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Research

Transcatheter Aortic Valve Implantation: A Tertiary Center Experience

Transkateter Aortik Kapak İmplantasyonu: Üçüncül Merkez Deneyimi

Fuad Fatullayev, Ersan Oflar, Cennet Yıldız, İbrahim Faruk Aktürk

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ABSTRACT

Objective: Transcatheter aortic valve implantation (TAVI), which is an actual treatment method for advanced aortic stenosis (AS) in high-risk patients, has been applied to many patients in our clinic. This study aimed to show whether the mortality and morbidity results of the TAVI procedure performed in our clinic were similar to those in the literature.

Methods: Patients who underwent TAVI for severe AS between April 2012 and February 2019 were evaluated. Society of Thoracic Surgeons scores were calculated. Biochemical parameters before and after the procedure and change in echocardiographic parameters after the procedure were determined.

Results: The mean age of the patients was 78 (62-89) years and 56.82% (n=25) were female. The average mean gradient of the patients before and after the procedure were 42 (20-72) and 10 (0-26) mmHg, respectively (p<0.001). In-hospital mortality rate was 9.09% (n=4), peri-procedural mortality rate was 15.9% (n=7). There was only one record of death in TAVI procedures performed after 2015. The Edwards Sapien valve was implanted in 50% (n=22) of patients, while Portico, Corevalve and Evolute valves were implanted in 25% (n=11), 18.18% (n=8) and 6.81% (n=3) of patients, respectively. Thirty (68.18%) patients had TAVI implantation without balloon pre-dilatation. There was no difference in peri-procedural mortality between patients who had and had not balloon pre-dilatation (n=2, 6.7%, and n=2, 14.3%; p=0.581, respectively). Two patients showed moderate aortic regurgitation after valve implantation. Eight patients had stage 1 acute kidney failure (AKF), whereas one patient had stage 2 AKF. One patient required permanent pacemaker implantation because of complete heart block.

Conclusion: When the studies in the literature were examined, the relatively high mortality rates were observed when TAVI procedure was first used. The number of deaths in our clinic has decreased significantly over the years, in accordance with the literature.

Keywords: Aortic stenosis, implantation, mean gradient

ÖZ

Amaç: Cerrahi açıdan yüksek riskli hastalarda ileri aort darlığı (AD) için geliştirilen güncel bir tedavi yöntemi olan transkateter aort kapak implantasyonu (TAVI) işlemi kliniğimizde de birçok hastaya uygulanmıştır. Bu çalışmanın amacı kliniğimizde uygulanan TAVI işleminin mortalite ve morbidite sonuclarının literatürdeki çalışmalar ile benzer olup olmadığının gösterilmesidir.

Gereç ve Yöntem: Çalışma kapsamında Nisan 2012-Şubat 2019 tarihleri arasında ileri AD sebebi ile TAVI uygulanan hastalar incelendi. Göğüs Cerrahları Dernek skorları hesaplandı. İşlem öncesi ve sonrası biyokimyasal parametreleri, ekokardiyografi sonuçlarının işlem sonrası nasıl değiştiği belirlendi.

Bulgular: Hastaların ortalama yaşı 78 (62-89) yıl idi ve %56,82'si (n=25) kadındı. Hastaların işlem öncesi ve sonrası ortalama gradiyenti sırası ile 42 (20-72) ve 10 (0-26) mmHg idi (p<0,001). Hastane içi ve periprosedurel mortalite oranı %9,09 olarak bulundu. Hastaların %50'sine (n=22) Edwards Sapien marka kapak, %25'ine (n=11) Portico, %18,18'ine (n=8) Corevalve, %6,81'ine ise (n=3) Evolute marka kapak takıldı. Otuz hastaya (%68,18) balon predilatasyonu uygulanmadı. Balon uygulanan ve uygulanmayan hastalarda periprosedurel mortalite açısından fark saptanmadı (sırası ile, n=2, %6,7 ve n=2, %14,3; p=0,581). İki hastada işlem sonrası orta derecede aort yetersizliği gelişti. Sekiz hastada evre 1 akut renal hasar, bir hastada ise evre 2 akut renal hasar gelişti. Bir hastaya tam kalp bloğu nedeni ile kalıcı pacemaker implante edildi.

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Sonuç: Literatürde yer alan çalışmalara bakıldığında TAVI işlemi ilk kullanılmaya başlandığında göreceli olarak yüksek mortalite oranları görülmüştür. Ancak ölüm sayısı literatür ile uyumlu şekilde yıllar içinde belirgin azalma göstermiştir.

Anahtar Kelimeler: Aort darlığı, implantasyon, ortalama gradient

INTRODUCTION

Calcific degeneration of the aortic valve is complicit in the pathogenesis of aortic stenosis (AS). It affects persons over the age of 75 years with an estimated prevalence of 3.4% (1). Classically, longstanding increase in pressure overload leads to left ventricular hypertrophy and ultimately heart failure. These patients are generally older and have comorbidities that can worsen their outcomes. If left untreated, symptomatic AS has an annual mortality rate of up to 25% (2,3).

Although surgical aortic valve replacement (SAVR) is the mainstay of treatment, transcatheter aortic valve implantation (TAVI) has become a novel treatment modality for the management of high risk patients with AS. First TAVI operation was performed in 2002, and in 2012, Food and Drug Administration approved TAVI for treating patients with AS who had a prohibitive risk of surgery. Clinical decision whether to perform TAVI or SAVR depends on various factors including patients' comorbidities, operator's experience and prosthesis factors. TAVI has been a non-inferior alternative of SAVR in extreme high-risk patient population; furthermore, it was found to be superior compared to medical treatment (4-6). Vascular-neurological complications and post-procedural aortic regurgitation (AR) are the most frequently reported complications in TAVI patients (4). After its promising results in high-risk patients, its performance in intermediate and low-risk patients have been evaluated. Nordic Aortic Valve Intervention trial assessed CoreValve transcatheter heart valve with SAVR using a sample of 280 patients with intermediate to low-risk profile. Primary endpoints, including myocardial infarction, stroke and mortality, were found to be similar in both groups. Acute kidney injury (AKI), bleeding was higher in SAVR, whereas paravalvular leakage and pacemaker implantation were higher in the transcatheter heart valve arm (7). A large-scale trial, Placement of Aortic Transcatheter Valves (PARTNER) IIA, compared balloon-expandable SAPIEN XT valve with SAVR in intermediate risk group patients. The primary end-point was similar in both treatment groups at two years follow-up. In this study transfemoral approach was superior to the surgical treatment (8). Furthermore, recent studies conducted in low risk patients with AS showed that TAVI was a non-inferior alternative to surgical treatment (9,10). Based on the results of clinical studies, current European valvular guideline advocates TAVI as class I indication for patients with AS who are older than 75 years have Society of Thoracic Surgeons Predicted Risk of Mortality (STS-PROM)/score EuroSCORE II greater than 8 or unsuitable for surgery (11). For the patients who are under 75 years of age and have a low surgical risk, SAVR is recommended. The remaining patients should be assessed according to their clinical and procedural features, including valve durability and life expectancy of the patient.

In our country, the number of TAVI procedures has increased since it was first carried out in 2009 (12). Although clinical trials and registries performed on patients with AS provide real world data in terms of procedural success, complications, short-and mid-term outcomes, patient selection and operator experience differ from center to center (13-15). It is well known that operator experience and center size have a positive impact on the outcome of patients (16). Therefore, obtaining homogenous data that are drawn from a single population may be important owing to non-uniform data collected from different centers. In this context, we evaluated whether mortality and morbidity results of TAVI procedure performed in our hospital were similar to those in the literature.

METHODS

This retrospective study included 44 patients who underwent TAVI procedure between April 2012-February 2019 in the cardiology department of a tertiary hospital in Türkiye. Patients' data, including age, comorbidities, presence of atrial fibrillation, beta blocker use, biochemical variables, pre and post-procedural systolic and diastolic blood pressure, electrocardiogram, implanted valve type and size were obtained from hospital records.

Echocardiography, Multislice Computed Tomography and Coronary Angiography

All the patients underwent echocardiographic examinations with the using of vivid 9 device, which had a sector transducer of 3.2 MHz (Horten, Norway). Echocardiographic assessments complied with the current guidelines (17). The diagnosis of severe AS was diagnosed if the patient had an aortic valve area of less than 1 cm^2, mean transaortic gradient of more than 40 mmHg, mean transaortic jet velocity of more than 4 m/s, or indexed aortic valve area of 0.6 cm^2/m^2. Pre-procedural and post-procedural aortic valvular gradient, presence and degree of AR, ejection fraction, left atrial, left ventricular diastolic and ascending aorta diameters

were assessed. Cardiac CT was performed for each patient to delineate the anatomical and functional properties of the aortic valve. Aortic root structural parameters such as perimeter, area and diameter; coronary hinge points, degree and distribution of aortic valve calcification, ascending aorta diameter were estimated. Coronary angiographic imaging of each patient was provided. If the patient had more than 70% stenosis in one of the major coronary arteries, that stenosis was classified as severe stenosis. For the left main coronary artery, severe stenosis was described as more than 50% occlusion of the artery.

STS scoring system was used for the operative risk assessment of the patients (18). Patients were high, intermediate and low risk if they had risk score of more than 8%, 4-8%, and less than 4%, respectively. The decision regarding performing SAVR or TAVI was made by heart team which included invasive cardiologist, cardiovascular imaging specialist, and a cardiovascular surgeon. If the patient had a high STS risk score and/or associated co-morbidities decision in favor of TAVI was made. If the patient had concomitant coronary artery disease, bicuspid aorta, moderate/severe AR, severe other valve disease, aortic annulus size less than 18 mm and/or more than 35 mm, the decision was made in favor of SAVR. Ethical Committee of University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital approved the study and it was directed in conformity with the declaration of Helsinki (decision no: 2019-05-12, date: 04.03.2019).

The suitability of the peripheral vasculature to the procedure was assessed by multi-slice computed tomography. The vascular acces route, tortuosity of the vessels, presence and extent of calcification in the peripheral arteries were determined. All procedures were done by transfemoral approach. Sheath sizes were adapted according to the valve size. The type of valve implanted was left to the operator's preference. Edwards Sapien, Portico, Corevalve and Evolute valves were implanted during the study period. All patients underwent temporary pacemaker implantation concomitant with TAVI operation under conscious sedation. At the end of the operation, percutaneous closure devices were used to provide femoral hemostasis. For assessing complications (mortality, neurological events, bleeding and renal complications) Valve Academic Research Consortium-3 was used (19). Periprocedural mortality was described as death within 30 days of valve implantation. In hospital and periprocedural mortality rates of the patients were calculated. Neurological events were classified as ischemic stroke, hemorrhagic stroke, or transient ischemic attack. AKI is classified into four stages. Stage 1 AKI was described as an increase in creatinine level 1.5-2 times the baseline within seven days or more than 0.3 mg/dL increase within 2 days after the procedure. Stage 2 AKI was described as 2-3 times increase in creatinine levels compared with baseline within 7 days after the procedure. Stage 3 AKI was described as an increase in serum creatinine levels 3 times of baseline within 7 days of procedure or serum creatinine level of more than 4 mg/dl with an increase of more than 0.5 mg/dL. Stage 4 AKI is described as temporary or permanent renal replacement therapy (19).

Statistical Analysis

Categorical variables were expressed as frequency and percentages. Normality testing of continuous variables was done by Kolmogorow-Smirnow test. Since all data showed a non-normal distribution, continuous variables were expressed as median, minimum and maximum. Comparison of pre-procedural and post-procedural variables was done by Kruskall-Wallis H test. Agreement between pre-procedural and post-procedural AR was evaluated by Fleiss Kappa analysis. Lastly, a comparison of STS scores of patients who deceased during the first 24 h of TAVI, deceased during indeterminate time from TAVI and living patients was made using Kruskal-Wallis H test. $P<0.05$ was considered as significant. All statistical analyses were done using Number Cruncher Statistical System, 2017 Statistical Software.

RESULTS

The average age of the study population was 78 (62-89) years, 19 of (43.18%) were male and 25 of (56.82%) were female, 29.55% were diabetic, 75% were hypertensive, 54.55% had ischemic heart disease. The demographic characteristics of the patients are given in Table 1. Mean STS score and mean-implanted valve size was 4 (2.49-11) and 26.5 (23-35) mm, respectively. Clinical characteristics are given in Table 2. Mean pre-procedural ejection fraction and aortic valve area were found to be 56.5 (20-65) and 0.8 (0.4-1.2) cm^2, respectively. Pre-procedural echocardiographic findings are given in Table 3. The Edwards Sapien valve was implanted in 50% (n=22) of patients, while Portico, Corevalve and Evolute valves were implanted in 25% (n=11), 18.18% (n=8) and 6.81% (n=3) of patients, respectively. Thirty (68.18%) patients had TAVI implantation without balloon pre-dilatation. We did not find any difference in peri-procedural mortality between patients who had and had not balloon pre-dilatation (p=0.581).

The mean aortic gradient decreased from 42 (20-72) mmHg to 10 (0-26) mmHg after TAVI procedure ($p<0.0001$) (Table 4). According to Fleiss Kappa analysis, there was no

significant agreement regard to the degree of AR before and after the procedure (Table 5). Four patients died or within 24 of the procedure. Two patients died from cardiac arrest and 2 patients died from ischemic stroke (in-hospital mortality=9.09%). Of the 4 deceased patients, three patients underwent TAVI procedure between 2012 and 2015, whereas one patient died in 2017. The mean STS score of the deceased patients was 5.63. Seven patients died within one month after the procedure (peri-procedural mortality n=7, 15.9%). Since we accessed deceased patients' information from government database system, we couldn't identify the cause of death. We found no differences with respect to STS scores between deceased and alive patients. Table 6 shows the in-hospital mortality of the study population.

Twenty-two patients had balloon expandable bioprostethic valve implantation. Of these patients 6 patients had mild, 4 patients had mild-to-moderate and only one patient had moderate AR before TAVI implantation. After valve implantation, only one patient who had moderate AR who mild AR before TAVI. No patient had severe AR after the procedure. Of 22 patients who underwent self-expandable valve implantation, 3 patient had mild, 6 patients had mild-to-moderate and 3 patients had moderate AR. After self-expandable valve implantation, only one patient had moderate AR, who had mild regurgitation before the procedure. No patient had severe regurgitation after self-expandable valve implantation.

Table 1. Demographic characteristics of the patients

	n	%
Male	19	43.18
Female	25	56.82
DM	13	29.55
HT	33	75
COPD	6	13.64
PCS	10	22.73
IHD	24	54.55
B-blocker use	24	54.55
AF	9	20.45

DM: Diabetes mellitus, HT: Hypertension, COPD: Chronic obstructive pulmonary disease, PCS: Previous cardiac surgery, IHD: Ischemic heart disease, AF: Atrial fibrillation

Table 2. Clinical characteristics of the patients

	Median (minimum-maximum)
Age (years)	78 (62-89)
Valve size (mm)	26.5 (23-35)
STS score	4 (2.49-11)
Hct (%)	32.65 (25.8-45.58)
Plt (×10^3/L)	207.5 (106-482)
MPV (fl)	9.17 (6.88-15.1)
Urea (mg/dL)	53.5 (22-158)
Uric acid (mg/dL)	6.47 (0-11.1)
TC (mg/dL)	192 (6.11-290)
LDL-C (mg/dL)	115 (50.8-226)
HDL-C (mg/dL)	49.5 (21-86)
TG (mg/dL)	101 (32-300)

STS: Society of Thoracic Surgeons, Plt: Platelelet, Hct: Hematocrit, MPV: Mean platelet volume, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride

Table 3. Echocardiographic parameters of the study population

	Median (minimum-maximum)
EF (%)	56.5 (20-65)
LA (mm)	41.5 (29-54)
IVS (mm)	13 (9-18)
PW (mm)	12 (8-14)
LVD (mm)	49 (35-70)
AVA (cm^2)	0.8 (0.4-1.2)
Pre-procedural maximum gradient (mmHg)	71.5 (38-150)
Pre-procedural mean gradient (mmHg)	41(20-72)
Ascending aortic diameter (mm)	35 (27-46)

EF: Ejection fraction, LA: Left atrium, IVS: Interventricular septum, PW: Posterior wall, LVD: Left ventricular end diastolic diameter, AVA: Aortic valve area

Table 4. Pre-procedural and post-procedural clinical variables of the patients

	Pre-procedural	Post-procedural
SBP	120 (100-163)	122.5 (83-172)
DBP	70 (54-90)	64 (42-91)
Hgb	10.7 (8.21-15.9)	10 (7.7-14.3)
WBC	7.11 (4.8-14.79)	10.15 (6.28-24.1)
Creatinine	1.13 (0.6-6.55)	1.21 (0.58-7.09)
Mean aortic gradient (p<0.0001)	42 (20-72)	10 (0-26)

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Hgb: Hemoglobin, WBC: White blood cell

Table 5. Comparison of pre and post-procedural aortic regurgitation

		Post-procedural aortic regurgitation					
		Absent (n,%)	Mild (n,%)	Mild-moderate (n,%)	Moderate (n,%)	Total (n,%)	p/Kappa
Pre-procedural aortic regurgitation	Absent	5 (38.46)	6 (46.15)	2 (15.18)	0 (0)	13 (100)	0.347/0.100
	Mild	2 (22.22)	4 (44.44)	1 (11.11)	2 (22.22)	9 (100)	
	Mild-moderate	2 (20.00)	8 (80.00)	0 (0)	0 (0)	10 (100)	
	Moderate	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)	
	Total	9 (25)	22 (61.11)	3 (8.33)	2 (5.56)	36 (100)	

Table 6. Peri-procedural mortality of the patients

	Death (n=16) (36.36%)	Alive (n=24) (54.55%)	In-hospital mortality (n=4) (9.09%)	p
STS score median (min-max)	4.46 (2.49-8.74)	3.54 (2.99-11.00)	4.19 (3.81-10.34)	0.558

STS: Society of Thoracic Surgeons, min: Minimum, max: Maximum

Eight patients had increase in creatinine level more than 0.3 mg/dL, indicating stage 1 AKF. Only one patient had more than 2 times increase in creatinine level compared with baseline (pre-procedural creatinine: 1.25 mg/dL versus post-procedural creatinine: 3.12 mg/dL). No patient required temporary or permanent renal replacement therapy. One patient required permanent pacemaker implantation because of complete heart block.

DISCUSSION

Since the first implantation in 2002, TAVI has become an established treatment for patients with severe AS who have a high or moderate surgical risk. When TAVI was first introduced to medical practice, its mortality rate was comparable to that of SAVR (6). Overtime, improvements in both operator experience and technical equipment have resulted in decreased procedural risk and mortality (9). Our in-hospital and periprocedural mortality rates were 9.09% and 15.9%, respectively and mean implanted valve size was 27.2 mm. The mean STS score of our study population was 4.99%; four patients died during or within 24 h after the procedure. Three patients died between 2012-2015 and one patient died in 2017, after that time no patient was lost during the hospital stay. There was no difference in STS score of deceased and alive patients.

