calcium absorption–excretion, parathormone level, and skeletal growth factors, directly reducing bone formation and increasing destruction. Tacrolimus, another immunosuppressive agent, prevents osteoclast formation and causes

osteoporosis.[20,21] These results suggest that immunosuppressive therapy is a factor that can cause scoliosis after LT. Therefore, the dosage and duration of the use of these drugs should be closely monitored. A more careful physical examination of the vertebral column should be performed to detect the possible development of scoliosis.

Incisional hernia is a frequent complication seen in 4-20% of patients after LT. Immunosuppression with end-stage liver disease, corticosteroid, and mycophenolate mofetil or sirolimus have significant effects on the development of incisional hernia.[22] In our study, 9 of the 17 patients with scoliosis had incisional hernias. The development of an incisional hernia may have caused a postural disorder in pediatric patients, leading to the development of co-sided scoliosis. Additionally, pain control after surgery is an important issue. The pain in the operation area may have triggered the formation of scoliosis with a similar posture disorder. Therefore, postoperative wound care and pain control should be performed carefully.

Physical examination for scoliosis should be performed with the patient completely naked in a bright room. The posture should be carefully examined from the front and side. The findings vary according to the severity of the deformity. The symmetry of the shoulders and nipples should be examined.[16] The basic imaging method in the diagnosis and evaluation of scoliosis is a standing radiograph. When considering a bone or neurological cause for scoliosis, CT or magnetic resonance imaging is used. It can also be used in planning, before CT scoliosis surgery.[23] In post-LT follow-up, CT is frequently performed, with different indications. In CT images, the spine and intra-abdominal organs can be evaluated. However, in the supine position, spinal rotation can be corrected spontaneously up to 30%.[24,25] Therefore, especially in cases with a low Cobb angle, false results may occur. Diagnosis and follow-up of patients with suspected scoliosis should be done by standing radiograph.

There were some limitations in our study, the most important being that we could not include physical examination findings of patients because this was a retrospective study. Additionally, as the side radiographs of all patients were not available, kyphosis evaluation could not be performed. In future prospective studies, it would be more appropriate to handle scoliosis in all aspects, clinically and radiologically. Another limitation was that our study was based on a single-center experience and a relatively small sample size.

CONCLUSION

In conclusion, although we could not find any specific risk factor for scoliosis development, we found that the prevalence of scoliosis after pediatric LT was higher than that in the general population. When following up with pediatric LT patients, caution should be exercised in terms of scoliosis, and a standing posterior–anterior radiograph should be taken when necessary. More patients are needed to make an exact conclusion in order to evaluate with comparable studies.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of İnönü University (Decision No: 2017/8-14).

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

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Original Article

Prenatal Dexamethasone Exposure in Male Rats Alters Gene Expression Patterns of Epigenetic Enzymes in Hippocampus and Cortex

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ABSTRACT

Objective: This study aimed to examine the effects of prenatal stress (PS) induced by dexamethasone exposure on gene expression levels of epigenetic enzymes in hippocampus and cerebral cortex of male rats through relative mRNA levels of histone acetyltransferases (activating transcription factor 2, P300), histone deacetylases (HDAC1, HDAC2), and DNA methyltransferases (DNMT1, DNMT3a, DNMT3b).

Methods: Pregnant rats were daily injected subcutaneously with dexamethasone (0.2 mg/kg) or saline during the third week of gestation. After birth, male rats were killed at 90 days of age (n = 5 for control and dexamethasone groups). Hippocampal and cortical tissues were used for gene expression analyses. The effects of dexamethasone on epigenetic mechanisms were investigated by real-time polymerase chain reaction through relative mRNA levels of DNMT1, DNMT3a, DNMT3b, activating transcription factor 2, P300, HDAC1, and HDAC2. Statistical comparisons were performed with Student's t-test.

Results: Prenatal dexamethasone exposure (PDE) caused increased DNMT1, DNMT3a, DNMT3b, activating transcription factor 2 and decreased P300 mRNA levels in hippocampus while increased DNMT3a, DNMT3b, activating transcription factor 2, P300, HDAC1, and HDAC2 mRNA levels were achieved in cortex. Furthermore, no significant differences were obtained in cortical DNMT1 and hippocampal HDAC1 and HDAC2 gene expression levels between control and prenatally stressed rats.

Conclusion: Our results emphasize the effect of prenatal dexamethasone exposure on gene expression levels of epigenetic enzymes involved in histone acetylation/deacetylation and DNA methylation in male rats and suggest that prenatal stress may lead to epigenetic dysregulation through alterations in hippocampal and cortical gene expression patterns of DNMT1, DNMT3a, DNMT3b, activating transcription factor 2, P300, HDAC1, and HDAC2.

Keywords: Dexamethasone, epigenetic enzymes, prenatal stress, rat

INTRODUCTION

The brain is vulnerable to stress factors during the perinatal life due to its high neuroplasticity. Prenatal exposure to environmental factors is assumed to change gene expression profiles throughout life and has been shown to have various negative effects on both health and cognition in animals and humans. The alterations in gene expression profiles can be caused by epigenetic mechanisms through histone or DNA modifications.[1,2] Moreover, prenatal stress has been proposed to affect the risk of developing mental or metabolic disorders such as anxiety disorder, depression, hypertension, and type 2 diabetes mellitus.[3-5]

Epigenetic mechanisms, which include histone modifications, non-coding RNAs, DNA methylation, and hydroxymethylation, are involved in the regulation of gene activity without modifying the DNA sequence.[6] The addition of acetyl groups to histones is catalyzed by histone acetyltransferases (HATs) while histone deacetylases (HDACs) catalyze the removal of acetyl groups.[7] Activating transcription factor 2 (ATF2) functions as HAT and associates with p300, which also works as HAT and transcriptional co-activator.[8] HDAC1 and 2, class I HDACs, are localized in hippocampus and cortex.[9] DNA methylation represses transcription and is catalyzed by DNA methyltransferases (DNMTs) including DNMT1, 2, and 3.[10]

The hypothalamic-pituitary-adrenal (HPA) axis plays an emerging role in stress adaptation. The hippocampus and the frontal cortex are involved in regulating the functioning of the HPA axis and are connected highly with hypothalamus.[11] Laboratory animals have been employed as prenatal glucocorticoid exposure (prenatal stress) models by administering corticosterone or its synthetic

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analogs throughout pregnancy. Dexamethasone (Dex), a synthetic glucocorticoid that is fat soluble, can pass through the placenta, and has been used to induce prenatal stress in rodents.[12] It has been demonstrated that the glucocorticoid receptor (GR) plays a critical role in modulating cellular responses to stress and circulating glucocorticoids. To regulate transcription and modify the structure of chromatin, GR works in conjunction with the epigenetic enzymes such as methyltransferases (DNMT1, DNMT3a, and DNMT3b), HATs (ATF2, p300), and HDACs (HDAC1, HDAC2).[13]

The present study examined for the first time how prenatal Dex exposure at a dose of 0.2 mg/kg during the last week of gestation affected the gene expression profiles of DNMTs (DNMT1, DNMT3a, and DNMT3b), HATs (ATF2, p300), and HDACs (HDAC1, HDAC2) concurrently.

METHODS

Animals and Treatment

The present study was approved by Ethics Committee on Animal Experiments of Ege University (May 24, 2017; Reference No. 2017-023). All efforts were made to minimize animal suffering. Six virgin female Sprague-Dawley rats, 210 ± 10 g, were housed randomly under a 12-hour light-dark cycle in plastic cages at 19-24°C and allowed continuous access to rat chow and water. Two female rats in proestrus were coupled with 1 male rat overnight. Pregnancies were confirmed by the vaginal smears and pregnant rats were divided into 2 groups as control or prenatal stress.[14] Rats were injected subcutaneously with Dex (Cat. No. D1159, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 0.2 mg/kg or saline of equal volume during the third week of gestation. At end of treatments, pregnant rats delivered normally. All offspring were weaned on postnatal day 21 (PN21). Prenatal Dex exposure at a dose of 0.2 mg/kg resulted in lethality in the offspring. For this reason, we had to limit the number of male rats in the experimental groups to 5. At PN90, male rats were killed by decapitation. Hippocampus and cortex were dissected immediately, frozen, and stored at −80°C.[12]

Total RNA Extraction, Reverse Transcription, and Real-Time Quantitative Polymerase Chain Reaction

The cortical and hippocampal samples were used for total RNA extraction with MasterPure Complete DNA and RNA Purification Kit (Cat. No. MC85200, LGC Biosearch Technologies, Wis, USA). The concentration and purity of RNA samples were measured using the CLARIOstar Plus microplate reader with the LVIS plate (BMG LABTECH, Ortenberg, Germany). The absorbance$_{260\ nm}$/absorbance$_{280\ nm}$ ratio of RNA samples was determined, and a ratio of 1.95-2.00 was accepted as pure for RNA samples. One microgram of total RNA was reverse transcribed using cDNA synthesis kit containing RevertAid Reverse Transcriptase, 5X Reaction Buffer, Random Hexamer Primer, dNTP Mix, RiboLock RNase Inhibitor, and nuclease-free water (Cat. No. K1622, Thermo Fisher Scientific, Mass, USA). The amplification protocol for reverse transcription consisted of 1 cycle of 25°C for 5 minutes, 42°C for 1 hour, and 70°C for 5 minutes.

Real-time quantitative polymerase chain reaction (PCR) experiments were performed with SYBR Green and AriaMx RT-PCR System (Agilent Technologies, Calif, USA). cDNA (1 µL), 250 nM forward and reverse primers (1 µL), and DNase- and RNase-free water (7 µL) were amplified with Master Mix (10 µL). Primer-BLAST was used for primer designs, and the sequences of primers were confirmed with in silico PCR amplification.[15,16] The primers used in this experiment were given in Table 1. The amplification protocol consisted of 1 cycle of 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 60 seconds. The dissociation curve analyses were performed at end of the amplification to control desired PCR products. The real-time PCR experiments were repeated 3 times. We determined the relative levels of mRNAs by comparative C_T method ($2^{-\Delta\Delta CT}$) and normalized to reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with Agilent software.[17]

Statistical Analysis

Data were analyzed with Statistical Package for the Social Sciences for Windows (SPSS 25.0, IBM SPSS Corp., NY, USA) using Student's *t*-test. Data were representative of 3 independent experiments and given as mean ± standard error. $P < .05$ was considered statistically significant.

Main Points

- Prenatal stress was shown to change mRNA levels of epigenetic enzymes involved in histone acetylation/deacetylation and DNA methylation.
- Prenatal dexamethasone (Dex) exposure altered the gene expression patterns of DNMT1, DNMT3a, DNMT3b, activating transcription factor 2 (ATF2), and P300 in hippocampus.
- Prenatal Dex exposure altered the gene expression patterns of DNMT3a, DNMT3b, ATF2, P300, HDAC1, and HDAC2 in cortex.

RESULTS

Effects of PDE on Relative mRNA Levels of DNMTs

To examine the effects of PDE on gene expression levels of DNMTs, we measured relative mRNA levels of DNMT1, 3a, and 3b in hippocampal and cortical tissues of PDE group and control (Figure 1). The Tm values of DNMT1, 3a, and 3b were found to be 80.5°C, 89.0°C, and 83.5°C, respectively. In hippocampus of control and PDE groups, relative mRNA levels of DNMT1 were 1.000 ± 0.023 and 1.221 ± 0.035, respectively. In cortical samples, relative mRNA levels of DNMT1 in control and PDE groups were 0.997 ± 0.012 and 0.992 ± 0.024, respectively. No significant differences were found in cortical DNMT1 mRNA levels between the groups but PDE increased hippocampal DNMT1 mRNA levels significantly when compared to control. We also found significant differences in DNMT1 gene expression levels between hippocampal and cortical tissues of PDE group (Figure 1, $^{a}P < .001$ vs. control in hippocampus and vs. Dex in cortex). The relative mRNA levels of DNMT3a in hippocampal samples of control and PDE groups were 0.999 ± 0.026 and 1.319 ± 0.028, respectively. In cortical tissues of control and PDE groups, relative mRNA levels of DNMT3a were found as 1.003 ± 0.018 and 1.316 ± 0.036, respectively. When compared to control group, DNMT3a mRNA levels of PDE group in hippocampus and cortex

Table 1. The Sequences for Forward and Reverse Primers Used in RT–qPCR

Primer	NCBI Ref. No	Primer Sequence	Position	Length
DNMT1	NM_053354.3	F: 5'–GAGGCACTGTCCGTCTTTGA–3' R: 5'–AAGTGACCGCGACTGCAATA–3'	1247	107 bp
DNMT3a	NM_001003958.1	F: 5'–ACGATAATACCTTCTCTGAAGCCC–3' R: 5'–CTTCCTTTCGATCATCCTCCCG–3'	88	150 bp
DNMT3b	NM_001396349.1	F: 5'–GATGAGGAGAGCCGAGAACG–3' R: 5'–CAGAGCCCACCCTCAAAGAG–3'	1488	128 bp
ATF2	NM_031018.2	F: 5'–GGATTGGTTAGGGCCCAGTC–3' R: 5'–CTCTTCTTCGACGGCCACTT–3'	1184	136 bp
P300	AB066220.1	F: 5'–AAGGTCTGGTAGTTCCCCCA–3' R: 5'–TGTGCCATTGGGCTTTTGAC–3'	254	129 bp
HDAC1	NM_001025409.1	F: 5'–CTCCATCTTCTCTCCAAGTCCC–3' R: 5'–GAGTTCTCCCAGTACCACTGC–3'	1480	150 bp
HDAC2	NM_053447.1	F: 5'–GGCCTCAGGATTCTGCTACG–3' R: 5'–CGGTCATCACGCGATCTGTT–3'	640	149 bp
GAPDH	NM_017008.4	F: 5'–AGTGCCAGCCTCGTCTCATA–3' R: 5'–AACTTGCCGTGGGTAGAGTC–3'	49	187 bp

GAPDH, glyceraldehyde–3–phosphate dehydrogenase; ATF2, activating transcription factor 2; HDAC, histone deacetylase; DNMT, DNA methyltransferase; RT–qPCR, reverse transcriptase quantitative polymerase chain reaction.

elevated significantly (Figure 1, $^{b}P < .001$ vs. control in hippocampus; $^{c}P < .001$ vs. control in cortex). The relative mRNA levels of DNMT3b in hippocampal samples were found as 0.998 ± 0.026 and 1.299 ± 0.031 in control and PDE groups, respectively. In cortical samples of control and PDE groups, relative mRNA levels of DNMT3b were 1.002 ± 0.029 and 1.250 ± 0.036, respectively. It was found that PDE led to significant enhancement of DNMT3b mRNA levels of PDE group in hippocampus and cortex when compared to control (Figure 1, $^{d}P < .001$ vs. control in hippocampus; $^{e}P < .001$ vs. control in cortex).