After all data were collected, we performed a review of literature to compare our results with already published studies. Surgical Replacement and Transcatheter Aortic Valve Implantation trial randomized 1660 intermediate patients with AS to TAVI or surgical procedure. Mean age and STS score of the enrolled patients were 79.8 years 4.5%, respectively. After 24 months, composite death from any cause or disabling stroke was 12.6% for the TAVI arm and 14% for the surgical arm. Peri-procedural mortality of the study population was 2.2% (20). In the PARTNER IIA study, 1011 and 1021 severe patients with AS with intermediate surgical risk were assigned to TAVI and SAVR, respectively. The mean STS score was 5.8%. The composite end point did not differ between two groups of patients. The incidences of all-cause mortality and disabling stroke at two years were 19.3% and 21.1% for TAVI and surgical arm, respectively. This study did not specifically note the peri-procedural mortality rate (8). Thourani et al. (21) compared intermediate risk patients with AS who received the third generation SAPIEN 3 valve with the surgical arm of the PARTNER IIA trial. In that study, the mean STS score was 5.2±1.7%, technical success was attained in 98% of patients. One year mortality and stroke rate was significantly lower with the use of third generation valve (p=0.0038) (21). Similarly, PARTNER III trials, which has been performed on low risk patients with AS (STS score: 1.9%) revealed that TAVI was superior to SAVR in terms of composite death, stroke and rehospitalization (9). Duran Karaduman et al. (22) reported their experience with 556 patients. The mean STS score of the patients was 6.0%±3.5%. In the hospital and one-year mortality of the study group were 3.9% and 12.3%, respectively (22). Dağdelen et al. (23) reported early and mid-term results of ten high-risk patients with AS who underwent Edwards Sapien bioprosthetic valve implantation. During early and mid-term follow-up no patient died or had stroke (23). Results

of randomized trials and observational studies showed that peri-procedural mortality of TAVI ranges from 13.30 (Engager) to 0.69 (SAPIEN 3) (24). The mortality rate at our hospital has declined in accordance with the literature. Our in-hospital and periprocedural mortality rates were 9.09% and 15.9%, respectively. Of the 4 patients who died during the hospital stay, two of them died from cardiovascular arrest and the other two died of a cerebrovascular event. There has been only one in-hospital death after 2015. This reduction in mortality was greatly due to decrease in sheath size and peripheral vascular complications, increased operator experience, and the use of second generation valves. In our study mean STS score was relatively low (median=4, minimum=2.49, maximum=11). That could be attributed to a long period of study time. Our study period began in April 2012 and ended in February 2019. During the initial period of study time, patients with high STS scores were underwent TAVI procedure, however with the operator experience and expansion of indications, patients with intermediate and even low STS scores underwent the procedure with favorable clinical results. Besides, our heart team evaluated each patient and not only the STS scores of the patients were evaluated but also other comorbidities were considered.

We also estimated post-procedural AR. Two patients who had no AR pre-procedurally, developed mild-moderate AR after the procedure. Likewise, 2 patients with mild AR, developed moderate AR after the procedure. Of the patients who developed moderate AR, one patient underwent balloon expandable valve and the other patient underwent self-expandable valve implantation. Mild-moderate AR regressed to mild AR in 8 patients and moderate AR mild AR in 4 patients. The Edwards Sapien valve was the most commonly implanted valve in our study, in addition we did not find any difference with respect to peri-procedural mortality in patients who underwent balloon pre-dilatation compared to those who did not undergo balloon pre-dilatation.

CONCLUSION

In conclusion, our TAVI experience was increased over time and the mortality rate of our clinic was decreased significantly in accordance with the literature. Mean STS score of our study group was 4 indicating an intermediate risk group of patients. TAVI is a reliable alternative of SAVR in high and intermediate risk groups of patients, moreover, it could have a future potential in low-risk patients.

The major limitations of our study were as follows; the study had a small sample size, we did not know the exact cause of death during long-term follow-up and the incidence of TAVI related para-valvular AR could not exactly estimated.

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ETHICS

Ethics Committee Approval: Ethical Committee of University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital approved the study and it was directed in conformity with the declaration of Helsinki (decision no: 2019-05-12, date: 04.03.2019).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: F.F., E.O., C.Y., İ.F.A., Design: F.F., E.O., İ.F.A., Data Collection or Processing: F.F., E.O., İ.F.A., Analysis or Interpretation: F.F., E.O., C.Y., İ.F.A., Literature Search: F.F., E.O., C.Y., İ.F.A., Writing: C.Y.

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Research

Serum Zonulin Levels in Patients with Hashimoto's Thyroiditis

Hashimoto Tiroditli Hastalarda Zonulin Düzeylerinin İncelenmesi

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ABSTRACT

Objective: Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis, is the most common autoimmune thyroid disease in children and adolescents. The exact mechanism that triggers autoimmunity in Hashimoto's thyroiditis is not fully known. Many pathologies, including the disruption of the intestinal epithelial barrier that causes the production of an inappropriate immune response by the interaction of submucosal immune cells with the antigens, have been accused. Zonulin is a protein that modulates the permeability of tight junctions between intestinal mucosal cells. Abnormal up-regulation of Zonulin causes increased intestinal permeability and is central to the development of autoimmune diseases.

Methods: A total of 53 adult patients with newly diagnosed Hashimoto's thyroiditis, 70 adult patients with previously diagnosed Hashimoto's thyroiditis, receiving thyroid hormone replacement therapy and 62 healthy controls were enrolled in the study. Patients with other autoimmune or systemic disorders were excluded. Hemogram parameters, biochemical variables and serum zonulin levels of the participants were examined.

Results: There was no significant zonulin elevation in the newly diagnosed Hashimoto's thyroiditis patient group or in the previously diagnosed Hashimoto's thyroiditis patient group currently receiving replacement therapy compared to the control group. Zonulin levels, which have an important place in the intestinal permeability modulation, can be affected by environmental factors, so they should be carefully evaluated. A statistically significant correlation was observed between zonulin levels and anti-thyroid peroxidase (anti-TPO) antibody levels in patient groups included in our study. There is also a significant correlation between zonulin levels and age, body mass index and cholesterol values.

Conclusion: A correlation between anti-TPO levels, an indicator of autoimmunity, and serum zonulin levels suggests that this molecule plays a role in the pathogenesis of Hashimoto's thyroiditis. The results of this study should be supported by larger-scale studies with longer follow-up periods.

Keywords: Zonulin, Hashimoto's thyroiditis, autoimmunity

ÖZ

Amaç: Hashimoto tiroiditi, diğer adıyla kronik lenfositik tiroidit, çocuk ve adölesanlarda en sık görülen otoimmün tiroid hastalığıdır. Hashimoto tiroiditinde otoimmünitenin nasıl tetiklendiği tam olarak bilinmemektedir. Bağırsak epitel bariyerindeki bozukluk submukozal immün hücrelerin antijenlere maruz kalmasına ve uygun olmayan immün cevabın oluşmasına sebep olur. Zonulin proteinin anormal up-regülasyonu bağırsak geçirgenliğinde artışa sebep olarak otoimmün hastalıkların oluşumunda önemli bir rol oynar. Tip 1 diabetes mellitus, çölyak ve Hashimoto tiroiditi ile ilgili yapılan çalışmalarda bağırsak geçirgenliğinin ve zonulin up-regülasyonun arttığını gösteren çalışmalar mevcut olmakla birlikte bağırsak geçirgenliği ve zonulin proteini ile hashimoto tiroiditi arasındaki bağlantıyı gösteren çok az çalışma mevcuttur.

Gereç ve Yöntem: Çalışmaya 53 yeni tanı Hashimoto tiroiditli erişkin hasta, 70 tiroid hormon replasman tedavisi alan erişkin hasta ve 62 sağlıklı kontrol alındı. Hashimoto tiroidit tanılı hastalar diğer otoimmün ve sistemik hastalıklar, kontrol grubu da herhangi otoimmün ve sistemik hastalıklar açısından dikkatli bir anamnez ile incelenerek çalışmaya dahil edildi.

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Bulgular: Çalışmamızda yeni tanı ve tedavi alan Hashimoto hastalarında kontrol grubuna göre anlamlı zonulin yüksekliği görülmedi. Ancak zonulin up-regülasyonu etkileyen birçok çevresel faktör olması bu verinin dikkatli incelenmesi gerektiğini düşündürmektedir. Çalışmamıza dahil edilen hastalarda anti-tiroid peroksidaz (anti-TPO) ve zonulin düzeyleri arasında anlamlı bir korelasyon izlenmiştir. Bununla birlikte zonulin proteininin yaş, vücut kitle indeksi ve kolesterol düzeyleri ile etkilendiği sonucuna ulaşılmıştır.

Sonuç: Otoimmünitenin bir göstesgesi olan anti-TPO düzeyi ile serum zonulin düzeyleri arasında bir korelasyon izlenmesi, Hashimoto tiroiditinin patogenezinde bu molekülün rolü olabileceğini düşündürmektedir. Bu çalışmanın sonuçlarının daha geniş ölçekli ve daha uzun takip süreli çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: Zonulin, Hashimoto tiroiditi, otoimmünite

INTRODUCTION

Hashimoto's thyroiditis is the most common cause of hypothyroidism (HT) in adults in areas with adequate iodine intake (1). Inadequate production of thyroid hormones in Hashimoto's thyroiditis causes symptoms that affect the quality of life of the patients and long-term functional insufficiency of the thyroid gland poses a risk especially for cardiovascular diseases (2).

Hashimoto's thyroiditis is in the group of autoimmune diseases. Both cellular and humoral immunity play a key role in the development of this disease. The damage in the thyroid tissue is caused by complex mechanisms because of T-cell-mediated and antibody-dependent cellular cytotoxicity. It develops because of complement activation and/or antibody-dependent cellular cytotoxicity mediated by antibodies against thyroid peroxidase (anti-TPO) and thyroglobulin (anti-TG) due to the effect of cytotoxic T-cells or due to cytokines such as interferon-gamma and interleukin-1. Antibodies against antigens in the thyroid tissue are released from B-cells after activation of T helper cells. It is considered that environmental factors and genetic factors are effective in the etiology of autoimmune thyroiditis (3). Subclinical *Yersinia enterocolitica* infection in Türkiye has been shown to be associated with autoimmune thyroid disease (4).

Recently, it has been observed that the intestines, through the epithelial barrier, play a role in controlling the entry of environmental antigens into the body, in the digestion and absorption functions. (5). The tight junctions (TJs) in the intestine provide the balance between tolerance to and/or immune response against non-self antigens, particularly since they are responsible for the paracellular passage of macromolecules (6). It has been shown that the protein called zonulin reversibly regulates intestinal permeability by modulating TJs. Exposure to bacteria and exposure to gluten in the small intestine has been identified as factors that trigger zonulin release (5). In celiac disease, it has been shown that zonulin levels increase with exposure to gluten. And it's shown that after removal of gluten, zonulin levels decrease and the intestines begin performing normal barrier function and the autoimmune response ends (7). It has been shown that zonulin levels are increased by 50% in patients with type 1 diabetes mellitus (DM), and this is associated with increased intestinal permeability (8).

The relationship between autoimmune diseases such as type 1 DM and celiac disease and zonulin up-regulation suggests that zonulin may play a role in the pathogenesis of Hashimoto's thyroiditis. It has been shown by some studies that intestinal epithelial morphology is impaired and intestinal permeability is increased in Hashimoto's thyroiditis. Intestinal microbiota is thought to play a role in the pathogenesis of Hashimoto's thyroiditis, as in other autoimmune diseases. There are also clinical studies suggesting that up-regulation of zonulin protein, causing increased intestinal permeability, plays a role in the pathogenesis of Hashimoto's thyroiditis (9,10). Our aim was to determine zonulin levels in Hashimoto's thyroiditis and to determine whether there is a relationship between autoantibody and zonulin levels.

METHODS

Patients who were admitted to the Internal Medicine Outpatient Clinic of University of Health Sciences Türkiye, Bağcılar Training and Research Hospital between March 1, 2017 and December 31, 2017 were included in the study. The purpose of the study was explained to each patient included in the study and a consent form was signed.

The study consisted of three groups. The first group consists of patients who were previously diagnosed with Hashimoto's thyroiditis and are currently receiving thyroid hormone replacement and are being followed up. The second group consists of patients that are newly diagnosed with Hashimoto's thyroiditis and the third group consists of healthy individuals. Patients included in the study were included by discussing their demographic data and comorbidities with the patients and their relatives and by examining their records. Ethical approval was obtained from the University of Health Sciences Türkiye, Bağcılar Training and Research Hospital Local Ethics Committee. Ethics committee date and approval number: 08.02.2017 and 2017/543.

Hashimoto's thyroiditis was diagnosed with the help of medical anamnesis, physical examination findings, serum anti-TPO and/or anti-TG level, and ultrasonography findings. Patients without any diagnosis or signs/symptoms of autoimmune disease other than Hashimoto's thyroiditis were included in the study. All individuals in the control group were negative for thyroid autoantibodies. Physical examinations of the patients were performed by the same physician. Body mass indexes (BMIs) of the patients were calculated using the body weight/height^2 (kg/m^2) formula.

Blood samples were obtained from the antecubital vein after 10-12 hours of fasting. Blood samples were centrifuged at 3000 rpm for 10 min and stored at -80 degrees until analyzed. Hemogram analysis was studied in EDTA containing tubes using an automatic hemogram device (XE-5000; Sysmex Corp, Kobe, Japan). Biochemical parameters such as serum glucose, urea, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase (ALT), lactate dehydrogenase, calcium, sodium, potassium, chlorine, total protein, albumin, total cholesterol, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c), triglyceride were studied by photometric method on a Siemens Advia 1800 device (Siemens Healthcare Diagnostics, Kobe, Japan). The hormone parameters such as insulin, C-peptide, and thyroid-stimulating hormone (TSH) were studied in a Siemens Advia Centaur device (XE-5000, Sysmex Corp. Kobe, Japan) a chemiluminescence immunoassay method. Hemoglobin A1c test was studied using high-performance liquid chromatography method (ADAMS A1c HA-8180V Kyoto, JAPAN).

Serum zonulin concentrations were measured using the Abbkine, INC brand ELISA kit. Samples removed from -80 °C were taken first to -20 °C and then to + 4 °C, and serums were gradually dissolved. After adding 150 uL of Standard Diluent to each tube; 150 µL of the stock standard tube were dispensed by serial dilution. Equal distribution was ensured by pipetting before each stage. The 30X Wash solution was diluted 1X with distilled water and this solution was used in the washing steps.

Statistical Analysis

In this study, statistical analyzes were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In addition to descriptive statistical methods (mean, standard deviation) in the evaluation of the data, One-Way analysis of variance in the intergroup comparisons of normally distributed variables, independent t-test in the comparison of paired groups, Kruskal-Wallis test in the intergroup comparisons of non-normally distributed variables, Dunn's multiple comparison test in subgroup comparisons. Mann-Whitney U test was used for the comparison of paired groups, the chi-square test was used for comparison of qualitative data, Pearson correlation test was used to determine the relationships of variables with each other. The results were evaluated at a significance level of $p<0.05$.

RESULTS

The patients included in our study were grouped as follows. Fifty three of them are newly diagnosed Hashimoto's thyroiditis patients, 70 of them are previously diagnosed Hashimoto's thyroiditis patients and are receiving thyroid hormone replacement therapy, 62 of them are in the healthy control group.

The mean age of the patient group who were previously diagnosed with Hashimoto's thyroiditis and are currently receiving treatment was significantly higher than the other 2 groups. No significant difference was observed between the groups in terms of other demographic data, such as BMI and gender distribution (Table 1).

Alkaline phosphatase (ALP) level, calcium, total cholesterol and LDL-c levels were significantly higher in the previously diagnosed and currently being treated patient group than in the other 2 groups (p=0.034 p=0.0001 p=0.0001 p=0.003 respectively) (Table 2).

The mean vitamin D, ferritin, and vitamin B12 levels of the groups were similar. The mean vitamin D, ferritin, and vitamin B12 levels of the patients in the previously diagnosed and

Table 1. Comparison of demographic data of Hashimoto individuals newly diagnosed and treated with the control group

		Control group n=62		Newly diagnosed group n=53		Treatment group n=70		p*
Age		37.32±11.13		36.51±11.22		42.76±13.17		0.006
Gender	Male	11	17.74%	4	7.55%	5	7.14%	0.098
	Female	51	82.26%	49	92.45%	65	92.86%	
BMI		27.31±6.44		27.25±5.91		29.37±6.89		0.105

*One-Way analysis of variance + chi-square test, BMI: Body mass index

treatment receiving group were nonsignificantly higher than those in the newly diagnosed group (Table 3).

Significantly higher zonulin levels were observed in the control group compared with the other two groups (p=0.007) (Table 4). The mean zonulin levels were similar between patients with different TSH levels [0.3-4.5 TSH, 4.5-10 TSH, and >10 TSH groups (p=0.833) (Table 5)].

We determined a significant correlation between zonulin levels and age, BMI, LDL, triglyceride and anti-TPO (respectively p=0.001, p=0.024, p=0.041, p=0.016, p=0.045) (Table 6).

No significant correlation was observed between zonulin values and ALT, albumin, ALP, iron, iron binding capacity, transferrin saturation, glucose, cholesterol, HDL-c, creatinine, potassium, sodium, phosphorus, calcium, urea, vitamin D, ferritin, anti TG, free T4, TSH, vitamin B12, hemoglobin, leukocyte, platelet values (p>0.05).

DISCUSSION

The common point of autoimmune diseases is that several pre-existing conditions initiate the autoimmune process. The first is that the host immune system has a genetic predisposition to recognize and possibly misinterpret an environmental antigen present in the gastrointestinal tract. And the antigen must be presented to the gastrointestinal immune system by passing through the intestinal lumen, which is normally protected by TJ integrity, into the intestinal submucosa (6).

The intact intestinal epithelial barrier prevents both pathogenic and non-pathogenic bacteria from entering the immunoreactive submucosa. Damage to the mucosal

Table 2. Comparison of the biochemical data of the control group and the newly diagnosed and treated Hashimoto individuals

	Control group n=62	Newly diagnosed group n=53	Tretment group n=70	p*
ALP	62.6±17.08	63.57±21.09	70.23±16.86	0.034
Cholesterol	177.33±41.06	183.28±41.43	207.07±38.79	0.0001
LDL-c	98.72±35.58	98.39±38.49	125.29±34.87	0.0001
Calcium	9.34±0.34	9.12±0.41	9.34±0.42	0.003

*One-Way analysis of variance, ALP: Alkaline phosphatase, LDL-c: Low-density lipoprotein-cholesterol

Table 3. Comparison of vitamin D, ferritin, and vitamin B12 levels of the groups

	Control group n=62	Newly diagnosed group n=53	Treatment group n=70	p*
Vitamin D	20.41±8.56	18.32±9.55	22.59±12.81	0.088
Ferritin	44.06±40.78	31.27±30.2	40.94±45.37	0.361
Vitamin B12	351.28±137.46	329.76±152.76	353.58±126.07	0.596

*One-Way analysis of variance

Table 4. Comparison of zonulin levels of the groups

		Control group n=62	Newly diagnosed group n=53	Treatment group n=70	p*
Zonulin	Mean ± SD	584.25±787.90	293.25±537.03	236.13±392.82	0.007
	Median (IQR)	96 (71.25-1241.75)	72 (60.5-192.75)	78 (61-116)	

*Kruskal-Wallis test. SD: Standard deviation, IQR: Interquartile range

Table 5. Averages of zonulin levels according to TSH levels

		0.3-4.5 TSH n=106	4.5-10 TSH n=47	>10 TSH n=20	p*
Zonulin	Mean ± SD	410.47±675.72	304.40±487.46	344.55±537.31	0.833
	Median (IQR)	80 (64.5-276.5)	76 (62-241)	90 (66-491)	

*Kruskal-Wallis test. SD: Standard deviation, IQR: Interquartile range, TSH: Thyroid-stimulating hormone

Table 6. Correlation analysis of zonulin level and demographic, biochemical and hormonal parameters

		Zonulin
Age	R	0.284
	p*	0.001
BMI	R	0.172
	p*	0.024
LDL-c	R	0.156
	p*	0.041
Triglyceride	R	0.184
	p*	0.016
Anti-TPO	R	0.153
	p	0.045

*Pearson correlation test. BMI: Body mass index, LDL-c: Low-density lipoprotein-cholesterol, Anti-TPO: Anti-thyroid peroxidase

barrier results in the exposure of submucosal immune cells to bacteria and dietary antigens, that may result in inappropriate immune response formation. Increased intestinal permeability has been shown to be a common feature in the pathogenesis of most of the autoimmune diseases. Increased permeability precedes the onset of the disease, and abnormalities in antigen presentation cause a multi-organ response and initiate the autoimmune process. As a result, this leads to the development of different autoimmune diseases (11,12).

There is evidence that many environmental factors, including infections, may trigger Hashimoto's thyroiditis in genetically susceptible individuals. Non-pathological symbiotic microorganisms in the gut may also affect the extra-intestinal immune response. For this reason, intestinal dysbiosis (microbial disorder) may cause a loss of tolerance to specific antigens (including thyroglobulin) and autoimmunity in the pathogenesis of Hashimoto's thyroiditis. Increased intestinal permeability and intraepithelial lymphocyte infiltration are risk factors for thyroid autoimmunity. However, there are few studies showing a correlation between the gut and Hashimoto's thyroiditis (13).

Although the mechanisms by which zonulin protein is stimulated and how it is secreted into the intestinal lumen have not yet been fully explained, it is thought that it binds to its receptor on the intestinal epithelial cell and opens TJs transiently and reversibly.

The potential role of zonulin in the pathogenesis of Hashimoto's thyroiditis was observed by Özışık (10). They found that serum zonulin levels in the patient group were significantly higher compared to control groups. However, they failed to determine a significant correlation between serum TSH, anti-TPO and anti-TG concentrations and zonulin levels in the patient group (10). Similarly, in our study, although no correlation was found between TSH and anti-TG levels and zonulin, instead a correlation was observed between anti-TPO levels and zonulin.

Many studies have proven that HT is a cause of secondary hyperlipidemia. The improvement in the lipid profile after treatment with HT is proof of this. Therefore, HT is considered a risk factor for atherosclerotic heart diseases (14). In some studies, while thyroid replacement therapy caused a significant decrease in LDL and total cholesterol levels, it did not cause a significant change in triglyceride and HDL levels (15). O'Brien et al. (14) performed a study on 268 patients with primary HT and 27 patients with secondary HT, and they observed that there was a significant correlation between hyperlipidemia and both primary and secondary HT, and there was a significant improvement in hyperlipidemia after HT treatment (14). We also determined higher LDL and total cholesterol levels in patients with Hashimoto's thyroiditis without finding a significant correlation between triglyceride and HDL levels.

Some studies have shown that iron deficiency anemia has effects on thyroid metabolism. Iron deficiency anemia lowers plasma total thyroxine (T4) and total triiodothyronine (T3) concentrations and may increase serum TSH levels (16-18). In a study by Beard et al. (16) on mice, blood transfusion to mice with anemia resulted in improvements in T3 and T4 levels. In another study by Beard et al. (19) on mice, it was shown that thyroid hormone metabolism was affected in iron-deficient mice. In this study, we established higher ferritin levels in the control group, compared to with patients newly diagnosed Hashimoto's thyroiditis. This can be explained by the decrease in iron absorption in Hashimoto's thyroiditis, or it can be attributed to the negative effects of iron deficiency on thyroid hormone metabolism (anemia and decreased oxygen transport). However, higher vitamin D and B12 levels are found in the patient group that were previously diagnosed with Hashimoto's thyroiditis and are currently receiving thyroid replacement compared with newly diagnosed Hashimoto's thyroiditis patients.

Regarding the studies examining the effect of intestinal permeability on autoimmune disorders, authors showed that increased bowel permeability exists before the development of autoimmunity, as shown in celiac disease (20,21). Meddings et al.'s (7) study with diabetes-prone biobreeding (BB) rats; showed that intestinal permeability increases before the development of autoimmune diabetes. Although it is difficult to experiment these findings on

human-beings; we know that even after successful treatment in celiac patients, the defect in intestinal permeability continues (22).

Moreno-Navarrete et al. (23) showed that zonulin levels are higher in obesity in their study on 33 obese and 90 nonobese patients. Similarly, obese children have higher zonulin levels in the study by Küme et al. (24). Moreover, Ohlsson et al. (25) determined a correlation between serum zonulin levels and obesity and hyperlipidemia. We also observed a correlation between serum zonulin level and BMI, LDL and triglyceride levels.

Although there is limited available data on the relation of zonulin level and age, Zhang et al. (26) in polycystic ovary syndrome and Moreno-Navarrete et al. (23) in obesity found no significant relation between age and zonulin. Contradictorily, we found a negative correlation between zonulin levels and age. The reason for this may be the fact that autoimmune diseases are more common at younger ages and their frequency decreases with age.

An unidentified trigger that is not discovered yet is thought to be responsible for zonulin secretion. Some studies suggest that dietary proteins may be the possible triggers. For example, it has been shown that the incidence of type 1 DM is reduced in BB diabetes prone mice fed with hydrolyzed food (7). High-fat diet is associated with metabolic disorders by changing the intestinal microbiota and permeability (27). There are many factors affecting intestinal permeability and zonulin up-regulation like dietary habits, showing us that the results of the study should be interpreted with caution. Our study suggests that there may be a relationship between the high zonulin level in healthy individuals and the dietary habits of the participants.

In some studies, deterioration in the morphology of the intestinal epithelium, intraepithelial lymphocyte infiltration and increase in intestinal permeability have also been shown in Hashimoto's thyroiditis, similar to type 1 DM (9,28). However, in our study, serum zonulin level was not increased in patients with Hashimoto's thyroiditis compared with healthy individuals. However, measuring intestinal permeability *in vivo* and revealing its relationship with tissue zonulin level may enable us to obtain more realistic results.

In a study, blood was drawn from celiac patients and healthy individuals at different times of the day, and zonulin and antibody levels against zonulin were measured. Because of the study, it was observed that zonulin levels fluctuated during the day, but IgA and IgG antibody levels against zonulin were stable (29). Based on this study, due to the fluctuation in zonulin levels, with the zonulin level, measurement of IgA and IgG levels against zonulin is also recommended. The fact that this fluctuation may be responsible for the low zonulin levels in patients with Hashimoto's thyroiditis in our study is another matter of discussion.

This study has some potential drawbacks. A low sample size was the main limitation which is related to the exclusion of cases with metabolic, renal or rheumatologic comorbidities. The reason for this is the exclusion of patients with concomitant pathologies such as DM, hypertension, chronic kidney disease or rheumatological diseases. Secondly, histopathological examination of tissue zonulin level can more clearly reflect zonulin activity and its relationship with other parameters, although it is an invasive procedure. This study was conducted on the Turkish population, and the lack of data on the dietary habits of the participants, which may alter serum zonulin levels limits us to generalize our results to other populations.

CONCLUSION

In conclusion, the relationship between the zonulin protein, which is responsible for TJ regulation, and autoimmune diseases is a topic that is currently and increasingly being researched. In this study, we predicted that zonulin levels would be higher in the patient groups compared with the control group, but in the study, no relationship was found between zonulin levels and patients with Hashimoto thyroiditis.

New and differently designed studies will increase our knowledge and experience in this subject. In addition to the increasing number of animal studies, humans studies may provide valuable data on the role of intestinal permeability in the development of autoimmune disorders, and may guide us for developing preventive medical approaches in the future.

ETHICS

Ethics Committee Approval: Ethical approval was obtained from the Bağcılar Training and Research Hospital Local Ethics Committee. Ethics committee date and approval number: 08.02.2017 and 2017/543.

Informed Consent: The purpose of the study was explained to each patient included in the study and a consent form was signed.

Authorship Contributions

Surgical and Medical Practices: Z.S.İ., E.G., Concept: H.S., A.E.A., Design: H.S., A.E.A., Data Collection or Processing: Z.S.İ., S.Y., E.G., S.H.K., Analysis or Interpretation: Z.S.İ., S.H.K., Literature Search: Z.S.İ., S.Y., Writing: Z.S.İ., A.E.A.

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Research

Which is Superior in the Treatment of Femoral Shaft Fracture? A Comparison of Talon Intramedullary Nailing and Conventional Locked Intramedullary Nailing

Femur Cisim Kırığının Tedavisinde Hangisi Daha Üstündür? Talon İntramedüller Çivileme ile Konvansiyonel Kilitli İntramedüller Çivilemenin Karşılaştırılması

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ABSTRACT

Objective: This study compared the clinical and radiological outcomes of screw and talon locking systems, which are distal nail locking systems, in adult femoral shaft fractures treated with intramedullary nails.

Methods: The data of patients who received intramedullary nails were retrospectively analyzed. The patients were divided into two groups: patients treated with conventional intramedullary nails (group 1) and those with a talon distal locking system (group 2). Both groups were assessed according to age, sex, fracture side, type of implant, length of surgery, fluoroscopy shot number of intraoperative fluoroscopy, length of postoperative follow-up, time to union, smoking, AO classification of fractures, and presence of malunion and nonunion. Additionally, patients were compared using the Knee Society score and Harris Hip score as clinical functional scores.

Results: Among 102 study patients, were 21 (20.5%) females and 81 (79.5%) males, with a mean age of 39.34 (18-65) years. When the time to union, fluoroscopy shots number and length of surgery were compared between the groups, the results were statistically significant ($p<0.05$). There was no statistically significant difference in malunion and nonunion rates between the groups ($p>0.05$). Results of clinical scores were similar in both groups and there was no statistically significant difference ($p>0.05$). Additionally, smoking, age, sex and subtype of fracture did not have a statistically significant association with time to union, and malunion and nonunion rates ($p>0.05$).

Conclusion: The talon system is a reliable method that provides an advantage in terms of radiation exposure and length of surgery compared to the conventional locking system. The union time was observed to be longer in patients using the talon system compared with the conventional method, and a joint decision should be made by the surgeon and the patient on the treatment method, by informing the patients about the advantages and disadvantages of this system before the operation.

Keywords: Femur shaft fracture, talon femoral nail, conventional femoral nail, distal locking

ÖZ

Amaç: Bu çalışmadaki amaç tedavide intramedüller çivi kullanılan erişkin femur diyafiz kırıklarında çivi distalindeki kilitleme sistemleri olan vidalı ve talonlu kilitleme sistemlerinin klinik ve radyolojik sonuçlarını karşılaştırmaktır.

Gereç ve Yöntem: İntramedüller çivi kullanılan hastaların verileri retrospektif olarak incelenmiştir. Hastalar konvansiyonel intramedüller çivi yapılanlar (grup 1) ve talonlu distal kilitleme yapılanlar (grup 2) olarak iki gruba ayrılmıştır. Her iki grup yaş, cinsiyet, taraf, implant tipi, ameliyat süresi, intraoperatif skopi sayısı, postoperatif takip süresi, union sağlama süresi, sigara kullanımı, AO kırık sınıflaması, malunion ve nonunion varlığına göre değerlendirilmiştir. Ayrıca hastalar klinik fonksiyonel skorlamalar olan Knee Society skoru ve Harris Hip skorlaması ile kıyaslanmıştır.

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Bulgular: Çalışma kapsamında değerlendirilen 102 hastanın 21'i (%20,5) kadın 781'i (%79,5) erkek olup yaş ortalaması 39,34 (18-65) idi. Gruplar arasında kaynama zamanı, radyasyona maruziyet süresi ve ameliyat süresi kıyaslandığında sonuçlar istatistiksel olarak anlamlıydı (p<0,05). Gruplar arasında malunion, nonunion gelişimi arasındaki ilişki istatistiksel olarak anlamlı değildi (p>0,05). Gruplar arasındaki klinik skorlamaların sonuçları benzerdi ve istatistiksel olarak anlamlı fark yoktu (p>0,05). Ayrıca sigara içimi, yaş, cinsiyet, kırık alt tipi ile kaynama zamanı, malunion ve nonunion gelişimi arasındaki ilişki istatistiksel olarak anlamlı değildi (p>0,05).

Sonuç: Talonlu sistem konvansiyonel kilitleme sistemine göre radyasyona maruziyet ve ameliyat süresi açısından avantaj sağlayan güvenilir bir yöntemdir. Talonlu sistem kullanılan hastalarda kaynama zamanı konvansiyonel yönteme göre daha uzun olarak görülmüş olup, ameliyat öncesi hastaların bu sistemin avantaj ve dezavantajları hakkında bilgilendirilerek, cerrah ve hasta tarafından tedavi yöntemi konusunda ortak karar alınmalıdır.

Anahtar Kelimeler: Femur şaft kırığı, talonlu femur çivisi, geleneksel femur çivisi, distal kilitleme

INTRODUCTION

Femoral shaft fracture is a common condition in adults that is caused by high-energy trauma and represents 4.6% of all adult fractures (1). Intramedullary nailing is accepted and used as a gold standard for treating adult femoral shaft fractures by several trauma surgeons since it allows early limb movement with stable fracture reduction (2-5). Although intramedullary nails are the gold standard treatment, the reported rate of nonunion after intramedullary nailing ranges from 0.5% to 12.5% in the literature (6-9). This rate, of course, may change due to reasons such as the mechanism of injury, the location of the fracture, and the amount of soft tissue affected (10-12). Additionally, intramedullary nails have changed in design along with the development of today's technology. A quick, safe and practical distal locking mechanism is of importance both in terms of saving time in surgery and stabilization of fracture fixation. The talon distal locking mechanism, in turn, is a new locking system that has become popular recently. With this system, stabilization is achieved via distal talon locks that can be deployed through the intramedullary nail.

Our aim in this study was to compare the clinical and functional results of two different locking systems (talon distal locking and conventional distal locking) in patients with femoral shaft fractures treated with a femoral nail.

METHODS

The study included 102 patients who were treated and followed up for femoral shaft fractures between 2017 and 2020. The data of the patients were analyzed retrospectively, and the data were collected by the Declaration of Helsinki. Adult patients who were aged ≥18 years, who had an isolated femoral shaft fracture, who were treated with antegrade nailing, and who had accessible 12-month follow-up data were included in the study. Patients with pathological fractures, open fractures according to the Gustilo-Anderson classification, an additional injury or a fracture involving a different extremity, who were treated with retrograde nailing, aged ≥65 years, previously operated for the same fracture, pregnant, using immunosuppressive drugs, and patients with a body mass index of ≥35, and chronic renal failure were excluded. The patients were divided into two groups according to the implants used in the treatment. Additionally, no specific criteria were used to determine which implant design should be used in which patient. Patient and implant design choices were made randomized. Based on two different distal locking designs, patients who were treated with intramedullary nails (Zimed Medical Türkiye, ZFN Multi-Purpose Femoral Nail Systems) were assigned to group 1 (Figure 1), and those who were treated with Talon distal locked IMN (Zimed Medical Türkiye, ZFN-Talon Lock Femoral Nail Systems) to group 2 (Figure 2). Patients' age, sex, length of follow-up, mechanism of injury, type of fractures according to AO classification, smoking, length of surgery, the number of intraoperative fluoroscopy shots, time to the union after treatment, postoperative infections, malunion and nonunion were recorded and the statistical difference between the groups was examined. Functional outcomes of the study group patients were assessed by the

Figure 1. Intramedullary antegrade conventional locking nail preferred in group 1 patients

Figure 2. Femoral cannulated intramedullary talon distal fixation nail preferred in group 2 patients

Knee Society score and Harris Hip score. For all patients, radiographic data on months 1, 3, 6, 9, and 12 at follow-up were used. Union was considered to be achieved in patients with no pain on weight-bearing and with callus formation on three of four cortices on radiographs at the follow-up (Figure 3, 4). An angulation of >5 degrees, a shortness of more than 2 cm, and a rotational deformity of >15 degrees in any plane were considered malunion (13). Patients were also evaluated in terms of limb length difference and the distance between spina iliaca anterior superior and medial malleolus was considered. The study was approved by the Gaziantep University Clinical Research Ethics Committee (decision no: 2021/53, date: 24.02.2021).

Surgical Technique

All patients were taken into the operating room after preoperative preparation. The same surgical procedure was performed in both the groups of patients. For prophylaxis, a second-generation cephalosporin (1 g was administered intravenously 45 min before the surgery and continued for one postoperative day. The patients included in the study were closed-reduced and the fracture line did not need to be opened. In all patients, a nail with the apex entrance of the trochanter major was preferred and a nail of appropriate thickness was placed in the medulla of each patient after femur reamerization. For distal locking with screws, the nail was locked manually with two screws under fluoroscopy and no dynamization was performed during follow-up. For the talon system, locking was completed by deploying the talons with the guide advanced into the nail. All patients

Figure 3. 19-year-old male patient. X-ray images at 6th and 12th months of post-traumatic treatment

Figure 4. 55-year-old male patient. X-ray images at 4th and 12th months of post-traumatic treatment

were postoperatively administered low-molecular-weight heparin. In the postoperative rehabilitation, weight-bearing was initiated at a tolerable level with early postoperative movement in both groups.

Statistical Analysis

The descriptive statistics of the variables analyzed in the study were expressed as mean ± standard deviation, median (minimum-maximum), and nominal variables as n (%), represented with appropriate charts. A comparison of two independent groups was analyzed by Mann-Whitney U test and t-test in accordance with Shapiro-Wilk normality test. In all statistical analyses, the level of significance was set at $p<0.05$. IBM SPSS version 22.0 (IBM Corp, Armonk, NY, USA) software was used for data analysis.

RESULTS

Among 102 study patients, were 21 (20.5%) females and 81 (79.5%) males, with a mean age of 39.34 (18-65) years. Additionally, the fracture was on the right side in 41 (40%) and left side in 61 (60%) patients. There were 37 (36.2%) non-smoker patients, while 65 (63.7%) patients were smokers independent of dose assessment. According to the AO classification of femoral shaft fractures, 62 (60.7%) patients had type A, 35 (34.4%) patients had type B, and 5 (4.9%) patients had type C fractures. The etiology of the fracture was a traffic accident in 51 (50%) patients and a fall in 51 (50%) patients. As the fracture fixation method, implants with a distal locking screw were used in 65 (63.7%) patients who received intramedullary femoral nailing and with a talon distal locking system in the remaining 37 (36.3%) patients. Mean follow-up time was 16.7 (12-44) months. During the follow-up of 102 patients, 6 (5.8%) patients were diagnosed with malunion and 3 (2.9%) patients with nonunion. No patient showed signs of infection during follow-up. Additionally, the demographic data of the study group patients are presented in Table 1.

There was a statistically significant difference between implant design and time to union ($p<0.05$). The implant design was also significantly difference with the length of surgery and duration of intraoperative fluoroscopy shots number ($p<0.05$). Regarding nonunion and malunion, the results were similar in both groups, to no statistical significance ($p>0.05$). The use of talon or screws for distal locking as implant design had similar effects on functional outcomes and there was no statistically significant difference ($p>0.05$) The mean values according to the groups are given in Table 2.

Fracture subtype did not have a statistically significant difference with nonunion, malunion and time to union ($p>0.05$). Our study could not establish any statistically significant difference in smoking, sex, age, and etiology of injury with time to union, malunion and nonunion ($p>0.05$) (Table 3).

DISCUSSION

The most important finding of this study is that the talon locking system provides the advantage of faster surgery and less radiation exposure compared to the conventional locking system.

As is known, intramedullary nailing is the gold standard for treating femoral shaft fractures caused by high-energy traumas. Many surgeons use the advantages of the nail fixed with the conventional locking method implantation intramedullary along with reamerization in intramedullary nailing, such as high rates of union, early weight-bearing with axial and rotational stability, short hospital stay, and short duration of surgery (14,15).