Effects of PDE on Relative mRNA Levels of Histone Acetyltransferases

To examine the effects of PDE on gene expression levels of HATs, we measured relative mRNA levels of ATF2 and P300 in hippocampal and cortical samples of PDE group and control (Figure 2). The Tm values of ATF2 and P300 were found to be 89.0°C and 83.0°C, respectively. The relative ATF2 mRNA levels in hippocampus and cortex of control were found as 1.002 ± 0.020 and 1.000 ± 0.022, respectively. In the PDE group, relative ATF2 mRNA levels in hippocampus and cortex were 1.602 ± 0.013 and 1.229 ± 0.028, respectively. When compared to control, the relative ATF2 mRNA levels were found elevated significantly in hippocampus and cortex of PDE group. We also found significant differences in ATF2 gene expression levels between hippocampus and cortex in the PDE group (Figure 2, $^{a}P < .001$ vs. control in hippocampus and vs. Dex in cortex; $^{b}P < .001$ vs. control in cortex). In hippocampal samples of control and PDE groups, relative mRNA levels of P300 were 0.999 ± 0.011 and 0.879 ± 0.008, respectively. The relative mRNA levels of P300 in cortical samples of control and PDE groups were found as 1.002 ± 0.016 and 1.350 ± 0.044, respectively. P300 gene expression levels of PDE group showed significant differences between hippocampus and cortex. We showed that P300 mRNA levels of PDE group significantly decreased in hippocampus, but were elevated in cortex compared to control (Figure 2, $^{c}P < .001$ vs. control in hippocampus and vs. Dex in cortex; $^{d}P < .001$ vs. control in cortex).

Effects of PDE on Relative mRNA Levels of Histone Deacetylases

To examine the effects of PDE on gene expression levels of HDACs, we measured relative mRNA levels of HDAC1 and 2 in hippocampal and cortical samples of PDE group and control (Figure 3). The Tm values of HDAC1 and HDAC2 were found to be 79.5°C and 81.0°C, respectively. In hippocampus and cortex of control group, relative mRNA levels of HDAC1 were found as 1.017 ± 0.040 and 1.014 ± 0.039, respectively. In PDE group, relative mRNA levels of HDAC1 in hippocampus and cortex were 1.131 ± 0.022 and 2.198 ± 0.083, respectively. We showed that HDAC1 mRNA levels of PDE group were significantly elevated in cortex, but no differences were found in hippocampus when compared to control. In PDE group, HDAC1 mRNA levels significantly differed between hippocampus and cortex (Figure 3, $^{a}P < .001$ vs. control in cortex and vs. Dex in hippocampus). The relative mRNA levels of HDAC2 were found as 1.000 ± 0.022 and 0.947 ± 0.024 in hippocampus of control and PDE groups, respectively. In cortical samples of control and PDE groups, the relative mRNA levels of HDAC2 were 0.999 ± 0.025 and 1.216 ± 0.011, respectively. We found that levels of HDAC2 mRNA were elevated significantly in cortex of PDE group compared to control. There were also significant differences in HDAC2 expression levels between hippocampus and cortex in the PDE group (Figure 3, $^{b}P < .001$ vs. control in cortex and vs. Dex in hippocampus).

DISCUSSION

The administration of dexamethasone, which is a fat-soluble synthetic glucocorticoid, during pregnancy has been used as a prenatal stress model in laboratory animals.[12] Through increased

Figure 1. Effects of prenatal Dex exposure on mRNA expression levels of DNMTs in hippocampus and cortex. The relative mRNA levels of DNMTs were determined with $2^{-\Delta\Delta CT}$ method and normalized to GAPDH. Data were expressed as mean ± standard error. $^{a}P < .001$ vs. control in hippocampus and vs. Dex in cortex; $^{b,d}P < .001$ vs. control in hippocampus; $^{c,e}P < .001$ vs. control in cortex (n = 5 for each group). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Dex, dexamethasone; DNMTs, DNA methyltransferases.

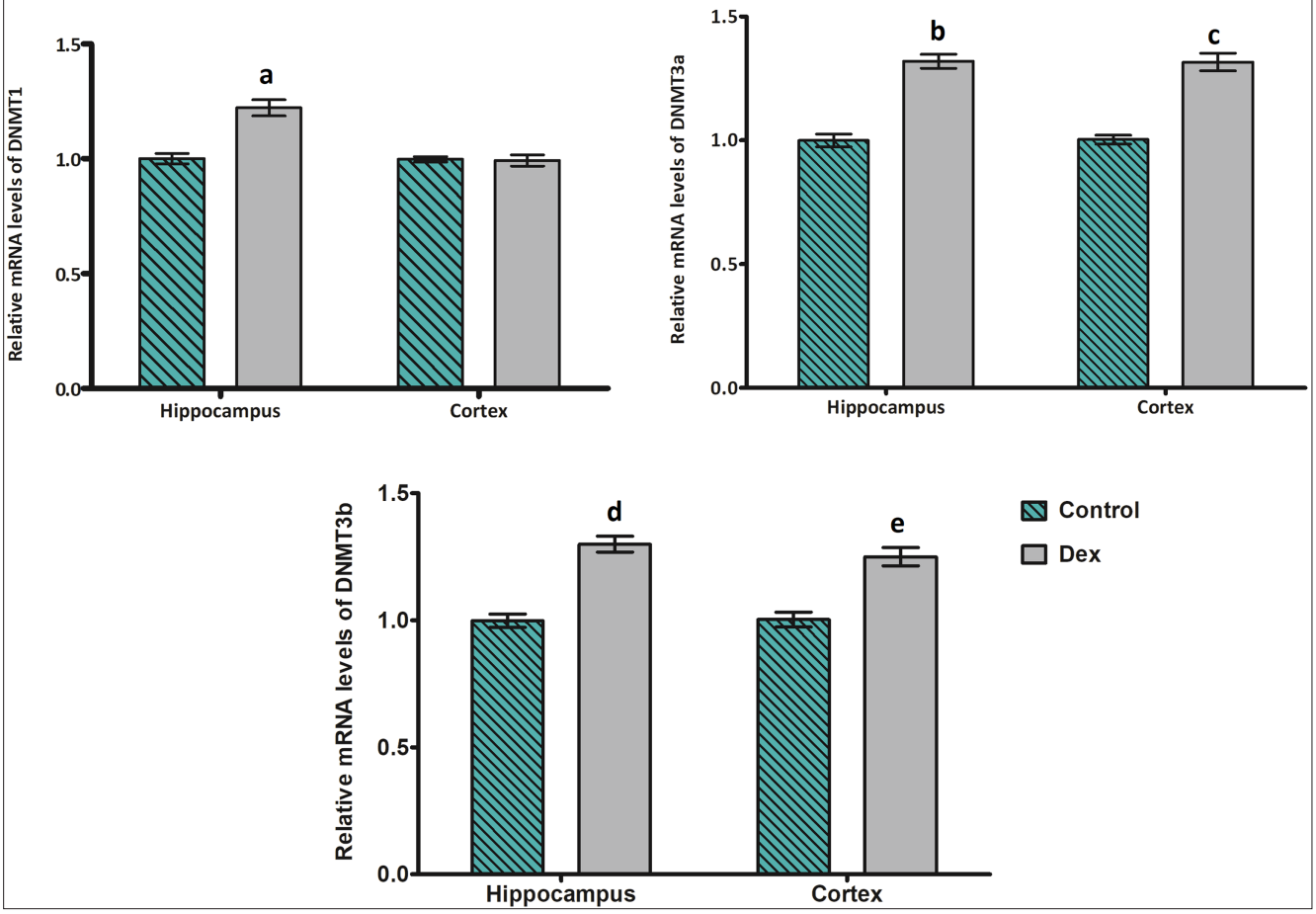


Figure 2. Effects of prenatal Dex exposure on mRNA expression levels of HATs in hippocampus and cortex. The relative mRNA levels of HATs were determined with $2^{-\Delta\Delta CT}$ method and normalized to GAPDH. Data were expressed as mean ± standard error. $^{a,c}P < .001$ vs. control in hippocampus and vs. Dex in cortex; $^{b,d}P < .001$ vs. control in cortex (n = 5 for each group). HATs, histone acetyltransferases; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Dex, dexamethasone.

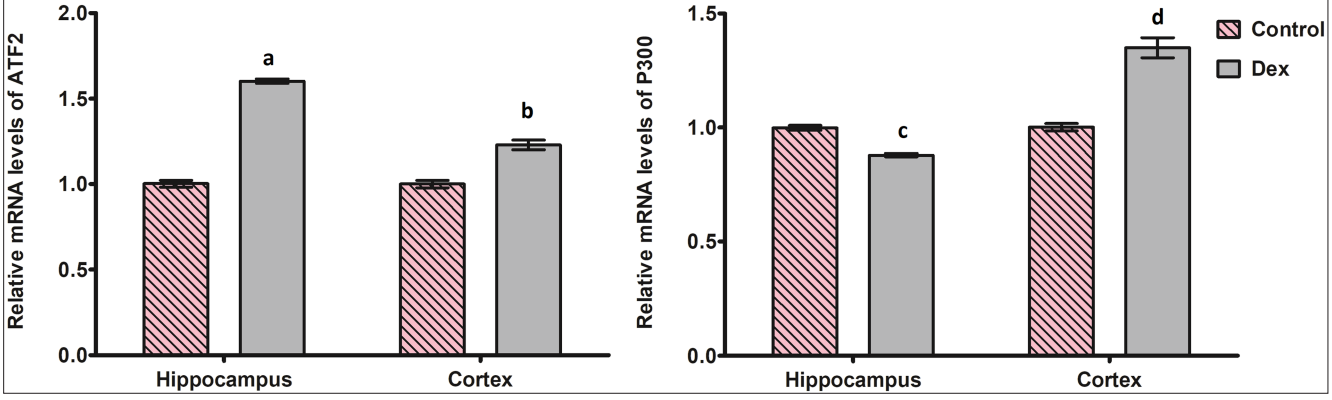
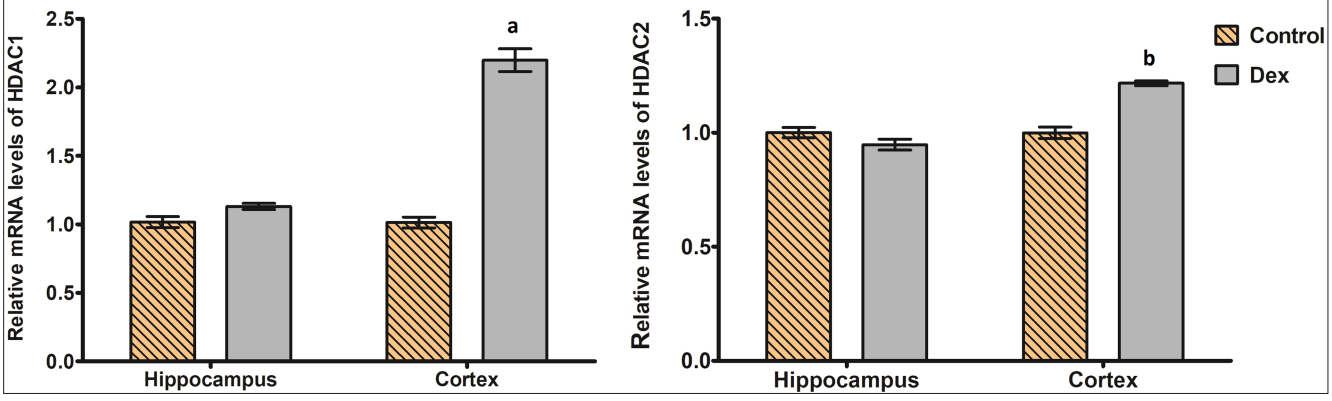