Despite these advantages, the use of intramedullary nails in the femur also has disadvantages, such as difficulty in inserting distal locking screws, increased radiation exposure time, prolonged surgery, risk of neurovascular injury, and soft tissue injury (16,17). Similar complications were reported by

Table 1. Demographic data by groups

		Group 1	Group 2	p-value
Sex	Female	13 (20%)	8 (21.6%)	0.82
	Male	52 (80%)	29 (78.4%)	
Age		38.98 (18-65)	39.97 (18-65)	0.85
Type of injury	Traffic accident	32 (49.2%)	19 (51.4%)	0.78
	Fall	33 (50.8%)	18 (48.6%)	
Smoking	No	24 (36.9%)	13 (35.1%)	0.82
	Yes	41 (63.1%)	24 (64.9%)	
AO type of fracture	A	40 (61.5%)	22 (59.4%)	0.72
	B	22 (33.8%)	13 (35.13%)	
	C	3 (4.6%)	2 (5.4%)	
Length of follow-up (month)		18.46 (12-26)	13.62 (12-44)	0.35
Infection		No	No	-
Malunion		4 (6%)	2 (5.4%)	0.74
Nonunion		2 (3%)	1 (2.7%)	0.87

Table 2. Correlations of type of implant with time to union, malunion and nonunion

Variables	Group 1	Group 2	p-value
Time to union (week)	14.2 (9-24)	19.4 (10-28)	0.008
Length of surgery (minute)	54.2 (±10.1)	44.2 (±8.2)	0.02
Fluoroscpy shot (number)	52 (32-150)	32 (20-60)	0.01
Knee society score (points)	92 (85-100)	91 (83-96)	0.88
Harris hip score (points)	92.50 (86-95)	90.5 (83-93)	0.8

Table 3. Correlations of smoking, sex, type of fracture and etiology of injury with time to union, malunion and nonunion

Variables		Time to union	Malunion	Nonunion
Smoking	p-value	0.06	0.182	0.809
Sex	p-value	0.806	0.433	0.887
Age	p-value	0.975	0.982	0.943
Type of fracture	p-value	0.388	0.31	0.32
Etiology of injury	p-value	0.785	0.198	0.304

studies on the use of distal locking screws in intramedullary nailing of the tibia, another long bone of the lower extremity (18,19).

According to the results of this study, the talon system for intramedullary femoral nailing resulted in a shorter duration of surgery and decrease fluoroscopy shots number. Biomechanical studies have shown that it improves the interfragmentary torsional and compression strength (20). In their study on proximal femoral fractures, Zehir et al. (21) found this system reduced the length of surgery and resulted in low cut-out rates. Similarly, Yapici et al. (22) showed that the talon nailing could be an alternative in proximal femoral fractures. Additionally, Çamurcu et al. (19) compared intramedullary nailing using conventional locking and talons in tibial shaft fractures and concluded that talon nailing was technically easy and safe.

However, the time to union was longer compared to the conventional distal locked tibial nailing. Besides, both techniques yielded successful outcomes in clinical and radiological terms.

Literature data show that the time to union may range from 5.4 months to 24 months in patients treated with conventional locking (6,23-26). According to our study data, the meantime to union was 14.2 (9-24) weeks in group 1, which agrees with the literature. For the talon system, the meantime was 19.4 (10-28) weeks. Even if there was a difference in the time to union, when we evaluate it together with the complication rates and clinical outcomes, both systems can be considered successful. A meta-analysis on nonunion reviewed 2,829 cases and reported a nonunion rate of 2.9% (27). In line with the data in the literature, our study identified a nonunion rate of 3% in patients treated with the conventional method and 2.7% in patients treated with the talon design, and the difference between the two methods was statistically insignificant.

Biomechanical studies report data indicating that new design expandable nails are weaker in resisting rotational and axial forces compared to conventional locked nails (28,29). Concerning the number of distal locking screws, studies are reported that two screws are superior to one screw against axial and angular deformities (30). When we evaluated the patient's radiographs, we concluded that callus formation was more prominent in patients who used a talon design. This result showed that a more stable fracture fixation was achieved against axial and rotational forces in cases where distal locking was performed with two screws. It is also possible to suggest that the talon system provided weaker stabilization in the fracture line due to the prolonged time to union.

The development of minimally invasive techniques in orthopedic surgery and traumatology creates the necessity of using fluoroscopy for a longer time. Nevertheless, various techniques have been developed to reduce the length of surgery and fluoroscopy shots number, depending on the distal locking mechanism of the intramedullary femoral nails (31,32). In this study, we assessed the length of surgery and the number of fluoroscopy shots for two different designs. We found approximately two times shorter radiation exposure with the talon system compared to the conventional method. The main advantage of the talon intramedullary femoral nail seems to be the reduction in radiation exposure by both the orthopedic surgeon and the patient by providing distal locking with six talons deployed distally. Thus, the length of surgery is reduced, suggesting a positive effect on the comfort of the surgery. When we look at the literature, this advantage of the talon design has been emphasized in studies on different long bone fractures (33,34). This study highlights that these advantages of the talon system apply to femoral shaft fractures. When we look at the literature, Yapıcı et al. (35) evaluated the data of 85 patients with femoral shaft fractures retrospectively, and compared the talon system with the conventional locking system. According to the results of this study, the talon system shortened the operation time and intraoperative radiation exposure time, however, clinical and functional results were similar for both systems (35).

When we made an overall assessment of the designs used in the study, we did not establish any significant differences in both clinical outcomes or complications. In our study, 6 patients were evaluated as malunion. these patients had an angulation of >5 degrees, but the angulation were <10 degrees. The patients refuse revision surgery, thinking that they did not have any problems in their daily lives. A total of 3 patients with the diagnosis of nonunion underwent revision with nail exchange and iliac autogenous graft, and union was observed in their follow-up. Although we expected an increase in the complication rate due to the lower stability of the talon system in terms of resistance to rotational and axial forces compared to the conventional method, the results revealed no significant difference. Additionally, we did not observe any reduction in limb length at the post-fracture follow-up. The point to be paid attention here is to ensure the contact of the talons with the femoral cortex while ensuring the central placement of the nail in the intramedullary area. Because of the width of the metaphysis, the talon system may be incapable of providing adequate stabilization, leading to instability in extra-articular fractures of the distal metaphysis.

There are factors that limit our study. First all, our study was a retrospective assessment. The number of patients can be considered another limitation. However, we ensured homogeneous results by including similar groups in the study while examining the outcomes of implant designs. Another factor is that we did not evaluate the effects of nail diameter. Despite the known effect of nail diameter on union, it was excluded in the assessment in this study.

CONCLUSION

In conclusion, intramedullary femoral nailing with a talon system can be considered an easier and safer option for femoral diaphyseal fractures compared with conventional locking design because of the reduction in the length of surgery and radiation exposure. Radiographic outcomes showed that the time to union might be longer with the talon system than with the conventional method, which should be considered during patient follow-up. We believe that this study is the first in the literature to compare the outcomes of two different implant designs for femoral diaphyseal fractures. We further believe that the minimum 12-month follow-up of our patients was a sufficient period for clinical assessment.

ETHICS

Ethics Committee Approval: The study was approved by the Gaziantep University Clinical Research Ethics Committee (decision no: 2021/53, date: 24.02.2021).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: A.M., S.B.T., Concept: A.M., Design: S.B.T., Data Collection or Processing: B.B., Analysis or Interpretation: A.M., Literature Search: S.B.T., Writing: B.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Research

Clinical Presentation of Celiac Disease in Children: A Single Center Experience

Çocuklarda Çölyak Hastalığının Klinik Prezentasyonu: Tek Merkez Deneyimi

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ABSTRACT

Objective: This study was an examination of the presenting complaints, clinical and laboratory data and biopsy reports of 174 celiac patients. The primary aims were to emphasize the presentation patterns of patients that certain complaints need to be queried even if they are not mentioned. Our secondary aim is to increase the awareness of investigating patients' first-degree family members, risk groups otherwise they have no complaints.

Methods: Presenting complaints, anthropometric data, biopsy reports were recorded by retrospectively examining the files of patients diagnosed with celiac disease (CD). In each diagnosis of CD, tissue transglutaminase IgA antibody, anti endomysium antibody titer and histopathological Marsh classification have been conducted.

Results: The prevalence of patients with weight and height below the 3rd percentile was 29.3%. In the order of frequency, the patients' most common presenting complaints were abdominal pain 64.4%, growth retardation 51.1%, diarrhea 36.8%, constipation 32.2% and halitosis 32.2%. It was found that 15.5% of the patients had no complaint. Physical examination was normal in 87.4% patients. When family members were questioned about the disease, it was determined that 20% of the patient families had one or more individuals with CD.

Conclusion: It was found that the rate of growth retardation is high in our population and increased with an increase in the duration of complaints. This shows the importance of early diagnosis and treatment for a quality of life. Additionally, particularly the first-degree relatives of CD patients should be questioned routinely, even if there are no complaints.

Keywords: Celiac disease, growth retardation, presentation, children

ÖZ

Amaç: Çalışmamızda 174 çölyak hastasının başvuru şikayetleri, klinik ve laboratuvar verileri ile biyopsi raporlarını inceledik. Çalışmadaki esas amacımız çölyak hastalarının prezentasyon şekillerini irdeleyerek, bazı şikayetlerin söylenmese de sorgulanması gerektiğini vurgulamaktır. Şikayeti olmasa da birinci derece aile bireylerinin ve riskli olan grupların tetkik edilmesi konusunda farkındalığı artırmaktır.

Gereç ve Yöntem: Çalışmaya dahil edilen hastaların dosyaları retrospektif olarak incelenerek başvuru şikayetleri, antropometrik verileri, biyopsi raporları kayıt altına alınmıştır. Çölyak hastalığı (ÇH) tanısında doku transglutaminaz IgA antikor, anti endomisyum antikor titresi ve histopatolojik olarak Marsh sınıflaması yapılmıştır.

Bulgular: Antropometrik veriler değerlendirildiğinde boy ve kilosu 3 persentil altında olan hasta oranı %29,3 idi. Hastaların en sık başvuru şikayeti sıklık sırasına göre, karın ağrısı %64,4, büyüme geriliği %51,1, ishal %36,8, kabızlık %32,2 ve halitozis %32,2 idi. Hastaların %15,5'inde hiçbir şikayet yoktu. Hastaların %87,4'ünde fizik muayenesi normal idi. Aile bireylerinde hastalık sorgulandığında %20'sinde ailede ÇH'si olan birey vardı.

Sonuç: Çalışmamızda hastaların şikayet süresi arttıkça büyüme geriliği oranları da artmaktadır. Bu da hastalığın erken tanı ve tedavisinin yaşam kalitesi üzerine olumlu etkisi ve tarama testlerinin önemini göstermektedir. Bazı riskli grupların ve özellikle ÇH olan bireylerin birinci derece akrabalarının şikayet olmasa da rutin sorgulanması gerekmektedir.

Anahtar Kelimeler: Çölyak hastalığı, gelişme geriliği, prezentasyon, çocuklar

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INTRODUCTION

Celiac disease (CD) is a chronic autoimmune enteropathy in genetically susceptible individuals that begins with the introduction of gluten into the diet, as well as the effects of environmental factors. It is characterized by the inflammation of the proximal intestine, and through the observation of clinical complaints associated with villus atrophy (1). The prevalence of CD has been gradually increasing with the screening of non-symptomatic risk groups (2). The prevalence of CD, which varied between 1:1000 and 1:3000 in Europe, increased to 1:150 and 1:300 after the tests began (3,4). Many prevalence studies were conducted on CD in European countries. When a systematic review was conducted, the prevalence of CD in Europe was found to be between 1/79 and 1/200 (5).

The results obtained in prevalence studies carried out in different regions of Türkiye were similar. In a study by Dalgic et al. (6), in school children, the prevalence of CD was reported to be 0.47%, in another study by Comba et al. (7), the prevalence was 0.46% in Çorum (1:216) and by Ertekin et al. (8) in the school-aged children in Erzurum, the prevalence of CD was found to be 1:115. For this reason, the study aimed to emphasize the importance of conducting research on CD, to make an examination of the presenting complaints and to query some findings, even if the patient does not report them.

In this study, we address certain parameters, in terms of early diagnosis, by examining the period that had elapsed between the first presentation complaints and diagnosis; some symptoms that the patient did not mention when questioned but which nevertheless existed, and the laboratory data and the endoscopic findings at the time of diagnosis. Our other aim is to highlight the need to evaluate the relatives of CD patients, even if they do not have any related complaints. To emphasize that to make a differential diagnosis, clinicians should consider CD when assessing and requesting etiological investigations for patients with non-specific gastric complaints.

METHODS

In this study, a retrospective analysis was made of the files of 174 patients diagnosed with CD who came for control to Adana City Training and Research Hospital, Pediatric Gastroenterology 1 Outpatient Clinic between June 1 and August 1, 2020. When patients initially present at the Pediatric Gastroenterology 1 Outpatient Clinic at Adana City Training and Research Hospital, a detailed file is started for each one of them. These patients are seen by me individually, as I do not have an assistant. After this study was planned, I have personally sought all information missing from the files from the patients directly during the two months of my involvement with them.

The patients' anthropometric data (height and weight values, percentiles) at the initial diagnosis, the age at diagnosis, gender, presenting complaints, some complaints questioned in detail [abdominal pain, combustion, heat (heart burn), halitosis, dyspepsia, presence of symptoms suggestive of reflux, vomiting, nausea, diarrhea, constipation, joint pain, fever, weight loss, growth retardation], duration of complaints, breastfeeding duration, months of first contact with gluten, additional diseases, the existence of CD in the family, socio-economic status, educational status of the mother and father, physical examination findings, the Marsh classification histopathological staging, *Helicobacter pylori* (Hp) positivity and some laboratory results (tissue transglutaminase IgA, serum IgA, anti endomysium antibody titer) in biopsy examination were obtained from the records in the file. The histopathological staging used for diagnosis was accomplished through the Marsh classification (9).

Among the patients who came to our outpatient follow-up, due to social or economic reasons, after being diagnosed in different centers, those without Marsh classification were additionally specified. Observer bias was an area that I have paid a considerable amount of attention to as well. I tried eliminating this by using the Marsh classification to standardize the results. These patients present with non-specific gastric complaints. Within the country's population Hp is very prevalent. I wanted to rule out other potential reasons and I wanted to prevent the patient from having to go through an other procedure. Therefore, I have followed the routine gastroscopy prosecures; taking 2 biopsies from the esophagus, the antrum, corpus, four biopsy from duodenum; and one biopsy was taken from the bulbus.

The diagnosis of those patients who were diagnosed in our center was performed in accordance with the items determined by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition Union Committee in 2012 (10). The percentile values of height and weight were assessed using the percentile curves suggested by Neyzi et al. (11). After examining the height/age and weight/age curves of the patients, those under the 3rd percentile were evaluated as the growth retardation group. All patients who presented at the clinic were included in the study within the given timeframe; however, the patients with the following criteria were excluded: Patients who had been diagnosed elsewhere who do not carry the genes for CD. patients whose endoscopic biopsy records could

not be accessed for any reason. Patients who don't have a differentiating diagnosis of either CD or gluten allergy specifically.

The values of the tissue transglutaminase IgA, anti-endomysium IgA and serum IgA titers at the time of diagnosis and before starting the diet were examined. Serum IgA was studied using the nepholometric method (Beckman Coulter IMMAGE 800, Model: 4800). Tissue transglutaminase IgA levels were measured using the enzyme linked immune sorbent assay (ELISA) method (Dynex-Dsx instrument Orgentec Diagnostic GmbH, ORG 540A, Mainz, Germany). Serum anti-endomysium IgA was determined by the ELISA method (Alisei device Radim Diagnostic GmbH, Deutschland). All the hospitals in Adana use the same private laboratory to process celiac antibody titer. In our study population, patients diagnosed in another city were excluded.

This study followed the principles of the Declaration of Helsinki. Additionally, ethical committee approval (date: May 20, 2020, decision no: 879) from Adana City Training and Research Hospital, Scientific Research Ethics Committee was granted for the study. Independent of this study, patients continued their gastroenterological routine follow-up every three months in the first year and every 6 months in the subsequent years. Further studies are possible from the results of these follow-up appointments; nevertheless, I want to assure you that their care planning has not been negatively impacted by this study.

Statistical Analysis

The SPSS 23.0 licensed software package was used for the statistical analysis of the data. Categorical data were expressed as numbers and percentages, while continuous data were expressed as mean and standard deviation (median, and minimum-maximum values, where required). The chi-square test and Fisher's Exact test were applied for the comparison of categorical variables. After checking the distribution of normality in the comparison of continuous variables between the groups, the Mann-Whitney U test in paired groups. and the Kruskal-Wallis tests in more than two groups were used for parameters without a normal distribution. The level of statistical significance was set as 0.05 for all analyses.

RESULTS

The mean and median of the patients' ages of diagnosis were 8.26±4.37 years and 93 months (12-218 months), respectively. The sex of the patients was 64.9% (113 patients) female and 35.1% (61 patients) male. The number of patients of Turkish ethnic origin was 163 patients (93.7%) while 11 patients (6.6%) were of other ethnicities. When the patients' ages of diagnosis were grouped, it was found that the diagnosis was the highest between the ages of 5-10 years with 36.8%. The rate of diagnosis was 33.9% at ages more than 10 years, 23% between the ages of 2-5 and 6.3% in the first 2-5 years of life (Figure 1).

When anthropometric data of patients were evaluated, the rate of patients with weight and height below the 3rd percentile was 29.3% (51 patients). In the order of frequency, the most common presenting complaints of the patients were abdominal pain 64.4% (112 patients), growth retardation 51.1% (89 patients), diarrhea 36.8% (64 patients), constipation 32.2% (56 patients), and halitosis 32.2% (56 patients) (Figure 2).

In the patient group, it was noteworthy that 15.5% of the patients (27 patients) had no complaints; some of those had been examined only because of the disease in their family members. When the disease was queried among the family members, it was determined that 20% patients had an individual with CD in first-degree relatives. The aforementioned 35% is for all extended family members including cousins. Physical examination was normal in 87.4% patients (Figure 3).

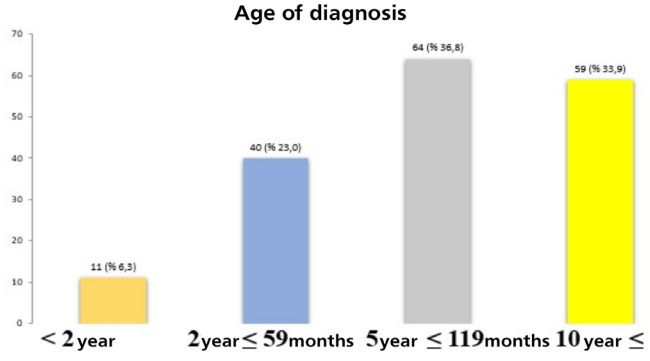

Figure 1. Age groups in which patients are diagnosed

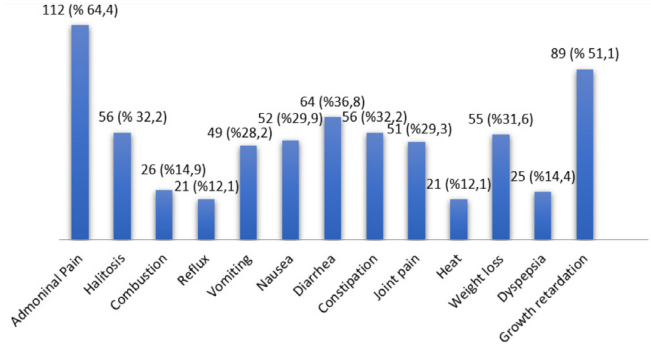

Figure 2. The percentage distribution of presenting complaints questioned in patients

When the biopsies of patients were evaluated, it was found that most biopsy results (45.6%) were compatible with Marsh 3b according to the Marsh classification (Figure 4). Patients with Marsh 0 and 1 were not excluded from the study because they have significantly high enough blood antibody titers (x10 upper limit of normal) to easily diagnose them. the gastroscopic examinations of those patients, we observed mucosal fissuring, villus destruction corresponding to CD. And we correlate with their genetic results that is HLA DQ2 and/or HLA DQ8 positive. The explanation for the difference between the macroscopic appearance and the biopsy is the patchy distribution pattern of the CD. No significant difference was found when comparing the complaint durations of patients with Hp positive and Hp negative in the biopsy material (p>0.05). The duration of the complaints was similar in both groups (12 months). In the evaluation of the material taken from the antrum and corpus area of the stomach by endoscopic biopsy, Hp was negative in 89 patients (70%), while it was positive in 38 patients (30%).