Figure 3. Effects of prenatal Dex exposure on mRNA expression levels of HDACs in hippocampus and cortex. The relative mRNA levels of HDACs were determined with $2^{-\Delta\Delta CT}$ method and normalized to GAPDH. Data were expressed as mean ± standard error. $^{a,b}P < .001$ vs. control in cortex and vs. Dex in hippocampus (n = 5 for each group). HDACs, histone deacetylases; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Dex, dexamethasone.



levels of adrenal corticotropin-releasing hormone and corticosterone, upregulation of corticotropin-releasing hormone in the hypothalamus, downregulation of mineralocorticoid receptors, and upregulation of 11-beta-hydroxysteroid dehydrogenase-1 in the hippocampus in adult male rats, prenatal Dex exposure has been shown to alter hippocampal drive on HPA axis activity.[18] Prenatal stress has been proposed to interact with genetics as well as epigenetics to alter the risk for various mental or metabolic disorders.[3-5]

The epigenetic regulation of gene activity, which includes DNA and histone modifications, is involved in various stages of neuronal function and are reported to have lifelong effects on mature neurons, neuroplasticity, and cognition.[6] The epigenetic processes causing changes in gene expression profiles during pre- and postnatal period may be responsible for development of the longer-lasting effects of prenatal stress.[19] Moreover, epigenetic mechanisms have attracted great attention as therapeutic strategy in diagnosis and treatment of neurodevelopmental disorder and neurodegeneration.[6,20,21] In our study, prenatal Dex exposure resulted in increased DNMT1, DNMT3a, DNMT3b, ATF2 and decreased P300 mRNA levels in hippocampus while increased DNMT3a, DNMT3b, ATF2, P300, HDAC1, and HDAC2 mRNA levels were found in cortex. Furthermore, there were no significant differences in cortical DNMT1, hippocampal HDAC1, and HDAC2 gene expression levels between control and prenatally stressed rats.

Activating transcription factor 2 regulates the expression of specific genes involved in inflammation, proliferation, apoptosis, transformation, and repair.[8] In the rat models of depression induced by lipopolysaccharide or chronic unpredicted mild stress, significant increases of ATF2 activation were obtained in hippocampus.[22] Neuroinflammation that occurs in particular brain regions in response to external stress stimuli is usually linked to depression. Chronic unpredicted mild stress-induced ATF2 activation in the hippocampus may be due to its role in inflammatory processes. Kucharczyk et al[11] showed activation of ATF2 in frontal cortex of male rats which were prenatally exposed to immobilization stress. In contrast to the frontal cortex, ATF2 activation has not been seen in the hippocampus.[11] In our study, PDE resulted in enhanced ATF2 mRNA levels both in hippocampus and cortex. The reason why we found increased expression of ATF2 in the hippocampus may be because we used a different prenatal stress protocol than Kucharczyk and colleagues.

P300 plays important roles during the neurodevelopmental stages and may involve in neuroplasticity of mature neurons.[23] The nuclear receptor interaction domain of the P300 interacts with the GR. P300 is required for transcriptional coactivation of target genes with glucocorticoid response elements.[13] In the study by Hu et al[24], pregnant rats were injected with Dex (0.2 mg/kg) from GD9 to 20 and PDE led to significant increase in P300 mRNA levels of fetal rats. P300 mRNA levels were found to be decreased significantly in hippocampus but increased in cortex of PDE group in our study. The differences in P300 expression pattern may be due to the fact that Hu et al used fetal hippocampal tissues in their study and we used hippocampal tissues from 3-month-old rats.

DNA methylation causes inhibition of transcription and is involved in regulation of gene expression and development.[10] Glucocorticoid receptor function can be regulated by DNMTs, and vice versa, GR can control the expression and activity of DNMTs.[13] Lui et al[25] induce PS using Dex at a different dose (0.1 mg/kg) from our study and demonstrated elevated DNMT1 mRNA levels in hippocampus of male rats. Boersma et al[2] demonstrated increased DNMT1 and 3a mRNA levels in hippocampus and amygdala of male rats exposed to different stressors including swim, social, and restraint stress during prenatal period.[2] In the study by Lei et al. PS induced by restraint stress caused enhancement in hippocampal protein levels of DNMT1 and 3a in female rats, but not in male offspring.[19] In the study by Grégoire et al.[26] prenatal restraint stress in male mice caused significant decrease in hippocampal DNMT3b and cortical HDAC1 mRNA levels whereas increase in hippocampal DNMT1 expression was

observed. In the present study, PDE caused enhanced mRNA levels of DNMT3a and DMNT3b both in hippocampus and cortex but DNMT1 mRNA levels increased only in hippocampus. Our findings supported previously reported studies describing increased DNMT1, DNMT3a, and DNMT3b expressions in the hippocampus as a result of prenatal stress.[2,25,26] The inhibition of HDAC activity plays important roles in cellular and molecular processes that are involved in neuroplasticity, oxidative stress, apoptosis, transcription, and neuroprotection through modification of histone acetylation levels.[27] Zheng et al[28] demonstrated that prenatal restraint stress increased DNMT1, HDAC1, and HDAC2 gene expressions in the hippocampal samples of male mice. Wei et al[29] discovered that GR occupancy was enhanced at the HDAC2 promoter glucocorticoid response element and enhances the transcription of HDAC2 in repeatedly stressed rats. We found that HDAC1 and HDAC2 mRNA levels elevated significantly in cortex but not altered in hippocampus of prenatally stressed rats. Using a different prenatal stress model and animal type from Zheng et al. may explain the difference in HDAC expressions.

There are some limitations in our study. We could obtain more effective results in gene expression analyses by increasing the number of animals in the experimental groups. We did not have the opportunity to analyze the protein levels of HATs, HDACs, and DNMTs. Detecting gene expression levels may not always give a precise result about the amount of a protein. We also did not separate subregions of cortical and hippocampal tissues. Therefore, protein quantification and microdissection techniques are required for a more detailed analysis.

CONCLUSION

Further studies detecting mRNA and protein levels of HATs, HDACs, and DNMTs throughout neurodevelopment and in later life are needed to determine the changes in epigenetic mechanisms. To the best of our knowledge, this study shows for the first time that prenatal Dex exposure at a dose of 0.2 mg/kg during the final week of gestation changed the gene expression profiles of DNMTs (DNMT1, DNMT3a, DNMT3b), HATs (ATF2, P300), and HDACs (HDAC1, HDAC2) concurrently in the cortex and hippocampus of male rats. Conclusively, our results will serve as an experimental contribution to understanding the neurodevelopmental effects of prenatal stress on epigenetic programming and suggest that prenatal stress may cause disruption of epigenetic regulation through changes in hippocampal and cortical gene expression patterns of DNMT1, DNMT3a, DNMT3b, ATF2, P300, HDAC1, and HDAC2 in male rats.

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Original Article

Polypharmacy and Depression Among Older Individuals

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ABSTRACT
Objective: The aim of the present study is to determine the frequency of polypharmacy and depression and the relationship between them in older adults.
Methods: We reviewed the files and electronic records of 863 patients aged 65 years and older admitted to our geriatric outpatient clinic. The presence of polypharmacy and depression was evaluated.
Results: The mean age of the participants was 73.3 ± 6.5 years. The proportion of female patients was 66.8%. While the frequency of polypharmacy was 47.1% in general, it was 80.5% in depressive patients. The proportion of patients diagnosed with depression was 15.9%. The presence of depression was found to be associated with a 3.3-fold increase in the risk of polypharmacy.
Conclusions: The frequency of polypharmacy was found to be high especially among depressed patients in our study. Early diagnosis of depressed elderly people seems to be an approach to prevent the occurrence of polypharmacy.
Keywords: Depression, older adults, polypharmacy

INTRODUCTION

Geriatric syndromes are common clinical conditions in older adults, including falls, frailty, cognitive impairment, delirium, urinary incontinence, depression, and polypharmacy.[1]

Polypharmacy is a global problem that particularly affects older adults, and its prevalence is higher in older adults.[2,3] Polypharmacy is most commonly defined as the use of 5 or more medications daily by an individual. Hyperpolypharmacy has been defined as using 10 or more medications. It is stated that approximately 30% of adults aged 65 and over take 5 or more drugs in developed countries.[4,5]

Polypharmacy is a complex geriatric syndrome that needs careful evaluation of its benefits and potential harms, especially in elderly patients. Complexity arises from conditions such as multimorbidity, sensory and cognitive impairment, drug-drug and drug-disease interactions, which are common in old age.[6] Prior studies have shown older adults to have multiple comorbidities associated with increased drug use.[7,8]

A very large cross-sectional study found that patients with depression are more likely to have multimorbidity.[9] In addition, the risk of having comorbidities increases by 40% in depressive disorders.[10] Little is known about the relationship between polypharmacy and depression in older adults. There is some evidence that increasing numbers of medications are associated with more depressive symptoms, but this area is less well understood.

This study aimed to determine the frequency of polypharmacy and depression and the relationship between them in older adults.

METHODS

Study Design and Patients

The subjects of this study were geriatric patients admitted to our outpatient clinic between November 1, 2020, and March 1, 2021. The files and hospital electronic record system data of 863 patients were reviewed. The patients' socio-demographic characteristics, chronic diseases, medications used, newly prescribed and discontinued drugs were examined. One hundred twenty-four patients with insufficient file data were excluded from the study. The study was completed with 739 patients. Polypharmacy, as defined most frequently, was accepted as using 5 or more drugs at the same time. The diagnosis of depression was made by an experienced psychiatrist or geriatrician.

Statistical Analysis

The normality of the distribution of continuous variables was tested by the Shapiro-Wilk test. Two independent groups

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Table 1. Participants' Socio-demographic Characteristics (n = 739)

Variables	Non-depressive (n = 621)	Depressive (n = 118)	*P*	Total (n = 739)
Gender				
Female	406 (65.4%)	88 (74.6%)	.055	494 (66.8%)
Male	215 (34.6%)	30 (25.4%)		245 (33.2%)
Age†	73.2 ± 6.5	73.7 ± 6.7	.486	73.3 ± 6.5
Age group				
65–74 years	399 (64.3%)	70 (59.3%)	.502	469 (63.5%)
75–84 years	179 (28.8%)	37 (31.4%)		216 (29.2%)
≥85 years	43 (6.9%)	11 (9.3%)		54 (7.3%)
Number of comorbidities†	2.5 ± 1.1	3.4 ± 1.0	.000*	2.7 ± 1.1
Comorbidities				
Hypertension	425 (68.4%)	87 (73.7%)	.277	512 (69.3%)
Diabetes mellitus	328 (52.8%)	74 (62.7%)	.055	402 (54.4%)
Coronary artery disease	127 (20.5%)	29 (24.6%)	.187	156 (21.1%)
Neurodegenerative diseases	37 (6.0%)	11 (9.3%)	.218	48 (6.5%)
Polypharmacy	253 (40.7%)	95 (80.5%)	.000*	348 (47.1%)
Number of medications used†	4.3 ± 2.4	5.8 ± 2.5	.000*	4.5 ± 2.5
Patients with increased medication	105 (16.9%)	29 (24.6%)	.047*	134 (18.1%)
Patients with decreased medication	71 (11.4%)	8 (6.8%)	.134	79 (10.7%)

*$P < .05$; †mean ± SD.

with a non-normal distribution were compared with Mann-Whitney *U* test. The relationship between categorical variables was assessed with the χ^2 test, and numerical variables with Spearman's rank correlation coefficient. Multicollinearity was checked, and multivariate binary logistic regression analysis was performed for independent predictors of polypharmacy. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows version 22.0 (IBM SPSS Statistics Corp., Armonk, NY, USA). A *P* value less than .05 was accepted as statistically significant.

Compliance with Ethical Standards

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval for the study was granted by Gaziantep University Medical Faculty Ethics Committee (Date: May 27, 2020, Decision no: 2020/115). The authors confirm independence from the sponsors, and the content of the article has not been influenced by the sponsors.

Main Points

- Almost half of the geriatric patients had polypharmacy.
- The frequency of polypharmacy was higher in depressive patients.
- The presence of depression increased the risk of polypharmacy.

RESULTS

The mean age of the 739 patients was 73.3 ± 6.5 years. The proportion of female patients was 66.8%. Almost half of the participants had polypharmacy. The proportion of patients who were previously or newly diagnosed with depression was 15.9%. The frequency of polypharmacy in depressive individuals was more than 80%, and also the rate of those with an increase in the number of medications was found to be higher in depressed patients. In addition, while 66 (19%) of 348 patients with polypharmacy had a decrease in the number of drugs, most of these patients were non-depressive patients. Other socio-demographic characteristics of the patients are shown in Table 1.

A statistically significant positive correlation between the number of medications used and comorbidities and also the number of medications used and medications discontinued are presented in Table 2.

Variance inflation factor was calculated, and the presence of hypertension and diabetes mellitus was excluded from models due to the collinearity problem. In multivariate binary logistic regression analysis, age, depression, and the number of comorbidities were found as independent variables for polypharmacy (Table 3).

DISCUSSION

The results of our study showed that polypharmacy is associated with depression and is more common in older adults with depression than in non-depressive adults.

Table 2. Correlation Analysis Results Between the Variables

		Age	Number of Comorbidities	Number of Medications	Number of Medications Newly Prescribed	Number of Medications Discontinued
Age	r	1.000	0.011	0.095	−0.060	−0.102
	P		.756	.010*	.485	.346
Number of comorbidities	r	0.011	1.000	0.730	0.104	0.271
	P	.756		.000*	.222	.011*
Number of medications	r	0.095	0.730	1.000	−0.045	0.479
	P	.010*	.000*		.597	.000*
Number of medications newly prescribed	r	−0.060	0.104	−0.045	1.000	0.661
	P	.485	.222	.597	1.000	.052
Number of medications discontinued	r	−0.102	0.271	0.479	0.661	1.000
	P	.346	.011*	.000*	.052	

r, Spearman rank correlation coefficient.
*Significant at .01 level.

Previous studies have shown that polypharmacy is very common in the elderly population and has a prevalence between 27% and 59% in primary care patients. In a recent study conducted with 34,232 elderly participants from 17 European countries, the prevalence of polypharmacy was found to be 32.1%.[11]

Depression is the most common psychological disorder in older adults that compromises health status, so individuals suffering from depression are more prone to polypharmacy.[12] Studies have shown that depression increases mortality alone or in combination with other diseases. Studies conducted in Turkey have reported that 10.2%-68.9% of the older individuals living in institutions and 29% of community dwellers had depressive symptoms.

There is a vicious circle between polypharmacy and depression. While depression can increase the number of drugs used, polypharmacy can also lead to depressive symptoms in individuals.

To date, few studies have investigated the relationship between depression and polypharmacy, and one of them claimed that depression is a better predictor of polypharmacy than other comorbid diseases.[13–15]

In some studies investigating the consequences of polypharmacy, depression has been addressed, but it has not been discussed sufficiently.[16,17] Vetrano et al[18] have shown that depressive symptoms were associated with polypharmacy. Furthermore, other studies have shown that depressive patients have higher odds for polypharmacy, and there was a positive association between polypharmacy and depressive symptoms in older women.[19,20]

Table 3. Multivariate Logistic Regression Analysis Results of the Independent Variables for Polypharmacy

	Polypharmacy	
Variable	**OR [95% CI]**	***P***
Age	1.04[1.01–1.07]	.016*
Gender (female vs. male)	1.01[0.67–1.51]	.970
Depression	3.30[1.88–5.78]	.000*
Number of comorbidities	5.49[4.25–7.10]	.000*

OR, odds ratio.
*$P < .05$ according to multivariate binary logistic regression analysis.

In the present study, the prevalence of polypharmacy has been found as 47.1% in older individuals. The high prevalence of polypharmacy in older adults can be explained by the exponential increase in the prevalence of chronic diseases and conditions associated with advancing age.[21] The fact that the frequency of polypharmacy in depressive individuals is more than 80% indicates the importance of evaluating the elderly in terms of the presence of depression. Also, the presence of depression was found to be associated with a 3.3-fold increase in the risk of polypharmacy.

Our findings showed that, compared to non-depressive older adults, depressive patients had a significantly higher number of comorbidities. Consistent with other studies, the number of comorbidities and depressive disorders were associated with the usage of more medications.[22,23] More importantly, the number of medications used for chronic diseases has increased in approximately a quarter of depressive patients who applied to our geriatric outpatient clinic. In addition, most of the patients with polypharmacy with a decreased number of drugs were non-depressive patients. This also forces geriatricians to fight against polypharmacy.

There are some limitations in our study. First is the retrospective design of the study. Second, the fact that the study was conducted in a tertiary healthcare institution increases the likelihood of depressive patients having more comorbidities. Third, the number of depressive patients was relatively low. Despite these limitations, our study has some strengths. There are few studies investigating the relationship between depression and polypharmacy. There was no heterogeneity among the study groups, so the relationship between depression and polypharmacy has been emphasized more clearly.

CONCLUSION

This study showed that polypharmacy is quite common in the elderly population, and defining variables, such as depression associated with polypharmacy, is important in monitoring the older individuals most vulnerable to this problem. Early diagnosis of depressed elderly people seems to be an approach to prevent the occurrence of polypharmacy. It is necessary to carry out further large-scale, multi-center, prospective studies to analyze this association better in older individuals.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Gaziantep University Medical Faculty University (Date: May 27, 2020, Decision no: 2020/115).

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Original Article

Evaluation of Relationship Between Sphenoid Sinus Septation and Onodi Cells Using Cone-Beam Computed Tomography

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ABSTRACT

Objective: This study aimed to evaluate the sphenoid sinus septation, the presence/absence of Onodi cells, and the relationship between these structures with each other in cone-beam computed tomography.

Methods: A total of 500 cases (250 males, 250 females) between the ages of 18 and 81 were included in this study. Sphenoid sinus septations were classified as intersphenoid, multiple, and absent, and Onodi cells as present or absent. The relationship between these parameters was also assessed. Chi square test was used to determine the relationship between sphenoid sinus septation and Onodi cells.

Results: Multiple septa seen in 451 (90.2%) of the cases is the most common type of sphenoid septation. Onodi cells were present in 327 (65.4%) of the cases. A statistically significant relationship was detected between Onodi cells and mean age ($P < .05$) but no significant difference was observed between the septation and mean age ($P > .05$). There was no significant difference between the septation or Onodi cells with gender ($P > .05$). Multiple septa were observed in all cases with Onodi cell. The relationship between sphenoid sinus septation and Onodi cell was statistically significant ($P < .001$).

Conclusion: This study revealed that there was a significant relationship between sphenoid sinus septation and Onodi cells. The presence of Onodi cells was observed only with multiple septa. Cone-beam computed tomography is an effective imaging method in the evaluation of sphenoid sinus anatomy and its variations and surrounding anatomical structures.

Keywords: Cone-beam computed tomography, Onodi cell, septation, Sphenoid sinus

INTRODUCTION

Paranasal sinus anatomy can be defined according to the development degree of each sinus, the pneumatization variations (additional extensions of air-filled cells from the ethmoid complex or from the sphenoid sinuses to the enclosing bone), and variations in the bony structure.[1] The sphenoid sinuses, the most posterior of the paranasal sinuses, are situated in the center of the skull base, in the body of the sphenoid bone. They are surrounded by some important structures such as the Vidian nerve in the sinus floor, the trigeminal nerve situated in the infero-lateral wall, the carotid artery in the mid-lateral sinus wall, and the optic nerve located in the superior lateral wall.[2,3]

Since the bony canal of the optic nerve and/or of the internal carotid artery may be associated with the sphenoid septations, great care should be taken during the removal of these septations in surgical procedures.[4] The sphenoid sinus septum is generally in the anterior midline, in line with the nasal septum, but the sphenoid septum can deviate from the midline to any side, curved, displace vertically, transversely, or obliquely. Thus, the 2 sinus cavities may not be equal to each other.[4,5] The septum can be S- or C-shaped, complete or incomplete. There may also be an accessory septum in the sinus.[4]

The optic nerve can be surrounded by an air cell named the sphenoethmoid or Onodi when the most posterior ethmoid air cells in superior location extend posterolaterally and the anterior wall of the sphenoid sinus is displaced. The optic nerve in the Onodi cell can be found without a bone cover and its surface can be unprotected. The Onodi cell is a significant anatomical guide in surgical interventions to the lateral sphenoid sinus and the posterior ethmoid cells due to its relationship with the optic nerve which is vulnerable.[4]

Because the Onodi cell and the sphenoid sinus share a common bony wall, the Onodi cell can mistakenly thought to be the

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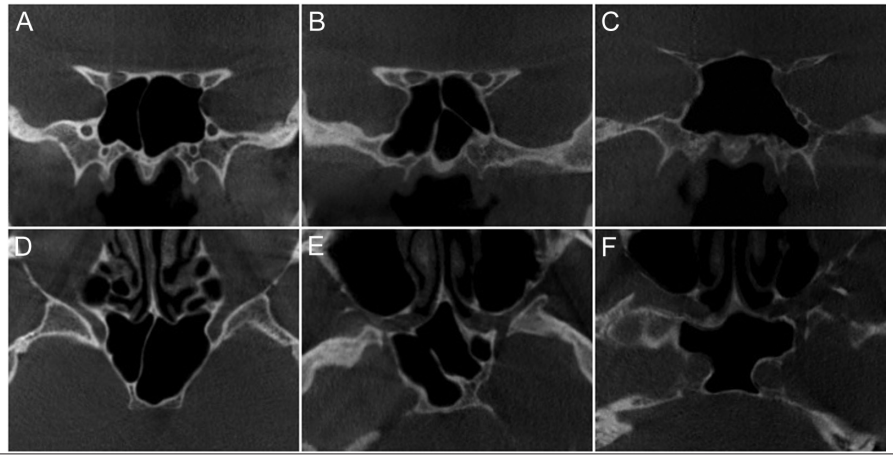
Figure 1. Sphenoid sinus septation types on coronal (A, B, C) and axial (D, E, F) CBCT sections: Intersphenoid septa (A, D), multiple septa (B, E), and absence of septa (C, F). CBCT, cone-beam computed tomography.



sphenoid sinus, but the sphenoid sinus is located inferiorly.[4] If there are unilateral or bilateral sphenoethmoidal air cells on coronal sections, there is a horizontal or cruciform septa because of air cell situated superiorly to the posterior choana (generally the sphenoid) that displaces the anterior wall of the sphenoid sinus horizontally, so that the sphenoid and the posterior ethmoid air cells can be distinguished from each other.[6] Because all sphenoid sinus septations are vertically directed, and if there are horizontally directed septa within the sphenoid sinus in sagittal sections, indeed, these septations belong to posterior ethmoid sinus.[5]

One of the methods used to provide multiplanar imaging for the maxillofacial region is cone-beam computed tomography (CBCT). Cone-beam computed tomography is a technical development in computed tomography (CT) imaging and suitable for use in situations limited with the head scanning because CBCT has relatively lower radiation dose and high isotropic spatial resolution compared to CT which is a large and expensive modality.[7,8] Cone-beam computed tomography is considered to be suitable for evaluation of paranasal sinus anatomy and surgical results and the imaging of intra- and perioperative osseous structures.[9]

Sphenoid sinus septations and Onodi cells are adjacent or associated with some neurovascular structures, and also Onodi cells are related to the walls and septations of sphenoid sinus. These structures and their relationships are important both during various sinus surgeries and in preoperative radiographic examinations. There are some investigations in the literature examining the sphenoid sinus septations and Onodi cells with CBCT or CT,[10-15] however, as far as we know, there is no study evaluating the relationship between these parameters. Therefore, the aim of this study was to assess the septation of sphenoid sinus, the presence/absence of Onodi cells, and the relationship between these structures with each other on CBCT.

Main Points

- The presence of Onodi cells was observed only with multiple septa.
- A relationship was detected between sphenoid sinus septation and Onodi cell.
- Cone-beam computed tomography is a suitable imaging method for the evaluation of sphenoid sinus.

METHODS

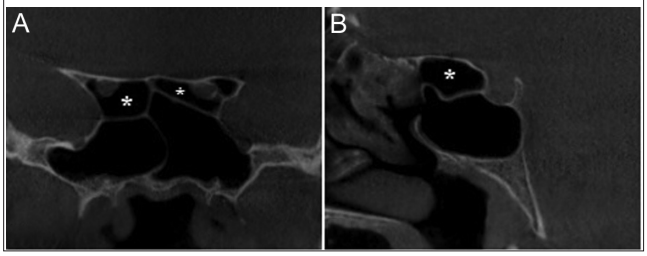
Study Design

Before the study, the ethical approval was obtained from Gaziantep University Ethics Committee (Decision No: 2020/245). The tomographical records in CBCT archive in Gaziantep University Faculty of Dentistry, Dentomaxillofacial Radiology Department were used and examined retrospectively. Images were acquired with Planmeca 3D Mid (ProMax, Helsinki, Finland) CBCT device, and Romexis software (Helsinki, Oy, Finland) was used for the analysis of the images. Multiplanar images with 16×16 and 16×9 cm field of view, a voxel resolution of 0.4 mm^3, and a slice thickness of 1 mm were used.

Sphenoid sinus septation and Onodi cells were examined according to mean age and gender in 500 cases (250 males, 250 females) between the ages of 18 and 81, and the relationship between these parameters with each other was also investigated. Images with fracture line due to trauma in the maxillofacial area or any maxillofacial lesion, sphenoid sinus disease, intracranial tumors, and craniofacial anomalies were not included in the study.

Sphenoid sinus septations were examined in coronal and axial sections, and they were classified as intersphenoid, multiple, or absent (Figure 1). If there was a single septa extending vertically from the superior to the inferior, located at or near the midline and dividing the sinus into 2 equal/unequal cavities, it was noted as the intersphenoid septa. If there was one or more complete or incomplete accessory septa in addition to the main septum, it was evaluated as multiple septa, and sinuses without septa were recorded as "absent."