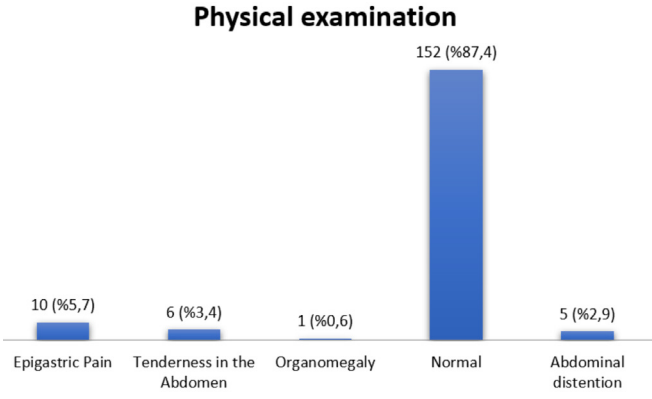

Figure 3. Physical examination results at admission

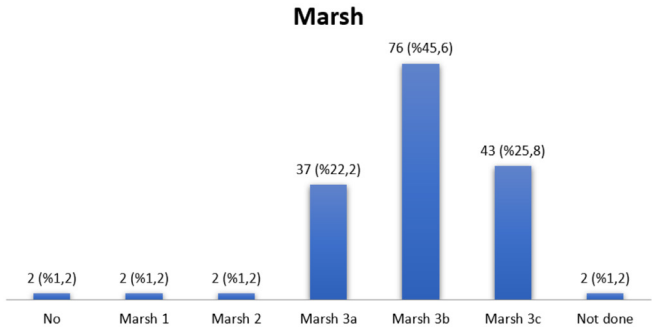

Figure 4. Marsh histopathological staging of the patients duodenal biopsy material

DISCUSSION

Over time, the presenting complaints of CD have changed from classic symptoms to nonclassical symptoms; the presentation has gradually increased, due to growth retardation; more advanced disease has been observed on histopathological examination; and the rate of autoimmune disease has decreased (12,13).

With CD, the presenting complaint of growth retardation is not rare. While the mechanism of growth retardation is not fully understood, it is considered to be caused by nutritional deficiencies, low-serum somatomedin activity, and a disruption in growth hormone release. In our study, when the anthropometric data of patients were evaluated, the rate of patients with growth retardation was found to be 29.3%. This result is consistent with other studies conducted in Türkiye (14).

When the growth retardation was associated with the age at diagnosis in our patients, the highest rate of growth retardatin (67.2%) was found in the group diagnosed between the ages of 5-10 years. Growth retardation was 64.4% in the patient group diagnosed after the age of 10 years. In contrast to the results reported in the literature, the rate of growth retardation was lower in our group of patients diagnosed in the first 2 years of life is 45.5%. When the relationship of growth retardation and the duration of complaints was evaluated, the complaint duration was 18 months in the patient group with growth retardation and 8 months in the patients without. As expected, as the duration of complaints increased, the rate of growth retardation also increased. This indicates the positive effect of early diagnosis and treatment on the quality of life and the importance of screening tests. Among our patients, the rate of patients with isolated short stature was 6.9%. When the results reported in the literature were examined, the rate of patients with isolated short stature was determined as 7.9% by Gokce and Arslantas (12) and 5.4% by Khatib et al. (15). Those results agreed with our data.

There are many studies in the literature, which support the claim that the age of diagnosis of CD has been increasing over the years (12,13,15,16). When compared, the age of diagnosis reported in the literature was found to be consistent with the age interval of our patients. Most diagnoses in our patients were in the 5-10 years old (36.8%).

While the age of admission in CD was gradually increasing, the symptoms related to the complaints of the gastrointestinal system, such as diarrhea, weight loss, abdominal pain and constipation dramatically decreased (14).

When the patient group was examined considering these studies, the most common presenting complaints of patients diagnosed before 2010 were abdominal pain (65.4%) and secondly, growth retardation (46.2%). The most common presenting complaints of the patients diagnosed after 2010 were abdominal pain (62.9%) and growth retardation (58.6%) respectively, similar to those of patients diagnosed before 2010. In a comparison of the patient groups with a CD diagnosis over the years, it was determined that the most important result was the reduction in heartburn and reflux. When these two groups were compared, while the heartburn and reflux symptoms were 22.1% and 18.3%, respectively in the patients diagnosed before 2010, the heartburn and reflux symptoms statistically and significantly decreased to 4.3% and 2.9%, respectively in the patients diagnosed after 2010 ($p<0.05$).

Because CD is an autoimmune disease, it should be considered that its prevalence increases in certain risk groups. There are many studies in the literature that have been conducted with these patient groups. In particular, Sari et al. (17) determined that 6.3% of patients with type 1 diabetes mellitus (DM) were found to have a CD association with the biopsy. In another study 4.9% of cases with autoimmune thyroiditis coexisted with CD (18). Similarly, the most common accompanying disease in our patient group was type 1 DM (7.4%) while autoimmune thyroiditis (2.2%) was the second most frequent disease. Other accompanying diseases were genetic syndromes (Turner syndrome, Down syndrome), cystic fibrosis, attention deficit and hyperactivity syndrome, and Familial Mediterranean fever, respectively.

In this study, 14.4% patients had an additional disease accompanying CD. In some studies, accompanying disease rates were found to be higher, such as 25.7% (12). According to the literature 4.6% of CD patients were found to have selective IgA deficiency, although no patient in our study group had selective IgA deficiency (16).

Foods containing gluten have an important place on the Turkish dining table and in Turkish cuisine. It is emphasized that breast feeding the infant during the first introduction of gluten and during the introduction period is important in terms of reducing CD risk (19).

In our patient group, the first introduction of gluten was mostly between the 4^{th} and 7^{th} month (69%). Then again, 28.7% of patients were introduced to gluten after reaching 7 months and 2.3% during the first 4 months of life. When it was questioned whether the patient received breast milk during the first contact with gluten, it was found that 75.9% patients were introduced to gluten while still being fed breast milk, whereas 24.1% were introduced to gluten after weaning.

In a study conducted in the Ankara region in 2015, the feeding of infants only their mother's milk was investigated. It was found that the rate of infants fed with only breast milk for 4 months was 49% and that of infants breastfed for 6 months was as low as 38% (20).

In our study, the duration of breast feeding in our patients was better, with an average of 13 months. It was concluded from this finding that increasing awareness by parents over time regarding the benefits of breast milk plays an important role.

While the prevalence of CD gradually increases over the years, the presenting complaints reduce and the severity eases. Approximately 25% of patients with no complaints can be detected with screening tests (14). In our study, while the rate of patients with no complaint was 15.5%, the rate of patients with one complaint was 47.1%. These results indicate the necessity of performing celiac screening tests on at-risk groups and those with a family history to prevent any delay in diagnosis.

The most common presenting complaints in our patient group were abdominal pain and growth retardation. Non-classical presenting complaints stated in the literature were not at the forefront. Khatib et al. (15) determined that the most common presenting complaints were abdominal pain (52.7%) and constipation (38.9%). These different results indicate that regional differences and genetics is applicable in presenting complaints. When the rate of growth retardation was compared according to the year of diagnosis in our patients, it was observed that while the growth retardation was detected in 30.8% of patients diagnosed before 2010, the rate of growth retardation was higher (44.3%) in patients diagnosed after 2010. No statistically significant difference was detected between the groups ($p>0.05$).

CONCLUSION

The prevalence of CH is known as 0.5-1%. The risk of CD increases in certain risk groups, particularly the first-degree relatives of individuals with CD and the people with autoimmune liver disease, autoimmune thyroiditis, type 1 diabetes, Down syndrome, Turner syndrome and Williams syndrome. With easy access to serological tests at the present time and their escalating use, the diagnosis of CD has been gradually increasing. In the existence of an individual with CD in the family, even if there is no complaint, other members of the family should be routinely examined. As we also specifically showed in our study, the early diagnosis of CD can be possible by screening first-degree family members, even if they have no complaints.

ETHICS

Ethics Committee Approval: Additionally, ethical committee approval (date: May 20, 2020, decision no: 879) from Adana City Training and Research Hospital, Scientific Research Ethics Committee was granted for the study.

Informed Consent: Retrospective study.

Financial Disclosure: The author declared that this study received no financial support.

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Research

Efficacy of Multilevel Botulinum Toxin Type A Injections Applied to the Lower Limb in a Single Session for Children with Spastic Cerebral Palsy

Spastik Serebral Palsili Çocuklarda Alt Ekstremiteye Tek Seansta Uygulanan Çok Düzeyli Botulinum Toksin Tip A Enjeksiyonlarının Etkinliği

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ABSTRACT

Objective: Dynamic spasticity may develop into static contractures in children with cerebral palsy. The study aimed to evaluate the efficacy of multilevel botulinum toxin type A (BtA) in children with spastic cerebral palsy, when combined with casting, followed by physical therapy and orthotics.

Methods: We retrospectively evaluated changes in physical examination and walking in 12 children treated at our institution between January and December 2014 using three-dimensional gait analysis at baseline, and 3 months and 6 months after treatment. We administered BtA 6-8 IU/kg to four points in gastrocnemius and 4-6 IU/kg BtA to two points in the hamstrings. A long-leg cast was applied for 10 days after the injections, after which an ankle-foot orthosis was supplied to all carers and an intensive physical therapy program was undertaken.

Results: Three months after injection, we observed significant improvements in cadence, stride time and velocity, and knee flexion at initial contact and maximum knee extension during the stance phase, but these had returned to baseline by 6 months. However, significant improvements from baseline in ankle plantar flexion at initial contact, maximum ankle dorsiflexion during the stance phase and maximum ankle plantar flexion during the terminal stance phase persisted for 6 months.

Conclusion: A single session of multilevel BtA treatment combined with casting, intensive physical therapy and orthosis use appears to be an effective means of preventing static contractures in children with cerebral palsy for up to 6 months.

Keywords: Botulinum toxin type A, cerebral palsy, kinetic analysis, kinematic analysis, gait analysis

ÖZ

Amaç: Serebral palsili çocuklarda dinamik spastisite statik kontraktürlere dönüşebilir. Çalışmanın amacı, spastik serebral palsili çocuklarda çok seviyeli botulinum toksini tip A (BtA) enjeksiyonu ile birlikte alçı uygulaması ile ardından fizik tedavi ve ortez ile kombine edilen tedavinin etkinliğini değerlendirmektir.

Gereç ve Yöntem: Ocak-Aralık 2014 tarihleri arasında kurumumuzda tedavi edilen 12 çocukta başlangıçta ve tedaviden 3 ay ve 6 ay sonra üç boyutlu yürüme analizi kullanarak fizik muayene ve yürümedeki değişiklikleri geriye dönük olarak değerlendirdik. Gastroknemiusta dört noktaya 6-8 IU/kg BtA, hamstringlerde iki noktaya 4-6 IU/kg BtA uyguladık. Enjeksiyonlardan sonra 10 gün boyunca uzun bacak alçı uygulandı, ardından tüm hastalara ayak bileği ortezi ve yoğun bir fizik tedavi programı uygulandı.

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Bulgular: Enjeksiyondan üç ay sonra, duruş fazı sırasında dakikadaki adım sayısı, çift adım süresi, hızı ve ilk temasta diz fleksiyonunda ve maksimum diz ekstansiyonunda önemli iyileşmeler gözlemledik, ancak bunların 6 ayda başlangıç seviyelerine döndüğünü saptadık. Bununla birlikte, ilk temasta ayak bileği plantar fleksiyonunda, basma fazı sırasında maksimum ayak bileği dorsifleksiyonunda ve basma sonu fazı sırasında maksimum ayak bileği plantar fleksiyonunda başlangıca göre önemli gelişmeler 6 ay boyunca devam etti.

Sonuç: Alçı, yoğun fizik tedavi ve ortez kullanımı ile birlikte tek seans çok seviyeli BtA tedavisi, 6 aya kadar serebral palsili çocuklarda statik kontraktürleri önlemenin etkili bir yolu gibi görünmektedir.

Anahtar Kelimeler: Botulinum toksin A, serebral palsi, kinetik analiz, kinematik analiz, yürüme analizi

INTRODUCTION

Cerebral palsy (CP) is characterized by a persistent disorder of motor control and posture because of non-progressive brain injury, and multilevel dynamic spasticity is common in early childhood (1). Equinus gait and excessive knee flexion during the stance phase are the most frequently observed walking disorders in children with CP (2,3).

The injection of botulinum toxin A (BtA), casting, orthotics, and intensive physical treatment are used to manage multilevel spasticity in children with CP (4,5). Boyd and Graham (5) and Molenaers et al. (4) have stressed the importance and objectivity of computed gait analysis for the functional assessment of walking in CP.

The therapeutic benefits of BtA are generally measured by comparing the range of motion or by analyzing gait before and after treatment. Investigators who have used these methods as outcome measures have reported improvements in ankle dorsiflexion of approximately 3 months' duration after injection into gastrocnemius (6-11), but the role of multilevel injections (4,5), including to the hamstring (12), has also been examined. Corry et al. (12) used gait analysis to document a significant improvement in knee extension after BtA therapy that was most evident 2 weeks after injection but was beginning to wear off after 12 weeks. Cosgrove et al. (13) used electrogoniometric measurements of sagittal plane angle kinematics at the hip, knee and ankle to identify significant improvements in ankle and knee positions after BtA treatment that lasted approximately 6 months.

We hypothesized that a single session of multilevel BtA therapy would improve walking function in children with CP-related spasticity in the lower extremities for up to 6 months, when administered as part of a multidisciplinary program that also included casting, physical therapy and orthotics. We undertook a retrospective analysis of spatiotemporal parameters and kinematic data obtained by computed gait analysis performed in a cohort of children with CP affecting the lower limb.

METHODS

Participants

The conduct of the study was approved by the Ethics Committee of Baltalimanı Metin Sabancı Bone Diseases Training and Research Hospital (decision no: 23, date: 21.04.2015). Patients with CP attending the pediatric orthopedic clinic of our hospital routinely undergo gait analysis before and after BtA treatment. We retrospectively identified a group who had undergone multilevel BtA injection for preventing spasticity in the lower extremity between January and December 2014. We included those with Gross Motor Function Classification System (GMFCS) level I-III disability, with complete three-dimensional gait analysis records obtained before BtA administration and at 3 months and 6 months afterwards (14). We excluded those with GMFSC level IV or V disability, static equinus deformity or knee flexion contracture, severe athetoid movements in the lower extremities, or who had undergone surgery to the foot, ankle, or leg.

Contracture Prevention Program

All BtA injections were administered in the operating room under general anesthesia and aseptic conditions. BtA 100 IU (Botox, Allergan, Irvine, CA, USA) was reconstituted in sterile 0.9% NaCl solution and injected using an insulin syringe with a 26-G needle. The maximum dose per muscle was 50 IU and the maximum total dose was 20 IU/kg. Injections were administered to three points in the medial head and one point in the lateral head of gastrocnemius, and two points 2 cm apart in the medial third of the hamstrings in all patients (15), as described by Cosgrove et al. (13) We administered 6-8 IU/kg BtA to gastrocnemius and 4-6 IU/kg to the hamstrings. We also administered BtA to the psoas, tibialis posterior and soleus in three cases, the adductor muscles in six cases and the rectus muscles in four cases.

Once injections were complete, stretching plaster of Paris casts were applied for 10 days to maintain the ankle joint in a neutral position and the knee in semi-flexion (5°-10°). After that, carers were provided with a non-articulated ankle-foot orthosis to be used in the shoe when walking. Patients also underwent 60-minute intensive physical therapy sessions

three times a week after cast removal (8), focused on active and passive stretching of the flexor muscles, strengthening of the extensors, functional mobility and gait training.

Outcome Measures

All patients underwent physical examination at baseline and 3 months and 6 months after injection. We recorded the Modified Ashworth scale (MAS) (16), hip extension, popliteal angle and ankle dorsiflexion using standard procedures (4,5,12,17). All examinations were conducted by the same physical therapist.

Gait Analysis

Three-dimensional gait analysis was performed before BtA injection, and 3 months and 6 months after injection. Walking was analyzed using the Vicon Bonita System (Oxford Metrics, Oxford, UK) using eight 100 Hz infrared cameras and two Bertec power platforms (Bertec, Columbus, OH, USA). Sixteen retroreflective markers were positioned at anatomic points according to the Modified Helen Hayes model (18-20). Patients were instructed to walk barefoot along a 9-m walkway at a normal speed. Data were processed using the Vicon Nexus 1.8.2 program. (Vicon Motion Systems, Oxford, UK) Time-distance parameters and kinematic graphs were obtained using Polygon 4.0.1 software (Vicon Motion Systems). Walking cycles when both feet were placed fully on the pressure plates one after the other, were accepted as normal. We recorded data for two to four walking cycles from each affected extremity. The most representative cycle was selected and used for statistical assessment.

Time-distance variables recorded were step width, stride length, stride time, cadence and walking speed, kinematic variables recorded were pelvic tilt, pelvic rotation, pelvic obliquity, hip abduction, maximum hip flexion at initial contact (H1), maximum hip extension during the stance phase (H2), maximum hip flexion during the swing stage (H3), knee flexion at initial contact (K1), maximum knee extension during the stance phase (K2), maximum knee flexion during the swing phase (K3), ankle plantar flexion after initial contact (A1), maximum ankle dorsiflexion during the stance phase (A2) and maximum ankle plantar flexion during the terminal and pre-swing phases (A3).

Statistical Analysis

Data are presented as the mean (± standard deviation) or the median (range). Data from one limb were subject to analysis, the right limb in diplegic children and the affected limb in hemiplegic children. The distribution of variables was assessed using the Kolmogorov-Smirnov test. Repeated measures analysis of variance (ANOVA) and the least significant difference (LSD) was used for pairwise comparisons of parametric data, and the Friedman and Wilcoxon tests were used for pairwise comparison of non-parametric data. The significance level for ANOVA and the Friedman test was $p<0.05$. Pairwise comparisons using the LSD and Wilcoxon tests were subject to the Bonferroni correction; for these analyses, the significance level was $p<0.016$. We used the SPSS statistical program for all analyses (version 22.0; IBM, Armonk, NY, USA).

RESULTS

Patient Cohort

Twelve patients met the inclusion criteria, four girls and eight boys. Their mean age was 6 years 3 months (range 4 years 7 months to 9 years 2 months); seven were diplegic and five hemiplegic. A physical examination identified dynamic gastrocnemius and hamstring spasticity in all cases at baseline.