Figure 2. Onodi cells on CBCT sections: coronal (A; asterisks) and sagittal (B; asterisk). CBCT, cone-beam computed tomography.



The presence or absence of Onodi cells was investigated in the coronal and sagittal planes (Figure 2). Sinuses with at least 1 Onodi cell were classified as "present" in these images, and sinuses without any Onodi cells were classified as "absent."

All CBCT images were assessed by the same maxillofacial radiologist. In order to determine the intra-observer calibration, the evaluations made by the observer were repeated in 100 randomly selected CBCT images (20% of all images), 2 weeks after the initial assessment, and intra-observer calibration was examined.

Statistical Analysis

Intraclass correlation coefficient was used to calculate intraobserver agreement. The conformity of the data to normal distribution was evaluated by Shapiro Wilk test. Chi square test was used to determine the relationship between sphenoid sinus septation and Onodi cells. As descriptive statistics, mean ± standard deviation for numerical variables and number and % values for categorical variables were given. Statistical Package for the Social Sciences software (version 24.0) (Armonk, NY, IBM SPSS Corp.) was used for statistical analysis, and *P* value < .05 was considered statistically significant.

RESULTS

Of the total 500 CBCT images between the ages of 18 and 81 (mean 46.11 ± 15.90), 250 were male (50%), 250 were female (50%), and the mean age was 47.02 ± 15.78 and 45.19 ± 16.01, respectively. The intraobserver reliability coefficient for all evaluations was found to be almost perfect (0.91).

When the distribution of sphenoid sinus septation was investigated, 44 (8.8%) cases had intersphenoid septation, 451 (90.2%) had multiple septa (Table 1). The sphenoid septum was absent in 5 (1%) cases. The relationship between the mean age and septation was also assessed. The mean age was 46.36 ± 17.65 for intersphenoid septa, 46.14 ± 15.75 for multiple septa, and 40.60 ± 15.66 for absent. No significant difference was determined between the septation and mean age ($P > 0.05$).

When the relationship of Onodi cells with mean age was examined, the mean age of 327 (65.4%) cases with Onodi cells was found to be 47.34 ± 15.32, and the mean age of 173 (34.6%) cases without Onodi was 43.77 ± 16.76 (Table 1). A statistically significant relationship was detected between Onodi cells and mean age ($P < .05$).

Table 1. Distribution of Sphenoid Sinus Septation and Onodi Cell According to Mean Age

		N (%)	Age (Mean ± SD)	*P*
Sphenoid sinus septation	Intersphenoid	44 (8.8)	46.36 ± 17.65	.751
	Multiple	451 (90.2)	46.14 ± 15.75	
	Absent	5 (1.0)	40.60 ± 15.66	
Onodi cell	Present	327 (65.4)	47.34 ± 15.32	.033*
	Absent	173 (34.6)	43.77 ± 16.76	

SD, Standard Deviation.
*$P < .05$.

The distribution of sphenoid sinus septation and Onodi cell according to gender was shown in Table 2. In males, 27 of the cases (10.8%) had intersphenoid, 220 (88.0%) had multiple septa, while 3 (1.2%) had no septa. In females, intersphenoid septa were in 17 (6.8%) cases, multiple septa in 231 (92.4%), and 2 (0.8%) had no septa. The frequency of Onodi cells in males and females was 156 (62.4%) and 171 (68.4%), respectively, and the absence of Onodi cells in genders was 94 (37.6%) and 79 (31.6%), respectively. There was no significant difference between the septation or Onodi cells with gender ($P > 0.05$).

The presence of Onodi cells was observed only with multiple septa (Table 3). The cases without Onodi were found together with multiple septa in 24.8% and with intersphenoid septa in 8.8%. The rate of cases with the absence of both Onodi cells and septa together was 1%. When the sphenoid sinus septation and Onodi cell were compared, a statistically significant relationship was observed ($P < .001$).

DISCUSSION

In this study, sphenoid sinus septation and Onodi cells were investigated according to mean age and gender on CBCT images and the relationship between these parameters was evaluated. In the literature, few studies have been found investigating sphenoid sinus septation and Onodi cells in CBCT,[13-15] but to the best of our knowledge, there is no published study assessing the relationship between these parameters. In this study, a statistically significant relationship was found between sphenoid sinus septation and Onodi cells. Multiple septa were observed in all

Table 2. Distribution of Sphenoid Sinus Septation and Onodi Cell According to Gender

		Male N (%)	Female N (%)	*P*
Sphenoid sinus septation	Intersphenoid	27 (10.8)	17 (6.8)	.254
	Multiple	220 (88.0)	231 (92.4)	
	Absent	3 (1.2)	2 (0.8)	
Onodi cell	Present	156 (62.4)	171 (68.4)	.158
	Absent	94 (37.6)	79 (31.6)	

Table 3. Relationship Between Sphenoid Sinus Septation and Onodi Cell

		Sphenoid Sinus Septation				
		Intersphenoid N (%)	Multiple N (%)	Absent N (%)	Total N (%)	*P*
Onodi cell	Present	0 (0)	327 (65.4)	0 (0)	327 (65.4)	.000*
	Absent	44 (8.8)	124 (24.8)	5 (1.0)	173 (34.6)	
Total N (%)		44 (8.8)	451 (90.2)	5 (1.0)	500 (100)	

*$P < .05$.

cases with Onodi cells. The most common sphenoid septation type was multiple (90.2%). Onodi cells were present in 65.4% of the cases.

When the sphenoid sinuses are well developed, structures such as the vidian (pterygoid) canal, foramen rotundum (maxillary nerve), optic nerve, and internal carotid artery can be defined by their indentations in the sinus cavity. These anatomical associations can be a potential surgical risk factor because fracture or removal of any sphenoid septa or indentations can cause injuries to surrounding nerves or vessels. The location and number of the sphenoid sinus septations are highly changeable and they usually attach to the osseous canal of the internal carotid artery and/or optic nerve, which extends into the posterolateral sphenoid sinus.[4,5]

The sphenoid and posterior ethmoid cells are also interrelated. The posterior ethmoid cells invading the sphenoid in the posterior and superior direction will cause contact of these cells with the optic nerve.[5] Also, this posterior ethmoid cell, called the Onodi cell, shares a bony wall with the sphenoid sinus.[4] Therefore, it should not be passed to more posterior part of the sphenoid sinus which has become an important landmark in ethmoidectomies.[5]

Sphenoid sinuses usually have an intersphenoid septum.[12] In most of the studies evaluating septa, the incidence of single/main intersphenoid septum was found to be quite high. Idowu et al[16] found this rate as 95%, Hamid et al[12] 71.6%, ELKammash et al[17] 70%, Rahmati et al[18] 69.8%, Anusha et al[19] 53.7%, and Kayalioglu et al[20] detected it as 46% in their study with bony specimens. Although this rate was seen as 38%,[21] 29.6%,[22] 28.1%,[23] and 20%[24] in some studies, the incidence in our study was 8.8% and it was much lower than in others. When the intersphenoid sinus septation was evaluated according to gender, Kapur et al[25] found that this parameter was 68% and 77.9% in males and females, respectively, and Akgül et al[26] detected this rate as 48.1% and 43.7%, respectively. In the present study, intersphenoid sinus septa were found as 10.8% in males and 6.8% in females.

The prevalence of multiple sphenoid sinus septa has been observed at around 50% in some studies,[19,22,27] and in some[12,17,28] at lower rates. This prevalence has been reported by Sareen et al[24] as 80% in their research on cadavers, Jaworek-Troć et al[29] as 78.04%, Seddighi et al[23] as 71.9%. In our research, this value was found to be 90.2%, consistent with other studies. When multiple septa were evaluated according to gender, Kapur et al[25] found this as 32% and 22.1% in males and females, respectively, and Akgül et al[26] found it as 51.9% and 56.3%, respectively. In this study, higher rates were detected to be 88.0% and 92.4%, respectively, compared to other studies.

Considering the studies with the absence of septa, the frequency in the control group in Yalçın's[22] study was 14.8%, 10.8% in the study of Hamid et al,[12] 7.5% in the study of Ngubane et al[28] 4% in the study of Wiebracht and Zimmer.[30] In the CT study of Sirikci et al,[31] in the anatomical and endoscopic study of Tan and Ong[3] on cadavers, and in the research of Seddighi et al[23] on pituitary adenomas on CT, there was no case without septa. Similarly, in the current study, the absence of septa was observed in only 1% of the cases.

In investigations with CT, the incidence of Onodi cells was reported by Wada et al[32] as 50.8% in the study group with a mean age of 55.6, Chmielik and Chmielik[33] as 39.8% in their study between the ages of 8 and 18, Hwang et al[34] as 32% in the study among persons aged 19-76 years, Ozturan et al[35] reported it as 16.6% in their study between the ages of 13 and 91. In the present study, the mean age was 47.34 ± 15.32 years and its incidence was higher (65.4%) than the others, and a significant relationship was detected between the mean age and Onodi cells. It was observed that the mean age of the patients with Onodi cells was higher than those without Onodi cells.

When the Onodi cells were assessed according to gender, Tomovic et al[36] on CT found this parameter as 62.2% and 63.5% in males and females, respectively, and Thanaviratananich et al[37] in the endoscopic study on cadavers detected this rate as 58.7% and 63.2%, respectively. Özdemir et al[38] on CT observed this parameter as 24.5% and 17.6% in males and females, respectively, and Avsever et al[15] on CBCT found this rate as 4.3% and 5.1%, respectively. In our study, Onodi cells were observed as 62.4% in males and 68.4% in females.

In the study of Driben et al[10] with CT and subsequent endoscopic dissection on adult human cadavers, the prevalence of Onodi cells detected was 7% and 39%, respectively, and significant difference was found between analyses. Weinberger et al[11] observed Onodi cells with a frequency of 8% in CT analysis and 14% in cadaver samples. In the CT study on different ethnicity of Hindi et al,[39] the relationship between the racial group and the incidence of Onodi cells was significant and they reported the incidence in the Chinese group higher than the others. Özdemir

et al[38] found the prevalence of Onodi cells on CT as 21.2% in the Turkish population. Tomovic et al,[36] in the study with different ethnic groups, found no statistically significant difference between the groups, and they detected Onodi cells in 57.0% of the African American population, in 62.7% of the Hispanic population, and in 73.1% of the White population. In the current study, consistent with these data, the incidence of Onodi cells was observed to be 65.4%.

Knowing the anatomical relationships of the sphenoid sinus is important because it can help explain the unusual symptoms caused by sphenoid sinus disease, as well as avoid surgical complications.[5] Cone beam computed tomography scanning of the paranasal sinuses provides useful information in evaluating the extent of the Onodi cell and detailed anatomy before the endoscopic sinus surgery.[40] Cone-beam computed tomography is an imaging method suitable for use in evaluating the bone structure of the head region and paranasal sinus anatomy and is relatively low-dose compared to CT.[7-9] Therefore, in our study, CBCT was used in the evaluation of sphenoid sinuses.

It has been reported that a horizontal septum crossing the sphenoid sinus lumen in coronal or sagittal plane images indicates the presence of Onodi cells.[5,6] This means that there is at least 1 septum in the sphenoid sinuses where Onodi cells are detected. In the present study, a statistically significant relationship was found between sphenoid sinus septation and Onodi cells. The fact that only multiple septations were found in cases that detected Onodi cells showed there was also another septa in addition to the horizontal septum. In accordance with the existing literature, in our study, if the sphenoid sinus septa were absent, Onodi cells were also absent. It can be thought that this relationship is due to the anatomical proximity of septa and Onodi cells and the variations during the development of these structures.

The difference between these results we obtained and the results of other studies may be due to reasons such as ethnicity, the study group consisting of living individuals/cadavers/dry bones, different sample numbers, or age groups. It may also be due to differences between study groups and imaging methods and using different classifications in the evaluation of sphenoid sinus septation.

The limitation of this study was that the medical history or systemic diseases of the cases were not known as the study was retrospective. Therefore, studies that are prospective or involve a particular pathological condition affecting the paranasal sinuses may be planned in the future. In addition, by increasing the number of samples, studies can be conducted in different populations and according to age groups.

CONCLUSION

In this study, the distribution of sphenoid sinus septation and Onodi cells according to mean age and gender was investigated, and a significant relationship was found between these parameters. Onodi cell was observed only with multiple septa. It is important to know these structures and their relationship with each other to define the variations in the surrounding neurovascular structures, planning and performing various sinus surgeries correctly, and avoid complications. Cone-beam computed tomography is an effective imaging method in the evaluation of sphenoid sinus anatomy and its variations and surrounding anatomical structures.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of Gaziantep University (Decision No: 2020/245).

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Author Contributions: Concept – E.D.Y.; Design – E.D.Y., C.B.; Supervision – E.D.Y.; Data Collection and/or Processing – C.B.; Analysis and/or Interpretation – E.D.Y., C.B.; Literature Search – E.D.Y., C.B.; Writing – E.D.Y., C.B.; Critical Reviews – E.D.Y., C.B.