Physical Examination

Statistically significant improvements in all parameters were identified after multilevel BtA injection (Table 1). Passive hip extension and hamstring and ankle MAS were found to have improved significantly at 3 months, but although they had deteriorated by 6 months were still significantly superior to the baseline. Popliteal angles had also improved significantly by 3 months, but had deteriorated to the extent that they were not significantly different from baseline by 6 months. Significant improvements in ankle dorsiflexion were maintained throughout the 6-month follow-up period.

Spatiotemporal Parameters

There were significant changes in cadence, stride time and walking speed after multilevel BtA injection (Table 2). Cadence had fallen significantly by 3 months, but had returned to baseline levels by 6 months. Stride time was significantly higher than the baseline at 3 months, but had returned to baseline by 6 months. Walking speed had fallen significantly from baseline by 3 months, but had returned to baseline by 6 months. There were no significant changes in any other spatiotemporal parameter.

Kinematics

There were no significant changes in the kinematic parameters measured in the pelvis or hip (Table 3, Figure 1 A-B), but there were significant improvements in knee flexion and extension (K1 and K2), and ankle plantar and dorsiflexion (A1, A2, and A3), throughout the walking cycle (Table 3, Figure 1 C-D). The significant improvements in knee kinematics observed at 3 months had returned to baseline by 6 months, but were still evident in the ankle at 6 months (A1, A2, and A3).

Table 1. Changes in physical examination variables before and after a single session of multilevel botulinum toxin type A injection

		Mean ± SD	Median	Range	p*
Hip extension (°)*	Pre BtA	14.6±6.2	15	0-20	0.042
	Post BtA 3 months	19.6±2.6	20	15-25	
	Post BtA 6 months	18.3±3.9	20	10-20	
Popliteal angle (°)*	Pre BtA	60.0±10.9	57.5	45-80	0.005
	Post BtA 3 months	50.0±6.0	50	40-60	
	Post BtA 6 months	54.2±7.9	55	35-65	
Ankle dorsiflexion (°)*	Pre BtA	-2.5±10.3	0	-25-5	0.001
	Post BtA 3 months	6.6±9.6	7.5	-10-25	
	Post BtA 6 months	5.4±12.5	10	-20-20	
Hamstring MAS†	Pre BtA	-	2	1-3	0.018
	Post BtA 3 months	-	1	0-2	
	Post BtA 6 months	-	1	0-3	
Ankle MAS†	Pre BtA	-	2	1-3	<0.001
	Post BtA 3 months	-	1	0-2	
	Post BtA 6 months	-	1	0-3	

SD: Standard deviation, BtA: Botulinum toxin type A, MAS: Modified Ashworth scale
*ANOVA test, †Friedman test; p<0.05 was considered statistically significant

DISCUSSION

We found that a single session of multilevel BtA treatment combined with casting, physical therapy and use of an orthosis was an effective treatment strategy for childhood lower limb spasticity in CP, improving various gait parameters for at least 3 months, and ankle movements for at least 6 months. These findings chimed with those of other investigators (4,6,7,9,21). An integrated approach to the treatment of dynamic spasticity is critical to success and should be timed correctly; patients should be selected for treatment according to objective measurement methods, appropriate doses of BtA should be administered at multiple levels, casting, orthoses and intensive physical therapy should be provided, and outcomes should be monitored regularly and objectively (4,5). Using this approach, we could achieve positive therapeutic outcomes for the patients in our cohort for up to 6 months, by which time the pharmacologic effects of BtA would likely have waned.

Gage et al. (2) described normal walking as having adequate stability during the stance phase, proper lifting of the foot away from the ground in the swing phase, proper positioning of the foot for initial contact at the end of the swing phase, and provision of adequate step length. Corry et al. (12) reported that cadence and walking speed had increased 2 weeks after BtA treatment, and although both had fallen by 12 weeks, they had not returned to baseline. We found that cadence and walking speed had decreased and stride time had increased 3 months after BtA treatment, but these changes were no longer evident 6 months after treatment. We interpret the changes that we observed at 3 months as an improvement in walking function, when taken together with a decrease in maximum knee flexion at initial contact (which depends on longitudinal extension of the hamstrings). In our opinion, the deterioration of spatiotemporal parameters seen 6 months after treatment was a consequence of knee function returning to baseline levels.

Few investigators have used objective outcome measures to evaluate the therapeutic effects of BtA injection on the proximal muscles of the lower extremities. Molenaers et al. (4) used gait analysis alone to examine the influence of BtA treatment in CP and reported improvements in pelvic stability, especially in the frontal and transverse planes, but found no changes in kinematic hip parameters. We found no significant changes in any pelvis or hip movements on gait analysis, despite improvements in hip extension detected on physical examination. Walking is a dynamic activity, and weakness in the trunk muscles combined with spasticity in the ankle and knee likely affects the hip joint and overloads the pelvis.

Table 2. Changes in spatiotemporal parameters before and after multilevel botulinum toxin A injection

		Mean ± SD	Median	Range	p*
Cadence (/min)	Pre BtA	141.3±21.2	144.5	99.0-185.0	0.006
	Post BtA 3 months	123.6±18.7	128.0	80.0-146.0	
	Post BtA 6 months	139.7±22.3	141.5	87.6-169.0	
Stride time (s)	Pre BtA	0.87±0.14	0.85	0.65-1.21	0.032
	Post BtA 3 months	0.99±0.18	0.94	0.82-1.50	
	Post BtA 6 months	0.89±0.17	0.85	0.71-1.37	
Single support	Pre BtA	0.36±0.06	0.36	0.28-0.50	0.218
	Post BtA 3 months	0.40±0.09	0.36	0.31-0.63	
	Post BtA 6 months	0.37±0.08	0.36	0.26-0.59	
Double support	Pre BtA	0.16±0.71	0.16	0.03-0.30	0.141
	Post BtA 3 months	0.20±0.06	0.20	0.11-0.35	
	Post BtA 6 months	0.21±0.19	0.16	0.07-0.82	
Stride length (m)	Pre BtA	0.85±0.092	0.82	0.75-1.04	0.438
	Post BtA 3 months	0.81±0.15	0.80	0.54-1.03	
	Post BtA 6 months	0.84±0.10	0.84	0.72-1.07	
Step width (m)	Pre BtA	0.15±0.12	0.12	0.01-0.53	0.742
	Post BtA 3 months	0.12±0.07	0.11	0.03-0.27	
	Post BtA 6 months	0.12±0.06	0.11	0.05-0.29	
Walking speed (m/s)	Pre BtA	0.99±0.14	1.01	0.72-1.21	0.019
	Post BtA 3 months	0.81±0.22	0.86	0.4-1.22	
	Post BtA 6 months	0.97±0.15	0.99	0.58-1.15	

SD: Standard deviation, BtA: Botulinum toxin type A, *ANOVA test, $p<0.05$ was considered statistically significant

BtA is the most effective in patients with dynamic contractures. Dynamic spasticity may develop into static contractures at older ages (6). Addressing spasticity between the ages of 1 and 6 years reportedly prevents the onset of contracture and delays the age at which surgery is required (7,21). Computerized gait analysis has some limitations in children. The proximity of the retroreflective markers makes analysis unreliable and children must be cooperative; consequently, the minimum height for gait analysis in our laboratory is 1 m and the minimum age is 4 years (22). We assessed gait in children aged <4 years visually and by physical examination. Consequently, we cannot generalize our findings to younger children, and the age of our cohort may have contributed to the limited changes in hip and pelvic parameters that we observed.

The specific goal of BtA injection is to increase stability during walking. To achieve this, foot contact during the stance phase must be optimized, along with proper positioning of the knee and ankle at the end of the swing phase for initial contact (2,4). We found that BtA injection combined with an integrated treatment strategy in children with multilevel spasticity-improved foot position at initial contact, and that the subsequent improvement in gait stability persisted for up to 6 months. However, although the knee extension at initial contact (K1) had improved statistically after 3 months, we found that the knee was still more flexed compared with reference data (23). Full knee extension could only be achieved in the mid-stance phase (K2), and improvements in knee movements had subsided to baseline by 6 months. Corry et al. (12) reported that improvements in hamstring function seen 2 weeks after BtA injection had subsided by 12 weeks, although not to baseline levels. Desloovere et al. (22) found no significant difference in the knee angle at initial contact between baseline and 3 months. Nevertheless, we observed a significant improvement in knee flexion at initial contact, possibly because we used an above-knee cast; Corry et al. (24) did not apply casts to their patients, and Desloovere et al. (22) used a below-knee cast. The short-term efficacy of BtA treatment to gastrocnemius is well recognized (6,7,9,11): Corry et al. (12) observed continuing

Table 3. Changes in kinematic parameters before and after multilevel botulinum toxin A injection

		Mean ± SD	Median	Range	p*
Pelvic tilt	Pre BtA	17.18±5.65	17.60	9.15-25.50	0.685
	Post BtA 3 months	16.26±6.60	15.60	6.73-27.70	
	Post BtA 6 months	16.34±5.71	14.95	8.31-28.30	
Pelvic rotation	Pre BtA	-1.28±6.70	-2.50	-8.54-12.20	0.150
	Post BtA 3 months	-1.57±5.52	-2.39	-9.11-9.04	
	Post BtA 6 months	0.97±5.97	3.64	-10.1-8.78	
Pelvic obliquity	Pre BtA	0.90±3.51	1.05	-5.49-7.29	0.194
	Post BtA 3 months	1.62±3.42	1.94	-3.01-7.64	
	Post BtA 6 months	2.48±2.51	2.20	-1.83-8.67	
Hip abduction	Pre BtA	3.51±4.19	3.65	-2.23-10.50	0.704
	Post BtA 3 months	4.53±4.69	4.59	-2.70-11.60	
	Post BtA 6 months	4.42±3.70	4.84	-0.37-9.17	
H1	Pre BtA	40.79±6.46	41.10	30.06-52.90	0.937
	Post BtA 3 months	40.83±5.84	38.95	34.40-55.00	
	Post BtA 6 months	41.25±7.39	42.30	26.80-53.30	
H2	Pre BtA	-3.06±3.05	-3.16	-8.19-2.59	0.062
	Post BtA 3 months	-4.96±6.09	-4.69	-17.80-7.09	
	Post BtA 6 months	-0.83±7.17	-1.42	-14.20-8.23	
H3	Pre BtA	48.38±8.88	48.45	34.10-59.70	0.176
	Post BtA 3 months	46.15±9.52	43.20	35.0-63.90	
	Post BtA 6 months	49.14±10.60	49.25	34.10-73.90	
K1	Pre BtA	28.74±7.16	29.35	19.60-41.10	0.003
	Post BtA 3 months	23.68±8.14	23.85	9.12-34.20	
	Post BtA 6 months	26.71±11.78	28.00	9.82-52.10	
K2	Pre BtA	6.01±7.42	7.52	-8.87-19.00	0.012
	Post BtA 3 months	-0.46±4.98	-0.69	-7.04-8.45	
	Post BtA 6 months	4.33±8.34	1.83	-6.89-24.20	
K3	Pre BtA	59.04±8.94	60.15	46.50-73.30	0.665
	Post BtA 3 months	60.15±9.46	58.40	45.50-77.20	
	Post BtA 6 months	61.67±8.98	62.50	43.90-72.10	
A1	Pre BtA	-8.17±9.77	-5.88	-26.70-3.98	0.018
	Post BtA 3 months	0.37±6.34	1.12	-14.50-7.83	
	Post BtA 6 months	1.64±4.46	1.35	-5.31-9.17	
A2	Pre BtA	-5.72±19.33	-2.32	-60.90-16.90	0.006
	Post BtA 3 months	10.15±15.71	13.60	-28.10-27.20	
	Post BtA 6 months	3.51±19.22	10.90	-53.90-19.10	
A3	Pre BtA	-34.92±24.58	-34.25	-81.90 - -1.03	0.002
	Post BtA 3 months	-19.33±24.66	-11.60	-76.60-4.40	
	Post BtA 6 months	-16.37±20.37	-11.15	-75.30 - -0.27	

SD: Standard deviation, BtA: Botulinum toxin type A, H1: Maximum hip flexion at initial contact, H2: Maximum hip extension during the stance phase, H3: Maximum hip flexion during the swing stage, K1: knee flexion at initial contact, K2: Maximum knee extension during the stance phase, K3: Maximum knee flexion during the swing phase, A1: Ankle plantar flexion after initial contact, A2: Maximum ankle dorsiflexion during the stance phase, A3: Maximum ankle plantar flexion during the terminal and pre-swing phases, *ANOVA test; $p<0.05$ was considered statistically significant

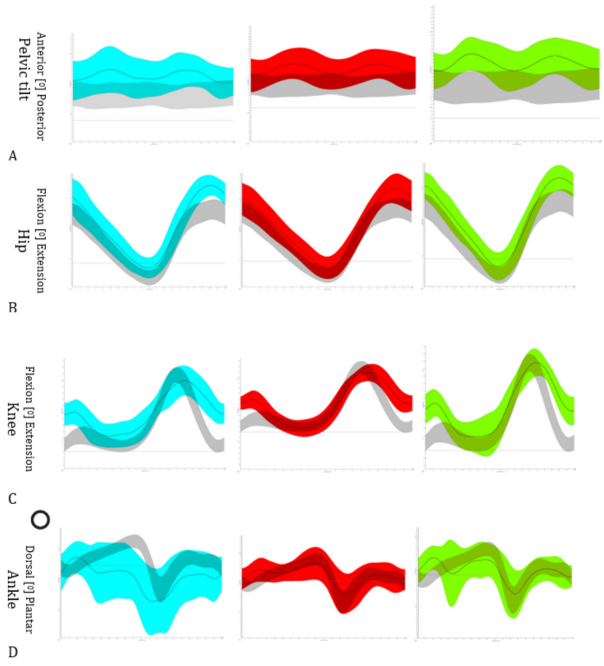

Figure 1. Mean sagittal plane kinematic mass graphs are shown for (A) pelvic tilt, (B) hip flexion, (C) knee flexion and (D) ankle dorsiflexion and plantar flexion for all patients at baseline (blue line), 3 months (red line) and 6 months (green line) after botulinum toxin type A injection, and for an age-matched control group of 19 subjects (gray area, ±1 standard deviation). Positive values indicate anterior pelvic tilt, hip flexion, knee flexion and ankle dorsiflexion

benefits at 12 weeks; Choi et al. (11) reported a therapeutic effect at 4 months; whereas we found that some benefits persisted for 6 months.

To enhance the statistical power of our study, we only analyzed data from one limb of each patient (the affected limb of in hemiplegic children and the right limb of diplegic children). Although our small sample size could be considered a limitation, it should be deemed a beta error; we used posteriori power analysis to ensure that the study had reached adequate statistical power.

CONCLUSION

We found that multilevel BtA treatment administered in a single session is an effective treatment for lower limb spasticity in children with CP. When combined with casting, intensive physical therapy and use of an orthosis, the most significant improvements were seen at the ankle joint, an effect that lasted 6 months. This is the first study to use computed gait analysis as an objective outcome measure over a 6-month follow-up period. We saw more short-lived improvements at the knee joint, but no improvements were observed in hip or pelvic locomotor function.

ETHICS

Ethics Committee Approval: The conduct of the study was approved by the Ethics Committee of Baltalimanı Metin Sabancı Bone Diseases Training and Research Hospital (decision no: 23, date: 21.04.2015).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: K.B., E.A., S.A., İ.A.B., K.A., M.F.Y., Concept: K.B., S.A., İ.A.B., K.A., M.F.Y., Design: K.B., E.A., İ.A.B., K.A., M.F.Y., Data Collection or Processing: K.B., E.A., S.A., K.A., M.F.Y., Analysis or Interpretation: K.B., E.A., S.A., İ.A.B., K.A., M.F.Y., Literature Search: K.B., E.A., S.A., K.A., Writing: K.B., İ.A.B.

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Research

The Retrospective Analysis of Interventional Procedures Performed in the Emergency Department Between 2017-2021: Türkiye Sample

2017-2021 Tarihleri Arasında Acil Serviste Yapılan Girişimsel İşlemlerin Retrospektif Analizi: Türkiye Örneklemi

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ABSTRACT

Objective: Many interventional procedures for diagnosis and treatment were being performed in the emergency departments. We examined interventional procedures, some of which we consider critical and some that we apply very frequently in emergency departments. We analyzed the correlation of interventions such as endotracheal intubation, central venous catheterization, tube thoracostomy, nasogastric tube placement, bladder catheterization, incision repairs applied in emergency departments with the admissions to the emergency departments, the quantitative changes over the years, and the effect of the pandemic process on both emergency admissions and these interventions.

Methods: In this study, which was planned as a retrospective descriptive study, the data of the patients who admitted to the emergency departments of the 2nd and 3rd level public hospitals in Türkiye, between March 2017 and March 2021 have been discussed. The annual mean value of the data between the 1-year pandemic period March 11, 2020 when the pandemic started in our country until March 11, 2021, and the data between March 11, 2017 and March 11, 2020, in the pre-pandemic period, were examined and these periods were compared. The data were recorded on the spreadsheet program and their percentage changes were calculated using the statistical formulas of the spreadsheet program.

Results: Between 2017 and 2020, there was a continuous increase in the emergency service examinations of 2nd and 3rd level public hospitals, and the number of examinations decreased to 93.5 million patients with the coronavirus disease-2019 (COVID-19) pandemic. The number of endotracheal intubations increased by 11.6% during the pandemic period. The number of central venous catheters applied during the pandemic period decreased by 16.2% compared with the 3-year average before the pandemic. Tube thoracostomy decreased by 0.13%, bladder catheter application by 3.2%, nasogastric tube application and incision repair decreased by 26.6% during the pandemic period.

Conclusion: In the first 1-year period of the COVID-19 pandemic, the average emergency service applications, incision repair, nasogastric tube application, bladder catheterization, tube thoracostomy and central venous catheterization applications decreased during the pandemic period compared to the 3-year period before the pandemic.

Keywords: Emergency medicine, endotracheal entubation, pandemic

ÖZ

Amaç: Acil servisler hayati önem arz eden tanıların konulduğu, tetkik ve tedavilerinin yapıldığı alanlar olmuştur. Acil servislerde tanı ve tedavi amaçlı birçok girişimsel işlem yapılmaktadır. Acil servislerde bir kısmını çok önemli gördüğümüz bir kısmını da çok sık uyguladığımız girişimsel işlemleri inceledik. Acil servislerde uygulanan endotrakeal entübasyon, santral venöz kateterizasyon, tüp torakostomi, nazogastrik tüp takılması, mesane sonda uygulamaları, kesi onarımları gibi girişimlerin acil servislere olan başvurularla korelasyonunu, yıllar içerisindeki niceliksel değişimlerini, pandemi sürecinin gerek acil başvurularına gerekse bu müdahalelere etkisini inceledik.

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Gereç ve Yöntem: Retrospektif tanımlayıcı planlanan bu çalışmada, Mart 2017-Mart 2021 tarihleri arasında Türkiye'de 2. ve 3. basamak kamu hastaneleri acil servislerine başvuran hastalara ait veriler ele alınmıştır. Ülkemizde pandeminin başladığı 11 Mart 2020 ile 11 Mart 2021 yılları arasındaki 1 yıllık pandemi süreci ile pandemi öncesi dönemdeki 11 Mart 2017-11 Mart 2020 yılları arasındaki verilerin yıllık ortalaması incelenmiş ve bu dönemler karşılaştırılmıştır. Veriler tablolama programı üzerine kaydedilmiş ve yüzdesel değişimleri tablolama programı istatistiksel formülleri üzerinden hesaplanmıştır.

Bulgular: 2017-2020 yılları arasında 2. ve 3. basamak kamu hastaneleri acil servis muayenelerinde sürekli artış görülmüş olup koronavirüs hastalığı-2019 (COVİD-19) pandemisiyle birlikte muayene sayıları 93,5 milyon hastaya düşmüştür. Endotrakeal entübasyon sayıları pandemi döneminde %11,6 oranında artmıştır. Pandemi sürecinde uygulanan santral venöz kateter sayıları pandemi öncesi 3 yıllık ortalamaya göre %16,2 oranında azalmıştır. Diğer uygulamalara baktığımızda tüp torakostomi %0,13 oranında, mesane sonda uygulaması %3,2 oranında, nazogastrik sonda uygulaması ve kesi onarımı %26,6 oranlarında pandemi döneminde azalmıştır.

Sonuç: COVİD-19 pandemisinin görüldüğü ilk 1 yıllık süreçte pandemi öncesi 3 yıllık döneme göre ortalama acil servis başvuruları, kesi onarımı, nazogastrik tüp uygulama, mesane sonda uygulaması, tüp torakostomi ve santral venöz kateterizasyon uygulamaları pandemi sürecinde azalmıştır. Pandemi döneminde, pandemi öncesi 3 yıllık sürece göre ortalama girişimsel işlem sayısı artan tek işlem endotrakeal entübasyon olmuştur.

Anahtar Kelimeler: Acil servis, endotrakeal entübasyon, pandemi

INTRODUCTION

In December 2019, a new coronavirus, the cause of which could not be found, was detected in the respiratory secretions of patients who had been admitted to hospitals with symptoms of lower respiratory tract infection in China. The World Health Organization (WHO) named this virus severe acute respiratory syndrome coronavirus-2 and the emerging infection was named as coronavirus disease-2019 (COVID-19) infection. As of March 11, 2020, WHO has declared a pandemic in the world (1).

Both during and before the pandemic, the most frequently admitted unit in the health facilities of our country was the emergency departments. The emergency departments have were areas where vital diagnoses were made, examinations and treatments were carried out. Many interventional procedures for diagnosis and treatment were being performed in the emergency departments.

We examined interventional procedures, some of which we consider critical and some that we apply very frequently in emergency departments. We analyzed the correlation of interventions such as endotracheal intubation, central venous catheterization (CVC), tube thoracostomy (TT), nasogastric tube (NGT) placement, bladder catheterization, incision repairs applied in emergency departments with the admissions to the emergency departments, the quantitative changes over the years, and the effect of the pandemic process on both emergency admissions and these interventions.

Our aim in the study is primarily to show the most frequently performed interventional procedures in emergency services, their changes according to years and their relationship with emergency room visits with current data.

METHODS

In this study, which was planned as a retrospective descriptive study, the data of the patients who were admitted to the emergency departments of the 2nd and 3rd level public hospitals in Türkiye, between March 2017 and March 2021 have been discussed. The annual mean value of the data between the 1-year pandemic period March 11, 2020 when the pandemic started in our country until March 11, 2021, and the data between March 11, 2017 and March 11, 2020, in the pre-pandemic period, were examined and these periods were compared.

The data were analyzed by obtaining the necessary permissions from the Ministry of Health. The study was initiated after obtaining the approval number E2-22-1620 of the Ankara City Hospital Clinical Research Ethics Committee (date: 30.03.2022). Our study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments.

Statistical Analysis

The data were recorded on the spreadsheet program and their percentage changes were calculated using the statistical formulas of the spreadsheet program.

RESULTS

When the emergency department admissions of the 2nd and 3rd level public health facilities between 2017 and 2020 were analyzed; we had determined that 107.4 million patients in 2017, 117.9 million patients in 2018 and 129.7 million patients in 2019 were examined in emergency departments. In 2020, there was an increase in emergency department patient admissions due to the COVID-19 pandemic and the number of examinations had decreased to 93.5 million patients (Figure 1). In the 3-year period before the pandemic, it was observed that there were a mean 118.4 million admissions per year and an annual increase of 9.6% were detected. During the 1-year pandemic period between 2020 and 2021, it was observed that the number of admissions decreased by 27.6% compared to the mean number of patients in the pre-pandemic period and the number of admissions decreased to 93.5 million.

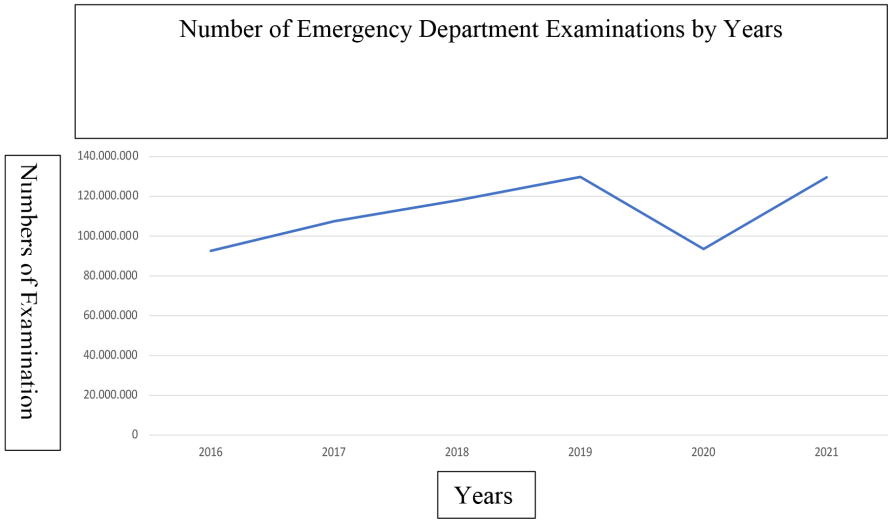

Figure 1. Number of emergency department examinations by years

When we considered the number of endotracheal intubations performed in the emergency department, it was seen that mean 86,447 endotracheal intubations were performed annually in the pre-pandemic period between 2017 and 2020, which corresponded to an annual increase of 9.7%. In the 1-year pandemic period between 2020 and 2021, an increase of 11.6% was determined compared with the mean endotracheal intubation in the 3-year period before the pandemic (Table 1).

When we considered the number of CVC inserted in the emergency department, it was seen that the annual mean value was 24,149 between 2017 and 2020 before the pandemic. It has been detected that the annual increasement ratio of these years corresponded to 18.5%. In 2020, it was determined that the number of insertions decreased to 20,225 and this decreasement was 16.2% compared with the mean value in the pre-pandemic period. In the pre-pandemic and pandemic periods, CVC insertions have changed proportionally with the emergency service admissions in these periods. When we considered the most frequently preferred veins for CVCs in our study, we had seen that the jugular vein was preferred most frequently with an annual mean of 11,124, which was followed by the femoral vein with an annual number of 11,041,5. The subclavian vein was preferred as the least with an annual number of 1,002,5 (Table 2).

When we examined the TT levels applied in the emergency department, it was seen that mean 7,212 chest tubes were inserted between 2017 and 2020, and it was determined that there was an annual increase of 3.2% in the pre-pandemic period. In 2020, it was determined that there was no significant change with the pandemic, and there was only a 0.13% decreasement in the 1-year period. We observed that the most frequently performed interventional procedure in the emergency department was incision repair. In the 3-year period before the pandemic, the annual mean number of incision repairs was 1,766,329, which has been determined as 1.52% among all emergency admissions (Table 1).

In the first 1-year period during the pandemic process, it was determined that incision repair decreased to 1,295,397 and the ratio among total emergency admissions was 1.38%. The annual increase ratio of the patients admitted for incision repair between 2017 and 2020 was determined as 8.5%. Since the 2020 pandemic, there was a significant decreasement in admissions and it decreased by 26.6% compared to the mean value of the pre-pandemic period. In the emergency department following incision repair, the most frequently performed interventional procedure was bladder catheterization. In the pre-pandemic period between 2017 and 2020, a mean urinary catheter was inserted into the bladder 805,686 times per year. During this process, a continuous increase was observed every year and the annual increase ratio was mean 11.5%. In 2020, 779,749 catheters were inserted and a decline of 3.2% was detected compared to the pre-pandemic mean value.

Another invasive procedure that has been frequently performed in the emergency department was the NGT placement. The mean number of NGT placements per year in the pre-pandemic period was detected as 269,770. This result corresponded to approximately 0.2% of emergency admissions. Although the number, which was 244,362 in 2017 during the pre-pandemic period, decreased in 2019, 4.9% annual mean increasement was observed in this 3-year period. In the first year of the pandemic, this number

Table 1. The number of emergency service admissions and interventional procedures over years

Column 1	2017	2018	2019	2020
The name of the process	The number of the process	The number of the process	The number of the process	The number of the process
Emergency department admission	107,491,580	117,982,971	129,764,460	93,567,880
Endotracheal intubation	75,520	86,113	97,708	96,529
Central vein catheterization	19,980	21,391	31,078	20,225
Tube thoracostomy	7,043	7,099	7,494	7,188
Incision repair	1,587,912	1,717,242	1,993,833	1,295,397
Bladder catheter insertion	715,597	824,634	875,938	779,749
Nasogastric tube placement	244,362	296,240	268,708	197,835

Table 2. The number of central venous catheters inserted in the emergency department by years

Column 1	2017	2018	2019	2020
The name of the process	The number of the process	The number of the process	The number of the process	The number of the process
Jugular vein catheterization	8,068	11,181	16,032	9,217
Femoral vein catheterization	10,861	9,204	13,939	10,162
Subclavian vein catheterization	1,051	1,006	1,107	846
Total	19,980	21,391	31,078	20,225

decreased to 197,835, and the ratio of decreasement was 26.6% compared to the number of NGTs that had been placed before the pandemic (Table 1).

DISCUSSION

When the data of the last four years were examined, emergency service admissions have increased continuously until 2020, when the COVID-19 pandemic had started and peaked, with an annual mean of 9.6%. The mean levels of all interventional procedures performed were on the rise in the pre-pandemic period. In 2020, with the effect of the COVID-19 pandemic, all interventional procedures along with the number of patients had received their share from the pandemic and had showed a significant decreasement.

Endotracheal intubation is a life saving procedure that is been commonly performed in the emergency room, intensive care unit, interventional radiology applications, pre-hospital patient transportation, or for critical patients in the hospital wards (2).

Airway management skill is one of the maximum important aids among all of the treatment methods and tools of the emergency physician at the time of emergency intervention requirement for the patient. The main purpose of airway management is to provide oxygenation and ventilation for the patients. The maintenance of ventilation and oxygenation for the patient can be done with a simple maneuver such as repositioning the patient's head, or it can be achieved with a complicated technique that requires opening a surgical airway to the patient (3).

In our study, we have determined that the rates of endotracheal intubation in emergency departments had increased continuously in correlation with the increase in admissions to the emergency department in the pre-pandemic period. Despite the 27.6% decreasement in admissions to the emergency department during the 2020 pandemic period, there was no significant increase in endotracheal intubation levels, and it increased by approximately 11.6% compared to the mean value of the last 3 years. It has been considered that the increase before the pandemic was due to excess patient population, and the high rates during the pandemic period were due to the high number of patients with respiratory failure secondary to COVID-19 pneumonia.

CVC is a commonly used method in patients undergoing both surgical and medical treatment. CVC; enables many procedures done such as haemodynamic monitoring, intravenous drug therapy, plasmapheresis, hemodialysis, and total parenteral nutrition in patients who present with cardiac arrest, in need of massive fluid

replacement, and in patients with no peripheral venous access (4).

In accordance with the increase in the number of emergency medicine specialists in our country, the CVC procedure has become a more feasible procedure in emergency departments. It is vital to provide an efficient and rapid intravenous route, particularly in unstable patients who are admitted to the emergency department. The CVC procedure can be performed accurately and rapidly and those complications can must be intervened once they occur (5).

In a study conducted in our country, it was determined that the most common cause of CVC insertion was hemodialysis (54%), and trauma had a ratio of only 5.6%. In terms of frequency sequence internal jugular vein, femoral vein, and subclavian veins were preferred in patients (5).

Similarly, in our study, the jugular vein was most frequently preferred, which was followed by the femoral vein and then the subclavian vein (Table 2). In the general overview of all CVC procedures by years, it has been determined that there was a decreasement when the previous years were compared with the period in which the cause of admission was COVID-19 infection. This reduction was considered the patients with COVID-19 infection were hospitalized or discharged before the need for CVC insertion.

NGT placement has been performed in thousands of patients who were being treated in the hospital every year for diagnostic, preventive, or therapeutic purposes as a complementary to medical and surgical procedures (6).

NGT placement has often been used for upper gastrointestinal bleeding, gastric emptying and lavage after suicidal and accidental intoxication, gastric outlet obstructions, intestinal obstructions, feeding the patients with difficult oral intake, to prevent gastric distension in some patients, to testing gastric contents, before surgery and after surgery for decompression (7). The use of NGT is increasing in hospitals and at homes due to the increase in the number of elderly patients and chronic diseases (8). Although there was no continuous increasement or decreasement before the COVID-19 period, there was an increase in total NGT placement. Along with the COVID-19 pandemic, a very significant decreasement in practice has been detected. It has been considered that this decreasement was related to the increase in the admissions of patients with chronic diseases and intoxication to the emergency departments.

Bladder catheter application; is the procedure of placing a catheter from the urethra to the bladder for diagnosis or treatment, which is required for many reasons such as urine output monitoring for critically ill patients, removal of urinary obstruction, surgical operations and palliative support.

This procedure increases the examination and follow-up possibility of clinicians and allied health personnel, and in some cases, it can increase the quality of life of the patient (9). Thus, it is one of a procedures that is widely performed in emergency departments and for the treatment and care of inpatients. In our study, it was determined that there were more bladder catheter applications than the increase in emergency service admissions in the pre-COVID-19 period, and there was an increase in the number of applications with the pandemic. When we consider the reasons for the cessation of elective surgical operations during the pandemic period, the decrease in hospitalizations other than COVID-19 infected patients, the application of catheters for palliative support with the help of units such as home care services other than the emergency department can be counted.

TT is one of the important surgical procedures frequently performed in emergency departments. This procedure can be applied to the patient in the operating room and at the bedside. The procedure is generally performed by thoracic surgeons, emergency medicine specialists and their assistants, interventional radiologists and intensive care specialists. There were many indications for TT. In the emergency department, the procedure was mostly performed due to spontaneous pneumothorax, traumatic pneumothorax and hemothorax (10).

The thoracic tube provides an evacuation of air or fluid (blood, lymph, pus and other) in the pleural cavity and expansion of the lungs (11,12).

Although TT is the most common surgical intervention performed by thoracic surgeons, it is a life-saving procedure especially in cases such as tension pneumothorax and massive pleural effusion. Therefore, emergency medicine specialists should accurately determine TT indications and perform TT techniques appropriately (11,12). In our study, when we consider TT procedures by years, a continuous increase was seen in the pre-COVID-19 period and although not at serious rates compared to the previous year, a slight increase was seen with the pandemic process. This may be once more due to the increase in accident and trauma cases, with lockdown restrictions.

Additionally, in this process, patients pleural fluid secondary to pneumonia or for any other reason and who needed pleurocan or TT were unkept waiting for a long time in the emergency departments and their procedures

were performed directly in the services or intensive care units.

Another group of procedures in the emergency department is incision repair. Incision repair is an important and urgent interventional treatment procedure applied to the patients with injuries.

The types of injuries of the patients who admitted to the emergency department can be seen in a wide range, from soft tissue injuries and simple cuts to complicated cuts (tendon, nerve injury) (13,14).

In different studies conducted, pre-COVID-19 emergency admissions by the ambulance were examined and it was determined that most of the trauma cases requiring TT and incision repair were transported by ambulance (15-17).

In the study by Oktay et al. (15), among the emergency department admissions, the first rank was trauma (33.1%), the second rank was cardiovascular system (CVS) diseases (18.5%), and the third rank was neurological diseases (14.2%). In the study of Zenginol et al. (16), 29.80% of the patients transported to the emergency department by ambulance were found to be trauma cases.

In the study by Önge et al. (17), admissions by ambulance were found to be 28.4% trauma, 16.4% neurological and 14.2% CVS diseases. In a study conducted during the COVID-19 pandemic period in 2021, it was observed that the rate of trauma cases transferred to the emergency department by ambulance was 17.7% (18). During the COVID-19 period the decreasement in trauma patients transported by ambulance, can be attributed to tthe decreasement in the number of patients in need of incision repair and TT.

The limitation of the study is the absence of data from private and university hospitals.

CONCLUSION

As a result, with a significant decrease in the number of examinations during the pandemic period, a decrease was observed in the number of interventional procedures except endotracheal intubation. The high number of emergency service visits and procedures has shown the importance of emergency services in our country's health system again.

ETHICS

Ethics Committee Approval: The study was initiated after obtaining the approval number E2-22-1620 of the Ankara City Hospital Clinical Research Ethics Committee (date: 30.03.2022). Our study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments.

Informed Consent: Retrospective study.

Authorship Contributions

Concept: A.B., H.C., Design: A.B., H.C., Data Collection or Processing: H.C., Analysis or Interpretation: A.B., Literature Search: H.C., Writing: A.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Research

Investigation of Potential Chest Computed Tomography Findings Associated with Incidental Gynecomastia in Adults

Erişkinlerde Saptanan Tesadüfi Jinekomasti ile İlişkili Olası Toraks Bilgisayarlı Tomografi Bulgularının Araştırılması

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ABSTRACT

Objective: To examine the incidence of gynecomastia detected by chest computed tomography (CT) in the adult male population and its association with fatty liver disease, abdominal wall, and retrorenal and subcutaneous fatty tissue thickness.

Methods: Chest CT scans of 1,191 patients were scanned retrospectively. One hundred fifty-eight cases with a fibroglandular tissue diameter (FGTD) of more than 2 cm were accepted as gynecomastia. Forty-five cases with FGTD less than 1 cm constituted the control group. Patterns of gynecomastia, FGTD, the subcutaneous adipose tissue thickness of breast (B-ATT), abdominal wall (AW-ATT) and retrorenal adipose tissue thickness (RR-ATT) were measured in mm. Additionally, the Hounsfield unit attenuation of the liver and spleen was obtained, and a liver-to-spleen (L/S) ratio below 0.8 was considered hepatosteatosis. The correlation between all these parameters was analysed by Kendall's Tau-b.

Results: The incidence of gynecomastia was calculated as 13.2% (158/1,191), observed bilateral in 84.2% (n=133) and unilateral in 15.8% (n=25) of the cases. The dendritic pattern was the most observed type, with 57.6% (n=91) of the cases. There was a significant difference between B-ATT, AW-ATT, and RR-AAT values and gynecomastia ($p<0.005$), but a weak relationship was observed ($r=0.137$, $r=0.132$, $r=0.098$, respectively). When the study population was divided into two groups according to the L/S ratio, no significant difference was found, regarding FGTD and all the adipose tissue thickness parameters ($p>0.05$).

Conclusion: Gynecomastia may signify visceral obesity rather than innocent hypertrophy. In clinical practice, incidental detection of gynecomastia by CT can play a useful role in case management.

Keywords: Gynecomastia, computed tomography, adipose tissue thickness, visceral obesity, hepatosteatosis

ÖZ

Amaç: Erişkin erkek popülasyonda toraks bilgisayarlı tomografisi (BT) ile saptanan jinekomasti insidansını ve yağlı karaciğer hastalığı, karın ön duvarı, retrorenal ve deri altı yağ doku kalınlığı ile arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Bin yüz doksan bir hastanın toraks BT görüntüleri geriye dönük olarak tarandı. Fibroglandüler doku çapı (FGTD) 2 cm'den fazla olan 158 olgu jinekomasti olarak kabul edildi. Bu kalınlık 1 cm'nin altında olan 45 olgu ise kontrol grubunu oluşturdu. Jinekomasti paternleri, FGTD, memenin deri altı yağ dokusu kalınlığı (B-ATT), karın ön duvarı (AW-ATT) ve retrorenal yağ dokusu kalınlığı (RR-ATT) mm cinsinden ölçüldü. Ek olarak, karaciğer ve dalak dansitesi Hounsfield birimi cinsinden elde edildi ve karaciğer-dalak (L/S) dansitesi oranının 0,8'in altında olması hepatosteatoz olarak kabul edildi. Tüm bu parametreler arasındaki korelasyon Kendall's Tau-b ile istatistiksel olarak analiz edildi.