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Original Article

The Importance of Ultrasound-Guided Manual Compression in Iatrogenic Pseudoaneurysm Treatment: The Sooner the Better

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ABSTRACT
Objective: Although ultrasound-guided manual compression is a safe and cost-effective method in the treatment of pseudoaneurysm, there are many factors affecting the success of the procedure. This study aimed to determine the factors affecting the success of ultrasound-guided manual compression.
Methods: The records of patients who developed iatrogenic femoral artery pseudoaneurysm in the cardiology department between 2017 and 2020 were retrospectively analyzed. Data regarding patients, procedural and aneurysm-related factors were evaluated by univariate and multivariate logistic regression analysis.
Results: Seventy-five patients who underwent ultrasound-guided manual compression were included in our study. In study population, the rate of successful ultrasound-guided manual compression is 72%. As a result of the univariate analysis, hypertension, diabetes mellitus, sheath size, the length of aneurysm sac, the width of aneurysm sac, compression duration, aneurysm detection time> 24 hours parameters were found to be significant predictors for failed ultrasound-guided manual compression. In the multivariate analysis, it was found that the independent predictors associated with failed ultrasound-guided manual compression were the aneurysm detection time longer than 24 hours and the length of aneurysm sac (odds ratio: 5.908; 95% CI 1.136-30.720; $P = .035$ and odds ratio: 1.042; 95% CI 1.008-1.100; $P = .045$). In receiver operating characteristic (ROC) curve analysis, the length of the pseudoaneurysm sac of 34 mm and above had 90% sensitivity and 57% specificity for failed ultrasound-guided manual compression.
Conclusions: Ultrasound-guided manual compression is an effective method in the treatment of pseudoaneurysm. However, early detection and size of pseudoaneurysm are important for the success of this treatment. Checking the operation site within the first 24 hours after catheterization is important for early diagnosis and treatment of possible complications. In addition, it should be considered that treatment success is low in length of pseudoaneurysm sacs longer than 34 mm.
Keywords: Compression, pseudoaneurysm, ultrasound

INTRODUCTION

Pseudoaneurysms are structures in which the integrity of the artery wall is impaired, where the outer wall of the aneurysmal sac is limited by perivascular tissue, blood clot, or a reactive fibrous tissue. They usually occur due to vascular trauma, infection, and iatrogenic reasons. Iatrogenic pseudoaneurysm is an important complication seen after percutaneous endovascular interventions performed for diagnostic or therapeutic purposes.[1,2]

Historically, surgical treatment was suggested for the first time for the treatment of pseudoaneurysms.[3] However, after surgical procedures, prolongation of hospital stay, increase in cost, and increase in morbidity and mortality have led to the search for less invasive methods.[4-6] Since 1991, the ultrasound-guided manual compression (UGMC) method has been used in the treatment of pseudoaneurysm.[7-11] Although the success rate of UGMC has been reported to be high in the literature, failure rates of 5%-30% have been reported in several case series.[7,12,13,14] Failed UGMC has been attributed to factors such as emergency procedure, interventional procedure, use of anticoagulants, aneurysm diameter, neck diameter, and track length.[12,15,16]

In the present study, we aimed to determine the factors affecting the efficiency and the success of the UGMC method in the treatment of iatrogenic femoral artery pseudoaneurysm (IFAP).

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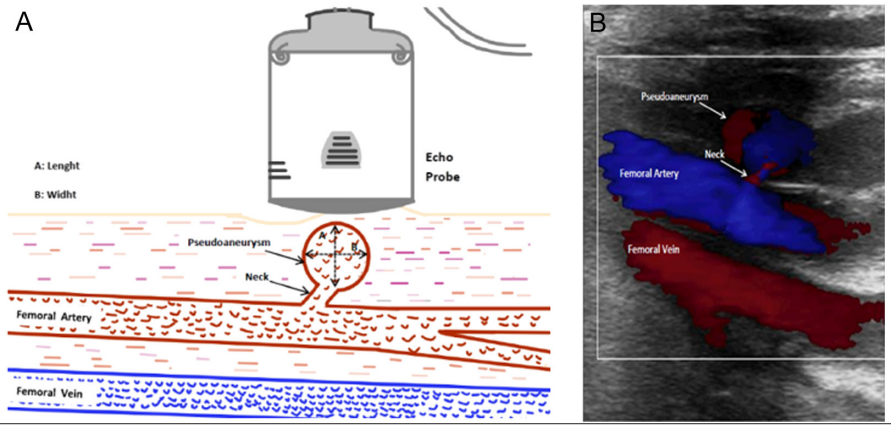
Figure 1. (A) Schematic image of PSA. (B) Ultrasound image of a PSA directly adjacent to the common femoral artery via a definable neck. Duplex scan demonstrates flow within the PSA and the femoral artery. PSA, pseudoaneurysm.



METHODS

The records of patients who developed IFAP after cardiac catheterization and who underwent UGMC in the cardiology clinic of our hospital between 2017 and 2020 were retrospectively analyzed. Procedures other than the femoral artery entry site were excluded. Patients were divided into 2 groups according to whether UGMC was successful or not. Demographic characteristics and comorbidities of the patients were analyzed. The intervened artery, sheath size, diagnostic/interventional procedure, emergency/elective procedure, catheterization procedure duration, pre- and post-catheterization anticoagulant and antiaggregant treatment use, and time from catheterization to the detection of pseudoaneurysm (pseudoaneurysm age) were recorded from the catheter laboratory reports. Approval was obtained from the local ethics committee for the study.

Cardiac catheterization was performed using a 6f or 7f sheath through the common femoral artery. While antiaggregant or anticoagulant therapy was not used in diagnostic procedures, dual antiplatelet therapy and intravenous (iv) heparin (70-100 u/kg) were used in emergency interventional procedures. After the diagnostic catheterization procedures, sheath was removed immediately after the procedure, and direct manual compression was applied for 15 minutes. Sheath was withdrawn approximately 4 hours after the interventional procedures and direct manual compression was applied for 15 minutes.

Main Points

- Latrogenic pseudoaneurysm is an important complication seen after percutaneous endovascular interventions performed for diagnostic or therapeutic purposes
- Since 1991, ultrasound-guided manual compression (UGMC), which is an effective and safe treatment method, has been used in the treatment of pseudoaneurysm that develops following interventional procedures.
- The aneurysm detection time longer than 24 hours and the length of the aneurysm sac were found as independent predictors associated with failed UGMC.
- Checking the intervention site within the first 24 hours is very important for the early diagnosis of a possible pseudoaneurysm and for the success of the UGMC treatment.

The diagnosis of pseudoaneurysm was confirmed by color Doppler ultrasound test performed in patients with pain, swelling in the groin after catheterization, and systolic murmur and pulsatile mass on physical examination. The identification of a pseudoaneurysm was confirmed by the classical triad of ultrasound findings including a hypoechoic sac in the vicinity of the parent vessel, a swirling high resistance flow on Doppler ultrasound within this mass, and a to and fro type waveform in the neck or in the sac close to the neck (Figure 1A and B). In all patients, aneurysm size, number of aneurysmal sacs, presence of arteriovenous fistula, and presence of partial thrombosis were recorded before the UGMC procedure.

Compression procedure under ultrasonographic guidance was performed by a cardiologist by determining the neck of the aneurysm with superficial ultrasound and applying pressure to this area. Three cycles of compression were applied to patients with aneurysm neck length over 8 mm with a US transducer (3.5-7 Mhz) for 15-20 minutes. Analgesic treatment (iv 1-2 mg midazolam hydrochloride) was administered to patients who felt pain during the procedure. The compression procedure was continued until the blood flow to the aneurysm sac was stopped while the blood flow continued in the main femoral artery. The blood flow to the sac was evaluated after a 10-minute break between each cycle. Compression tape was applied to the patients for 12 hours after the procedure. Successful treatment after the procedure was defined as the cessation of blood flow in the false lumen and the continuation of blood flow in the common femoral artery. Each patient who underwent successful compression underwent ultrasonography (USG) control 24 hours after the procedure. Patients whose aneurysm pouch filling could not be completely prevented were accepted as failed compression. Results were expressed using mean values and standard deviation.

Ethical committee approval was received from Antalya Education and Research Hospital Ethics Committee (Date: March 24, 2021, Decision no: 1/29, Protocol no: 2021/15).

Statistical Analysis

All statistical analyses (sensitivity, specificity, negative predictive value, and positive predictive value) were performed using MedCalc Statistical Software version v19.4.1 (MedCalc Software, Ostend, Belgium) and Statistical Package for the Social Sciences 25.0 (Armonk, NY: IBM SPSS Corp.). The data of the patients are expressed as median (quartiles) for distributed data and percentage for categorical variables. Shapiro Wilk test was used if the continuous variables were normally distributed. Student's *t*-test was used for parameters with normal distribution and Mann–Whitney U-test was used for parameters with non-normal distribution. Chi square test or Fisher's exact test was used in the analysis of categorical variables. Whether the width and length of the pseudoaneurysm sac measurements were effective in distinguishing successful compression patients from failed compression patients was evaluated by calculating the area under the ROC curve, and significant predictors were determined by binary logistic regression. $P < .05$ was considered statistically significant.

RESULTS

Seventy-five patients who received UGMC treatment for IFAP were divided into 2 groups, 54 (72%) as successful compression and 21 (28%) as failed compression. Some demographic characteristics of the patients such as age (65.5 ± 13.5 and 63.1 ± 10.0), male gender (50% and 42.9%), smoking, hyperlipidemia, chronic renal failure, and peripheral artery disease were found to be similar in both groups, whereas diabetes mellitus and hypertension differed significantly in the failed UGMC group (Table 1).

Comparing blood parameters such as international normalized ratio, hemoglobin, and platelets between the groups, no significant difference was found. In addition, there was no statistically significant difference between the use of antiaggregant and anticoagulant drugs in both groups.

Procedure- and aneurysm-related risk characteristics of the groups are presented in Table 2. There was no significant difference in both groups in terms of diagnostic/therapeutic procedure, emergency/elective procedure, type of procedure, and duration of the procedure that led to IFAP development. However, the use of large-scale sheath was found to be higher in the failed UGMC group (*P*:.048).

While the diameter of the pseudoaneurysm sac length (30.0 mm (20.0-45.2) and 47.0 mm (38.5-69.0) $P < .001$) and the width of the pseudoaneurysm sac (18.0 mm (12.0-30.0) and 30.0 mm (17.5-49.0) *P* .007) were found to be significantly larger; the pseudoaneurysm sac's being unilobuled/multilobuled or the presence of partial thrombosis in the sac were found to be similar in both groups.

The time until the detection of pseudoaneurysm was longer in the failed UGMC group (24.0 hours (21.0-48.7) and 35.0 hours (27.0-69.0) *P*: 0.011); especially pseudoaneurysms detected after the first 24 hours were found to be statistically significant for failed UGMC ($P < .001$). However, the UGMC duration (37.5 minutes (34.5-48.0) and 50.0 minutes (40.0-57.5)) was also found to be longer in the failed UGMC group.

Comparing the groups, the length and width of the pseudoaneurysm sac (30.0 mm (20.0-45.2) and 47.0 mm (38.5-69.0) $P < .001$; 18.0 mm (12.0-30.0) 30.0 mm (17.5-49.0) *P*: .007) in the failed UGMC group were found to be statistically significantly large.

As a result of the univariate regression analysis, parameters such as hypertension, diabetes mellitus, sheath size, the length of the aneurysm sac, the width of the aneurysm sac, compression duration, aneurysm detection time >24 hours were found to be significant predictors for failed UGMC ($P = .036$, $P = .014$,

Table 1. Demographic Characteristics, Clinical Features, and Laboratory Findings of Patients

Variables	Successful UGMC Group (n = 54)	Failed UGMC Group (n = 21)	*P*
Age, years	65.5 ± 13.5	63.1 ± 10.0	.345
Male, % (n)	50.0 (27)	42.9 (9)	.578
HT, % (n)	53.7 (29)	81.0 (17)	.030
DM, % (n)	22.2 (12)	52.4 (11)	.011
Smoking, % (n)	22.2 (12)	38.1 (8)	.163
Hyperlipidemia, % (n)	61.1 (33)	71.4 (15)	.403
CAD, % (n)	68.5 (37)	90.5 (19)	.050
PAD*, % (n)	9.3 (5)	14.3 (3)	.396
CRF*, % (n)	9.3 (5)	4.8 (1)	1.000
Antiaggregant therapy*, % (n)			
–None	5.6 (3)	9.5 (2)	0.507
–Single therapy	20.4 (11)	9.5 (2)	
–Dual therapy	74.1 (40)	81.0 (17)	
Anticoagulant therapy*, % (n)			
–None	77.8 (42)	81.0 (17)	0.440
–LMWH therapy	14.8 (8)	9.5 (2)	
–VKA therapy	5.6 (3)	0.0 (0)	
–NOAC therapy	1.9 (1)	9.5 (2)	
INR	1.06 (1.00–1.18)	1.09 (1.06–1.13)	0.200
Hemoglobin, g/dL	11.8 (10.6–14.1)	11.9 (9.8–12.8)	0.265
Platelet count, ($\times 10^3$ per µL)	209.0 (179.0–268.0)	204.5 (190.0–229.2)	0.166

*Fisher's exact test.
HT, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; PAD, peripheral artery disease; CRF, chronic renal failure; LMWH, low–molecular–weight heparin; VKA, vitamin K antagonist; NOAC, non–vitamin K antagonist oral anticoagulant; INR, International normalized ratio.

Table 2. Procedural and Aneurysmal Risk Characteristics of Patients

Variables	Successful UGMC Group	Failed UGMC Group	*P*
Procedure, % (n)			
Emergency procedure	22.2 (12)	28.6 (6)	.563
Elective procedure	77.8 (42)	71.4 (15)	
Intervention, % (n)			
Diagnostic	37.0 (20)	38.1 (8)	.932
Therapeutic	63.0 (34)	61.9 (13)	
Type of procedure*, % (n)			
CAG	90.7 (49)	90.5 (19)	.712
EPS	3.7 (2)	9.5 (2)	
TAVI	1.9 (1)	0.0 (0)	
PAG	3,7 (2)	0.0 (0)	
Duration of procedure (minutes)	17.5 (10.0–23.2)	19.0 (14.0–28.5)	
Sheat size*, % (F)			
6f	96.3 (52)	81. (17)	.048
7f	3.7 (2)	19.0 (4)	
Aneurysm sac diameter (length) (mm)	30.0 (20.0–45.2)	47.0 (38.5–69.0)	<.001
Aneurysm sac diameter (width) (mm)	18.0 (12.0–30.0)	30.0 (17.5–49.0)	.007
Unilobule/multilobule sac*, % (n)			
Unilobule	83.3 (45)	85.7 (18)	1.000
Multilobule	16.7 (9)	14.3 (3)	
Partial thrombosis of pseudoaneurysm, % (n)			
No	64.8 (35)	57.1 (12)	.600
Yes	35.2 (19)	42.9 (9)	
Duration of compression (minute)	37.5 (34.5–48.0)	50.0 (40.0–57.5)	.002
Time lag before diagnosis (hour)	24.0 (21.0–48.7)	35.0 (27.0–69.0)	.011
Time lag before diagnosis of aneurysm			
≤ 24 hours (n = 34)	57.4 (31)	14.3 (3)	<.001
>24 hours (n = 41)	42.6 (23)	85.7 (18)	

* Fisher's exact test.
CAG, coronary angiography; EPS, electrophysiological study; TAVI, transcatheter aortic valve implantation; PAG, peripheral angiography.

P = .047, *P* = .001, *P* = .029, *P* = .012, *P* = .002, respectively). In the multivariate logistic regression analysis performed with these parameters, it was found that the independent predictors associated with failed UGMC were the aneurysm detection time longer than 24 hours and the length of the aneurysm sac (OR: 5.908; 95% CI 1.136-30.720; *P* = .035, and OR: 1.042; 95% CI 1.008-1.100; *P* = .045) (Table 3).

In ROC curve analysis, the length diameter of the pseudoaneurysm sac of 34 mm and above had 90% sensitivity and 57% specificity for failed UGMC (Figure 2).

Major complications associated with UGMC such as rupture, distal embolization, skin necrosis, or neuropathy were not detected in our patients. However, transient hypotension developed during compression in 2 patients, and vagal reaction developed in 1 patient as a result compression was interrupted in these patients. Compression was continued after the patients were stabilized.

DISCUSSION

Ultrasound-guided manual compression, first described by Fellmeth et al.[7] in 1991, is a safe and cost-effective method; however, its success rate varies between 57% and 99% in various case series.[9,12,17] In our clinic, our success rate after UGMC was 72%, which was consistent with the studies in the literature. In studies where the success rate was higher, the presence of partial thrombosis after compression was considered successful compression, whereas pseudoaneurysms with large hematoma were considered not suitable for compression and were excluded from the study.[7,18] Partial thrombosis was not used as a criterion for success in our study, while the presence of a large hematoma was not considered a contraindication for UGMC.

Other studies in the literature report that anticoagulant and antiplatelet therapy cause higher failure and recurrence rates.[15-19] Although IFAP was detected more in those receiving single or dual antiplatelet therapy in our study (93%), taking antiplatelet or anticoagulant therapy was not found to be a predictor of failed UGMC. This may be related to the discontinuation of anticoagulant treatment before the procedure or the low number of patients in our study group.

The need for long compression times may also be associated with failed UGMC. While the average compression time in the literature is approximately 44 minutes, in some situations, there are cases with compression time up to 300 minutes.[18] There are also studies reporting that long compression times do not correlate with the success of UGMC. Eisenberg et al[15] explained this by the failure to perform effective compression for more than 1 hour due to patient comfort and operator fatigue. Although compression time was found to be longer in the failed UGMC group in our study, it was not found to be an independent predictor for failed UGMC in multivariate analysis. In our study, a statistically significant difference may not have been found between the compression time and failed UGMC due to the inability to continue compression for a long time due to patient and operator comfort.

Different results were obtained in studies investigating the relationship between pseudoaneurysm age and UGMC success. Shatnawi et al.[20] reported an association between aneurysm age >48 hours after catheterization and the rate of failed UGMC. Similarly, the aneurysm detection time was found to be longer

Table 3. Univariate and Multivariate Analysis of Factors Associated with Ultrasound-Guided Manual Compression Failure

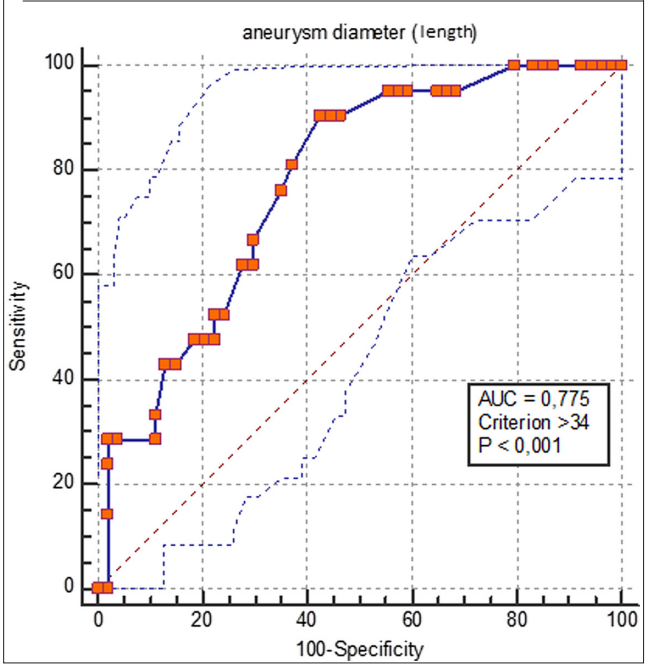
	Univariate		Multivariate	
Variables	**OR (95% CI)**	***P***	**Adjusted OR (95% CI)**	***P***
Age (years)	0.985 (0.946-1.026)	.464		
HT	3.664 (1.089-12.329)	.036	2.858 (0.529-15.445)	.223
DM	3.850 (1.321-11.224)	.014	2.188 (0.527-9.094)	.281
CAD	4.365 (0.912-20.899)	.065	6.344 (0.849-47.389)	.072
Sheath size (6f, 7f)	6.118 (1.028-36.405)	.047	5.204 (0.187-144.772)	.331
Aneurysm sac diameter (length)(mm)	1.058 (1.024-1.094)	.001	1.042 (1.008-1.100)	.045
Aneurysm sac diameter (width) (mm)	1.029 (1.003-1.056)	.029	1.029 (0.982-1.078)	.227
Compression time (minutes)	1.043 (1.009-1.078)	.012	1.032 (0.981-1.086)	.226
Time lag before diagnosis of aneurysm >24 hour	8.087 (2.126-30.760)	.002	5.908 (1.136-30.720)	.035

HT, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; OR, odds ratio.

in the failed UGMC group in our study, and aneurysm detection time of >24 hours was determined as an independent predictor of failed UGMC in the multivariate analysis. This situation may be explained by the fact that the pseudointima layer that develops in the aneurysm sac over time and endothelialization prevents thrombosis, which is necessary for successful compression.[15] Therefore, checking the intervention site within the first 24 hours is very important for the early diagnosis of a possible pseudoaneurysm and for the success of the UGMC treatment to be applied.

While there are publications showing that the size of the pseudoaneurysm sac is unrelated to failed UGMC,[20] many studies found that the size of the pseudoaneurysm sac was the limiting factor affecting the success of UGMC.[15,21,22] Our study has demonstrated that there is an inverse relationship between pseudoaneurysm size and compression success. This result may be explained by the fact that the flow through larger pseudoaneurysms tends to be greater than the flow in smaller pseudoaneurysms and therefore it is more difficult to stop it completely. In our study, it was also found that the diameter of the pseudoaneurysm was larger and that the success of UGMC was lower in patients with pseudoaneurysm after >24 hours. This result once again reveals the importance of early diagnosis in the treatment of pseudoaneurysm.

Figure 2. Receiver operating characteristic curves showing the AUC for aneurysm diameter (length). AUC, area under curve.



Limitations

The main limitations of our study were that it is a single-center study, that the operators performing the procedure had varying length of experience, and the body mass index of many patients were not recorded.

CONCLUSION

Ultrasound-guided manual compression is an effective and safe treatment method in the treatment of pseudoaneurysm that develops following interventional procedures. However, for this treatment to be successful, it is important to detect the pseudoaneurysm before 24 hours. Checking the intervention site within the first 24 hours after catheterization may increase the success of UGMC treatment by detecting possible pseudoaneurysm before it becomes too large. In addition, since the size of the pseudoaneurysm sac length also affects the success of UGMC, it should be considered that treatment success is low, especially in the length of pseudoaneurysm sacs longer than 34 mm.

Ethics Committee Approval: Ethical committee approval was received from the Antalya Education and Research Hospital Ethics Committee (Date: March 24, 2021, Decision no: 1/29, Protocol no: 2021/15).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.K, N.B. ; Design – Ş.A., G.Ç.; Supervision – Ş.S., G.Ç.; ; Materials – R.G., G.K.; Data Collection and/or Processing – E.C.O., R.G.; Analysis and/or Interpretation – R.G., E.C.O.; Literature Review – G.K., G.Ç.; Writing – G.K., N.B.; Critical Review – G.Ç., Ş.A.

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Original Article

Investigation of DEL22 Frequency with Fluorescent In Situ Hybridization Method in Children with Conotruncal Heart Anomaly

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ABSTRACT

Objective: Conotruncal heart defects represent 10%-15% of congenital heart diseases and mostly include tetralogy of Fallot, pulmonary atresia with ventricular septal defect, truncus arteriosus, and interrupted aortic arch.

Methods: This study aimed to investigate the prevalence of 22q11.2 deletion (Del22) with fluorescent in situ hybridization analysis among children followed with conotruncal heart defects. In 104 cases with conotruncal heart defects, the 22q11.2 region was screened for deletion through the fluorescent in situ hybridization analysis using a probe specific to this region.

Results: The fluorescent in situ hybridization analysis performed in patients with conotruncal heart defects showed that Del22 was present in 3 cases in Group I (70 pts) with isolated cardiopathy (4.3%), 2 cases in Group II (29 pts) with cardiopathy + dysmorphism (6.9%), 2 cases in Group III (2 pts) with cardiopathy + immunodeficiency + dysmorphism (100%), and 1 case in Group IV (3 pts) with cardiopathy + immunodeficiency (33.3%) ($P < .05$). Eight (7.5%) of 104 patients with conotruncal heart defects were found to have Del22.

Conclusions: The results obtained from the present study are compatible with the literature. The clinical manifestation of Del22 is extremely variable. With additional abnormalities such as immunodeficiency and dysmorphic features, Del22 positivity was increasing statistically.

Keywords: Conotruncal heart defects, Del22, FISH, 22q11.2

INTRODUCTION

Conotruncal heart defects (CTHD) include various defects, such as tetralogy of Fallot (TOF), tricuspid atresia, double outlet right ventricle, and transposition of the great arteries. These defects represent 5%-10% of congenital heart diseases and are required to be corrected in the neonatal or childhood period since they often cause severe cyanosis. Congenital aortic arch malformations occur in approximately 80% of all patients with Del22, suggesting that Del22 is an important risk factor for aortic anomalies.[1-3]

Del22 is a chromosomal disorder that causes congenital defects. The most common clinical manifestations are cardiac defects, palate disorders, dysmorphic face, growth disorders, and immunosuppression. The prognosis of Del22 is variable. The wide spectrum of the phenotypes of the syndrome has been previously divided into different sections (DiGeorge syndrome, velocardiofacial syndrome, cardiofacial syndrome, etc.); however, they are known to be etiologically similar and called 22q11.2 deletion or Del22 syndrome.[1-5]

METHODS

Patient Group

A total of 104 cases, who were followed in our hospital's Pediatric Cardiology Outpatient Clinic for CTHD, were divided into 4 groups according to the presence of dysmorphic features and immune system disorders.

Patients with CTHD were included in the study. Metaphase chromosomes obtained from the peripheral blood of the patients were investigated by the fluorescent in situ hybridization (FISH) method using the Del22 probe. This study was approved by the Ethics Committee of the Gaziantep University with number 341/2017.

Fluorescent In Situ Hybridization Analysis

For the FISH analysis, standard metaphase preparations of peripheral blood lymphocytes were used. Genomic DNA was extracted from peripheral blood following standard protocols. It is the 2-Mb commonly deleted region called DiGeorge Critical Zone and is seen in 90% of patients.[6-8] The critical region of a minimum

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of 300-480 kb containing several genes, including TUPLE1, TBX1, SLC25A1, and CLTD, was described within the region. The Del22 screening was performed via FISH method using TUPLE1 probe (Kreatech Diagnostics, Amsterdam, Netherlands) and P250-A1 kit (MRC-Holland, Amsterdam, Netherlands) in accordance with the manufacturer's instructions. The P250 kit consists of 30 different probes targeting the 22q11 region and can be used to detect common and atypical types of deletion.

Measurements and Parameters

Patients diagnosed with CTHD by imaging methods and parents of the younger patients were informed about the subject of the study. A 2-cc blood sample was collected to analyze with the FISH method, technical details of which were given earlier.

Evaluation of the Data

Statistical analysis was performed using MedCalc version 19.6.4 software. Demographic characteristics of the participants were expressed as frequency and percentage. The one-tailed chi-square test (χ^2) was used for detecting Del22 prevalence between groups. A *P* value of <.05 was considered statistically significant.

RESULTS

A total of 104 patients, ages ranging from 10 days to 18 years, were included in the study. The mean age of the patients was 6.3 ± 5.1 years (range 10 days-19 years). The diagnosis of CTHD was made by the pediatric cardiologist on the basis of echocardiography, cardiac catheterization, and cardiac computed tomography or cardiac magnetic resonance results (Table 1). All cases included in the study were evaluated for Del22 using FISH method. Of the cases, 39 were female and 65 (62.5%) were male. The cases, whose peripheral blood samples were studied, were divided into 4 groups according to the cardiac findings, dysmorphic features, and immune system disorders accompanying Del22. Group I, cases with congenital cardiopathy alone; group II, cases with cardiopathy + dysmorphic findings; group III, cases with cardiopathy + dysmorphic findings + immunodeficiency; and group, cases with cardiopathy + immunodeficiency. The classification of patients according to the phenotypic findings is presented in Table 2.

As seen in Table 2, the largest group in the study was group I, the isolated cardiopathy group with 70 cases, which was followed by group II, cardiopathy + dysmorphism group with 29 cases, group III, cardiopathy + dysmorphism + immunodeficiency group with 2 cases, and group IV, cardiopathy + immunodeficiency group with 3 cases. The FISH analysis performed showed that Del22 was present in 3 cases in group I, 2 cases in group II and III, 1 case in group IV. The ratio of male patients was higher in all groups.

Main Points

- Conotruncal heart disorders carry risk of increased 22q11.2 deletion (Del22) frequency.
- Increased Del22 frequency is more prominent with increased multisystem involvement such as conotruncal heart defect, dysmorphic face, and immune disorders.
- 22q11.2 deletion problem is not rare; therefore, it should be considered for every single patient.

Table 1. Conotruncal Heart Disease Type and Number of Patients

Conotruncal Heart Diseases	Number of Cases
Tetralogy of Fallot	85
Isolated tetralogy of Fallot	(55)
With right aortic arch	(17)
With pulmonary atresia	(13)
Double outlet right ventricle	6
Tricuspid atresia	3
Isolated perimembranous ventricular septal defect	3
Others*	8

*Taussig Bing anomaly, atrioventricular septal defect, dilated ascending aorta, right aortic arch, patent ductus arterious, ventricular septal defect, major aortopulmonary collateral arteries, hypoplastic left heart syndrome.

The classification of patients undergoing Del22 analysis according to the type of cardiopathy and accompanying findings and the rate of Del22 detection are given in Table 3. In the group consisting of 67.4% cases with cardiopathy alone, the most common cardiopathies were TOF. Del22 positivity with FISH analysis were 4.3%, 6.9%, 100%, and 33.3% in groups ($P = .029$, χ^2).

The characteristics of 8 cases with Del22 are given in Table 4. Four (50%) of these 8 patients with cardiac defects had TOF, 1 (12.5%) had TOF + right aortic arch, 1 (12.5%) had perimembranous ventricular septal defect, 1 (12.5%) had TOF + pulmonary atresia, and 1 (12.5%) had major aortopulmonary collaterals. A total of 75% of patients with Del22 were found to have TOF. Four cases were accompanied by immunodeficiency and 3 were accompanied by dysmorphic findings.

DISCUSSION

Del22 is a chromosomal disorder that causes congenital defects. The most common clinical manifestations are cardiac defects, palate disorders, dysmorphic face, growth disorders, and

Table 2. Classification of the Cases, Whose Del22 Analysis Was Performed, According to the Phenotypic Findings and Detection Rates of Del22

	Total Number (Female/Male)	*Del22 + Number
Group I	70 (67.4%) (28–42)	3 (4.3%)
Group II	29 (27.9%) (10–19)	2 (6.9%)
Group III	2 (1.9%) (0–2)	2 (100%)
Group IV	3 (2.8%) (1–2)	1 (33.3%)
Total	104 (39–65)	8 (7.5%)

Group I, cardiopathy alone; group II, cardiopathy + dysmorphic findings; group III, cardiopathy + dysmorphic findings + immunodeficiency; and group IV, cardiopathy + immunodeficiency.
*$P = .0029$, chi-squared test between groups.

Table 3. Detection of Del22 According to the Type of Cardiopathy and Accompanying Findings in Patients for Whom Del22 Analysis Was Performed

	Group I		Group II		Group III		Group IV	
	n	Del22+	n	Del22+	n	Del22+	n	Del22+
Tetralogy of Fallot	45	1	5		1	2	2	1
Tetralogy of Fallot with right aortic arch	10	1	7					
Tetralogy of Fallot with pulmonary atresia	4		9	1	1			
Double outlet right ventricle	3		3					
Isolated perimembranous ventricle septal defect			2	1			1	
Truncus arteriosus	3							
Others*	5	1	3					
Total	70		29		2		3	

Group I, cardiopathy alone; group II, cardiopathy + dysmorphic findings; group III, congenital cardiopathy + dysmorphic findings + immunodeficiency; and group IV, cardiopathy + immunodeficiency.
*Taussig Bing anomaly, atrioventricular septal defect, dilated ascending aorta, right aortic arch, patent ductus arterious, ventricular septal defect, major aortopulmonary collateral arteries, hypoplastic left heart syndrome.

immunosuppression. The prognosis of Del22 is variable. Palatal abnormalities causing hypernasal speech, feeding, and swallowing difficulties can be seen in more than 75% of the cases. Most patients are admitted to the hospital with mild dysmorphic facial and vertebral defects. Immunodeficiency is observed in the majority of cases due to thymic aplasia or hypoplasia increasing susceptibility to viral infections. Furthermore, these individuals are more likely to develop autoimmune diseases such as idiopathic thrombocytopenic purpura and juvenile idiopathic arthritis. Neonatal hypocalcemia is seen in half of the patients. It often recovers spontaneously but can reappear in a certain period of life or after several conditions such as an infection, surgery, and pregnancy. Other clinical features include gastrointestinal disorders, hearing loss, renal and dental defects, learning disorders, and psychiatric problems. The wide spectrum of the phenotypes of the syndrome has been previously divided into different sections (DiGeorge syndrome, velocardiofacial syndrome, cardiofacial syndrome, Shprintzen syndrome, conotruncal cardiac anomaly, and CATCH 22); however, they are known to be etiologically similar and called 22q11.2 deletion syndrome or shortly Del22 at present.[1-5]

The FISH method is the most frequently used method to screen syndromes with common pathogenesis that occur due to loss of genetic material in the eleventh region of the long arm of chromosome 22.[5] The loss of genetic material due to deletion and haploinsufficiency increases the variety of clinical findings and causes different phenotypes in generations in the same family.[5-6] In 93% of the cases, the deletion emerges "de novo" or may be inherited in autosomal dominant manner in 6%-25% of the patients.[5]

Goldmuntz[7] reported that the patients with Del22 had cardiac anomalies and most of these defects were CTHD or aortic arch anomalies. Among these, TOF and interruptic aortic arch are the most frequent ones, similar to our study. The prevalence of Del22 in children with CTHD was reported to be 30%.[2] On this basis, Del22 examination is recommended in the routine clinical practice in all rare conotruncal anomalies such as interruptic aortic arch and truncus arteriosus. The age of diagnosis of the syndrome varies from center to center.[2,7]

Alikaşifoğlu et al[8] conducted a study involving 32 patients with clinical features of TOF, truncus arteriosus, outlet ventricular septal defect, transposition, pulmonary atresia, vascular anomalies, and facial dysmorphism and reported the rate of deletion as 6.3%. In a study by Giray et al.,[9] involving 36 patients with congenital heart defects, the rate of patients with Del22 was found

Table 4. Findings of Eight Cases with Del22

	Cardiopathy	Dysmorphic Findings	Immunodeficiency
1	Tetralogy of Fallot	Hypertelorism	Yes
2	Tetralogy of Fallot	Hypertelorism, low-set ears, small mouth	Yes
3	Tetralogy of Fallot	No	Yes
4	Tetralogy of Fallot	No	No
5	Tetralogy of Fallot with right aortic arch	No	No
6	Tetralogy of Fallot with pulmonary atresia	Hypertelorism, small mouth	No
7	Perimembranous ventricle septal defect	No	Yes
8	Major aortopulmonary collaterals	No	No

to be 19.4% with the FISH method. This rate constitutes 14.2% of patients with isolated CTHD and 30.4% of all patients with CTHD.[9]

In a study by Halder et al.[10] in which 146 patients with congenital heart defects requiring surgical treatment were examined with FISH method for Del22 frequency, typical clinical features of Del22 were detected in 87 cases and 59 cases were observed to have isolated heart defects. The authors further detected hemizygous Del22 in 9 (6.16%) of 146 patients whereas no Del22 was found in patients with isolated heart malformation.

Beauchesne et al[11] investigated the frequency of DiGeorge syndrome in 103 patients by FISH analysis and detected TOF in 77 patients, pulmonary atresia in 23 patients, and truncus arteriosus in 3 patients. Six patients (5.8%) were found to have a Del22, similar to our results.

The FISH technique has many advantages over classical cytogenetic techniques in detecting Del22. It is more precise than traditional karyotyping or using high-resolution banding techniques and requires less effort than karyotyping, DNA dosage analysis, or restriction fragment length polymorphism. The FISH technique has been further reported to be useful in prenatal diagnosis using amniotic fluid cells.[12]

The frequency of cardiac malformations in patients with DiGeorge syndrome ranges from 49% to 83%. The CTHDs are the most commonly seen heart diseases, suggesting that the incidence of TOF in these patients may be high. In the study by Fomin et al.[13] the rate of TOF was found to be 50%. In the present study, 83 of the patients with CTHD had TOF. Del22 should be investigated in all patients with heart malformations such as TOF, interruptic aortic arch, septal defects, and truncus arteriosus.

In our study, the results were found to be similar to the literature. An important point in our study is that with added abnormalities such as immunodeficiency and dysmorphic features (e.g., group III), Del22 positivity was increasing. But in every groups had different rate Del22 patients. Such as some cardiac malformations for example the right-sided aortic arch, are not prominent cardiac anomalies and have no pronounced clinical symptoms. Therefore, diagnosis can only be made when genetic testing is performed.[14]

Population-based screening is required to determine the true incidence and prevalence of this syndrome. However, it may be highly expensive to screen a large population, and it is unlikely as it will be ethically questionable. Therefore, it would be more acceptable to screen certain risk groups. Tobias et al.[15] suggested several guidelines to facilitate the early detection of Del22. Several criteria have been recommended by the International Primary Immunodeficiency Diseases Classification Committee to help diagnose DiGeorge syndrome. These criteria mainly refer to immune defects and include the most common clinical features such as immunodeficiency, hypoparathyroidism, CTHD, facial abnormalities, and 22q11.2.[16] Increased awareness of Del22 syndrome, diagnostic guidelines, and a long follow-up period are the most important factors in diagnosing the disease.

CONCLUSION

In conclusion, the clinical presentation of Del22 is highly variable. It is obvious that the probability of being able to diagnose the disease increases if the test is administered to patients with at least one other symptom of the syndrome in addition to the conotruncal anomaly. However, this can prevent the diagnosis of some isolated cases.

Clinical follow-up of the patients positive for Del22 must be carried out through multidisciplinary teamwork.[7,15] Risks such as hypocalcemia, immunity, vascular anomalies must be taken into consideration before surgical operations. Necessary changes in diet and vaccinations must be taken into consideration. Treatment of infections should not be delayed since some patients are more likely to develop recurrent infections due to immunodeficiency. Furthermore, motor, behavior, and speech developmental processes must be followed closely. Improved life quality for the patients can be only possible with the success of well-organized teamwork.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Gaziantep University (Decision No: 341/2017).

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

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