Bulgular: Jinekomasti insidansı %13,2 (158/1.191) olup, olguların %84,2'sinde (n=133) iki taraflı ve %15,8'inde (n=25) tek taraflı idi. En çok gözlenen tip olan dendritik patern olguların %57,6'sında (n=91) saptandı. B-ATT, AW-ATT ve RR-AAT değerleri ile jinekomasti arasında anlamlı fark ($p<0,005$) saptanmakla birlikte zayıf bir ilişki gözlendi (sırasıyla $r=0,137$, $r=0,132$, $r=0,098$). L/S oranına göre ayrılan iki grup arasında FGTD ve tüm adipoz doku kalınlık parametreleri açısından anlamlı fark bulunmadı ($p>0,05$).

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Sonuç: Jinekomasti, masum bir hipertrofiden ziyade viseral obezite gibi ciddi patolojileri işaret ediyor olabilir. Klinik pratikte, BT ile tesadüfi olarak jinekomasti saptanması olguların yönetiminde faydalı bir rol oynayabilir.

Anahtar Kelimeler: Jinekomasti, bilgisayarlı tomografi, adipoz doku kalınlığı, visseral obezite, hepatosetatoz

INTRODUCTION

The most common pathology of the male breast is gynecomastia (1). In the autopsy series, up to 55% rates have been reported (2). Gynecomastia is a benign breast pathology characterized by the proliferation of involutioned ducts and stroma. As a result, an increase in the breast tissue volume is expected. Three peaks have been defined for gynecomastia: neonatal period, puberty and sixth (3) decade. Its etiology is highly variable, and is primarily based on the disruption of the balance between estrogen and androgen levels. It may develop physiologically during puberty and old age or may occur secondary to various endocrine, systemic, tumoral, or toxic causes (4). In the presence of obesity, the increase in estrogen level due to the effect of the aromatase enzyme released from the adipose tissue also causes gynecomastia. It is considered idiopathic if gynecomastia persists for at least 18 months without any underlying cause (5). This condition may occur in middle or elder ages. Clinically, gynecomastia is easy to diagnose, on physical examination, it usually presents as a painful, mobile hard mass behind the nipple. It is generally bilateral and asymmetrical since the etiology is systemic. However, it can also occur unilaterally (4). Three well-defined gynecomastia patterns have a typical appearance on mammography (MG) and ultrasonography (USG). The fluoride phase corresponding to the reversible phase is observed in a nodular pattern. In this phase, it is disc-shaped, well-defined opacity in the retro-areolar area on MG and a hypoechoic mass or area surrounded by adipose tissue on the USG. The chronic phase, which is irreversible, is called the dendritic pattern. This phase is observed as a wedge-shaped heterogeneous density with radiating extensions toward the fatty tissue, with the apex facing the nipple on the MG. The USG equivalent is an irregularly shaped, heterogeneous hypoechoic area with no sharp borders in the same location. The third pattern is a heterogeneous increase in density filling the entire breast on MG and USG, resembling the female breast. It is crucial to exclude any type of malignancy in the differential diagnosis of gynecomastia.

With the increasing frequency of radiological imaging and widespread use of thoracic imaging, gynecomastia has become one of the most common pathologies encountered incidentally in chest computed tomography (CT) (6). However, there is limited knowledge in the literature investigating the clinical significance of incidentally occurring gynecomastia. In this study, we investigated the incidence of gynecomastia detected by chest CT in the adult male population and its association with fatty liver disease in addition to the abdominal wall, retrorenal and subcutaneous fat tissue thickness.

METHODS

This retrospective study was approved by the Non-Interventional Clinical Research Ethics Committee of the İstanbul University-Cerrahpaşa (decision no: E-83045809-604.01.01-406516, date: 13/06/2022). Chest CT images of 1191 adult male cases obtained in our hospital for various reasons and archived in the picture archiving and communication system (PACS) between January 2019 and December 2021 were scanned. Contrast-enhanced scans with insufficient technical quality, women cases, men with a history of breast surgery and patients under the age of 18 years were excluded. Additionally, conditions that may be in the etiology of secondary gynecomastia, such as malignant cases, cases with alcohol or specific drug use, or systemic diseases like cirrhosis, chronic renal failure, hypothyroidism, were excluded. Non-enhanced sequences of angiographic CT scans were evaluated. As a result, 203 adult male cases aged between 18 and 88 were included in the study. The generally accepted cut-off value for fibroglandular tissue diameter for gynecomastia in the literature is 2 cm (7-9). In consequence, while 158 cases were accepted as gynecomastia, the control group consisted of 45 cases with a diameter of less than 1 cm.

According to CT findings, 158 cases constituted the gynecomastia group, while 45 cases formed the control group.

In 91 of these cases, no known disease could cause comorbidity. Additionally, various known chronic diseases such as hypertension (HT), cerebrovascular disease and diabetes mellitus in 47 cases, inflammatory processes such as coronavirus disease-2019, pancreatitis, and sepsis in 23 cases, a trauma in 28 patients and emergency vascular pathology-like pulmonary embolism, or dissection in 14 cases were recorded. The demographic data and variables related to gynecomastia are summarized in Table 1.

All thoracic images were obtained by one of the two CT scanners with 128 detectors (SOMATOM Definition AS, Siemens Healthcare, Forchheim, Germany and Revolution

Table 1. Patient population and gynecomastia variables

Variables	Gynecomastia (n=158) n (%)	Normal (n=45) n (%)
Age mean ± SD	48.79±19.98	53.44±18.92
Medical records		
None/not known	75 (47.5)	16 (35.6)
Yes		
Chronic diseases	29 (18.3)	18 (40)
Acute vascular conditions	14 (8.9)	-
Acute infectionous diseases	19 (12)	4 (8.9)
Trauma	21 (13.3)	7 (15.5)
Gynecomastia involvement		
Unilateral	25 (15.8)	-
Bilateral	133 (84.2)	-
Gynecomastia pattern		
Nodular	56 (35.4)	-
Dendritic	91 (57.6)	-
Diffuse	11 (7)	-

SD: Standard deviation

HD, General Electric Systems, Waukesha, WI, USA). CT scan parameters for the thoracic imaging protocol were as follows: 100 kV tube voltage, 200 mA, matrix 512x512, slice thickness of 1 mm.

All CT scans were retrospectively evaluated via the PACS system by two radiologists (E.M.Ö. and E.Y.Ö.) in consensus who were blinded to the any clinical information of the patient. In case of discrepancy, one of the board-certified radiologists (S.A.K. or Y.K.) with more than ten years of experience in radiologic imaging was consulted. First, the fibroglandular tissue diameter (FGTD) and the pattern of gynecomastia were recorded. For FGTD, the mediolateral length of the fibroglandular density was measured in mm. Measurements of higher than 20 mm in the axial plane were accepted as gynecomastia (Figure 1). Gynecomastia patterns were recorded separately for each case, divided into three types: nodular, dendritic, or diffuse (Figure 2).

Then, the subcutaneous fatty tissue thickness of the breast and abdominal wall and the adipose tissue thickness of the retrorenal space were measured and recorded in mm (Figure 3). The breast adipose tissue thickness (B-ATT) was measured at the nipple level, where it was thickest. For the abdominal wall adipose tissue thickness (AW-ATT), the distance from the central part of the rectus muscle to the skin was recorded at the umbilicus level. Retrorenal adipose

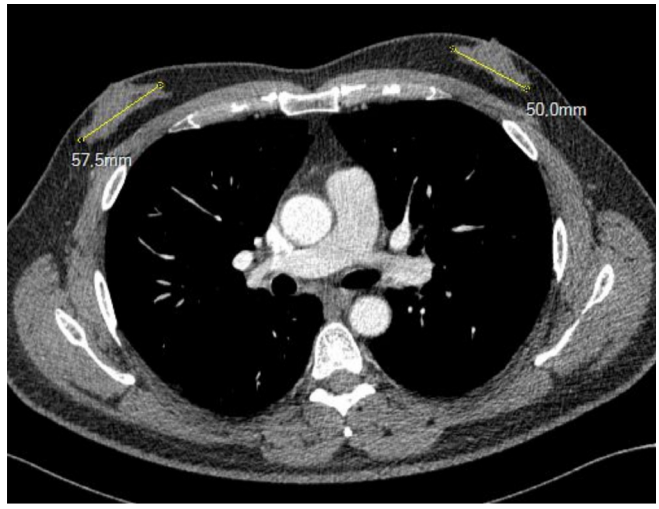

Figure 1. FGTD measurement in the transverse plane in a 48-year-old case with gynecomastia of dendritic pattern on both sides
FGTD: Fibroglandular tissue diameter

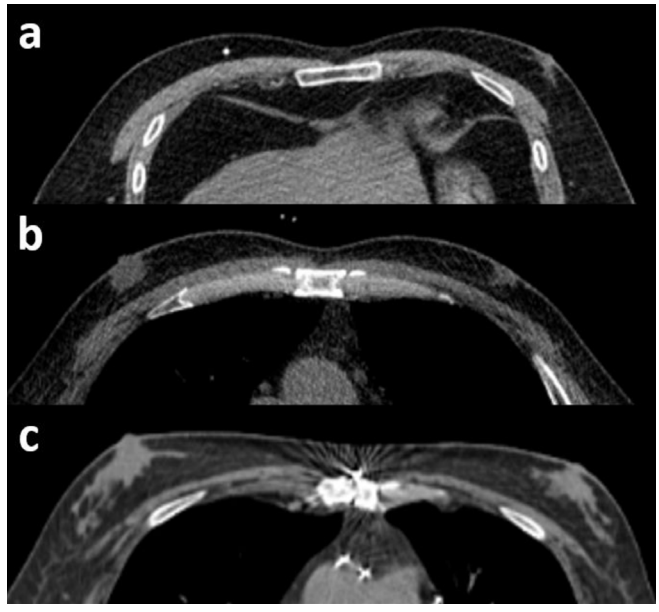

Figure 2. Patterns of gynecomastia, (a) nodular, (b) dendritic, (c) diffuse

tissue thickness (RR-ATT) was obtained by measuring the widest distance of fatty tissue in the retrorenal space at the level of the renal hilum. Finally, the mean Hounsfield unit attenuation of the liver and spleen on CT was recorded (Figure 4). For this purpose, a circular region of interest was used in axial sections passing through the upper abdomen. A liver-to-spleen (L/S) ratio below 0.8 was considered significant for hepatosteatosis.

Statistical Analysis

SPSS version 25.0 was used for all statistical analyses. After descriptive analysis, the patient population was divided into two groups according to the presence of gynecomastia and the L/S ratio. The correlation between all variables

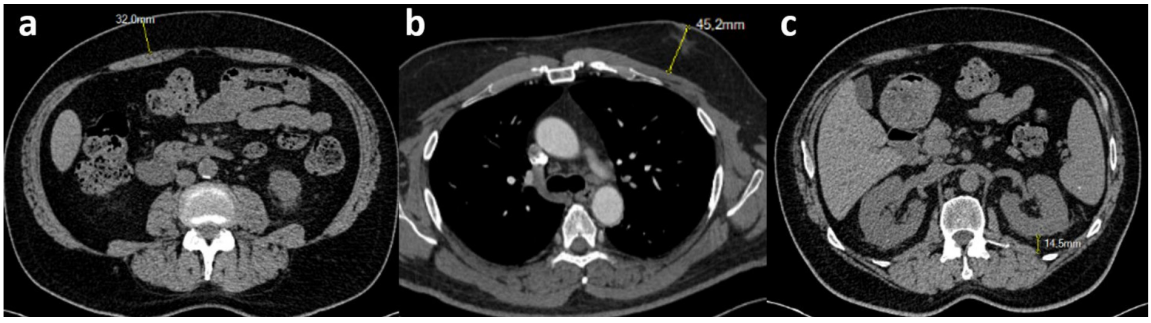

Figure 3. Adipose tissue thickness measurements in a 58-year-old man with dendritic gynecomastia. (a) Retrorenal adipose tissue thickness. (b) Abdominal wall adipose tissue thickness. (c) Breast adipose tissue thickness

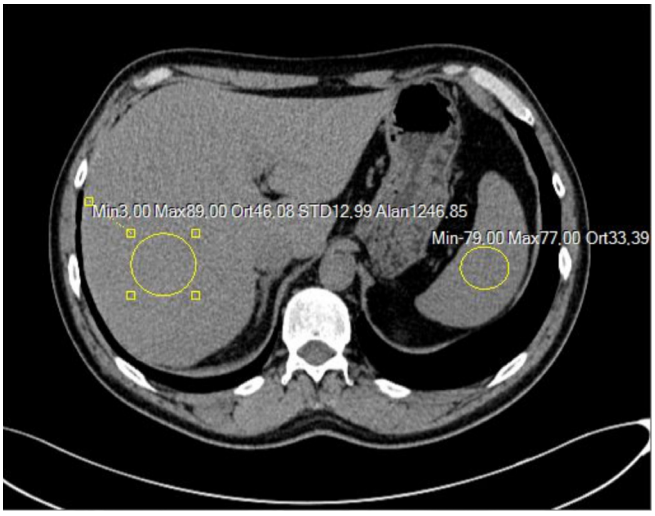

Figure 4. HU measurements of the liver and spleen
HU: Hounsfield unit

was analyzed by Kendall's Tau-b. A p-value of <0.05 was accepted as significant.

RESULTS

The incidence of gynecomastia among scanned CT scans was 13.2% (158/1191). The mean age was 48.79±19.98 in the gynecomastia group, and 53.44±18.92 in the control group. Gynecomastia was bilateral in 84.2% (n=133) and unilateral in 15.8% (n=25) of the cases. The most frequently observed pattern was the dendritic pattern, indicating the chronic phase, 57.6% (n=91). While the nodular pattern was observed in 35.4% (n=56) of the cases, the diffuse pattern was recorded in only 7% (n=11). The mean FGTD was 28.59∓10.17 mm.

No significant difference could be found between the group with gynecomastia, and the control group in terms of age, adipose tissue measurements and L/S ratio (Table 2). However, a significant difference between B-ATT, AW-ATT, and RR-AAT values and gynecomastia (p<0.005), a weak relationship was observed by Kendall's Tau-b (r=0.137, r=0.132, r=0.098 respectively). A moderate association was found between B-ATT and AW-ATT, RR-ATT (r=0.559, r=0.394 retrospectively, p<0.001).

When the patients were divided into two groups according to the cut-off value of 0.8 for the L/S ratio, no significant difference was found in terms of age, FGTD, and all the adipose tissue thickness parameters (p>0.05) (Table 3).

The results of the correlations are summarized in Table 4.

DISCUSSION

Our study found a weak but significant relationship between gynecomastia and B-ATT, AW-ATT and RR-ATT. To the best of our knowledge, the relationship between gynecomastia and retrorenal adipose tissue was investigated for the first time in the literature. Additionally, hepatosteatosis was not associated with gynecomastia or adipose tissue thickness in our patient population.

Gynecomastia, which is defined as the benign proliferation of male breast tissue, is seen with a frequency of 32%-65% in the general population (10). This rate is 60%-90% in the neonatal period, 50%-60% in adolescence, and around 70% in the elderly (3). It is thought that the underlying cause of gynecomastia is the imbalance between estrogen and androgen activity. Various studies have reported that maternal estrogen in the neonatal period, rapid increase in estrogen production in puberty, and decreased androgen production in old age are responsible for gynecomastia. (4,10,11). In addition to idiopathic and physiological causes, gynecomastia can occur in systemic diseases such as chronic liver disease and chronic renal failure, endocrine diseases such as hypothyroidism and hypogonadism, Klinefelter syndrome, the presence of gonadal, adrenal and pituitary tumors, hepatocellular cancer, the use of various drugs, or chronic alcoholism (4). The amount and activity of the aromatase enzyme, which converts testosterone to estrogen in adipose tissue, increase in the presence of obesity. The increased leptin production in obesity also contributes to the development of fibroglandular tissue. The most critical steps in diagnosing gynecomastia are anamnesis

Table 2. Variables according to the presence of gynecomastia

	Gynecomastia (n=158)	Control (n=45)	p-value	95% CI	
				Lower limit	Upper limit
Age	48.79±19.98	53.44±18.92	>0.05	-11.23	1.92
B-ATT mm	19.11±9.32	18.87±11.39	>0.05	-3.03	3.50
AW-AAT mm	18.45±9.11	18.27±10.41	>0.05	-2.95	3.31
RR-AAT mm	9.31±8.66	9.51±9.61	>0.05	-3.15	2.76
L/S ratio	1.20±0.40	1.22±0.42	>0.05	-0.15	0.12

AW-AAT: Abdominal wall adipose tissue thickness, B-ATT: Breast adipose tissue thickness, CI: Confidence interval, RR-AAT: Retrorenal adipose tissue thickness, L/S ratio: Liver-to-spleen ratio

Table 3. Variables according to L/S ratio

	L/S ratio >0.8 (n=160)	L/S ratio <0.8 (n=43)	p-value	95% CI	
				Lower limit	Upper limit
Age	50.56±20.46	47.04±17.05	>0.05	-3.18	10.22
FGTD mm	25.88±12.35	24.64±9.75	>0.05	-2.77	5.26
B-ATT mm	18.79±9.9	20.05±9.37	>0.05	-4.58	2.05
AW-ATT mm	17.95±9.43	20,14±9.13	>0.05	-5.37	0.97
RR-ATT mm	9.07±8.69	10.42±9.45	>0.05	-4.35	1.65

AW-AAT: Abdominal wall adipose tissue thickness, B-ATT: Breast adipose tissue thickness, CI: Confidence interval, FGTD: Fibroglandular tissue diameter, RR-AAT: Retrorenal adipose tissue thickness, L/S ratio: Liver-to-spleen ratio

Table 4. Correlation analysis of measurement parameters in gynecomastia

Parameters	Coef.	Std. Err.	p-value	95% CI	
				Lower limit	Upper limit
B-ATT	0.137	1.68326	0.005	-5.37163	0.97896
RR-ATT	0.098	1.52238	0.046	-4.54283	1.84257
AW-ATT	0.132	1.61032	0.006	-4.58726	2.05098

AW-AAT: Abdominal wall adipose tissue thickness, B-ATT: Breast adipose tissue thickness, CI: Confidence interval, RR-AAT: Retrorenal adipose tissue thickness, Std. Err.: Standard error, Coef.: Coefficient

and physical examination. The primary imaging methods used for diagnosis are MG and USG. The widespread use of CT recently has increased the prevalence of incidentally detected gynecomastia (6). The mean age was 48.79 years in our study, and it points to that the cases mostly belonged to the middle age group. No comorbidity factor or pathology that could cause gynecomastia was detected in 47.5% of the cases. However, since clinical and radiological follow-ups of these cases were not performed individually, further interpretation could not be made about whether there was idiopathic gynecomastia.

Obesity is accepted as excessive energy accumulation in adipose tissue, and its regional deposition is closely associated with morbidity and mortality (12). The intraperitoneal adipose tissue is the visceral fat area that could be measured by CT scans for decades. Until date, the increase in the ratio of this tissue to subcutaneous adipose tissue has been accepted as visceral obesity in various studies (12-14). Visceral obesity is closely associated with metabolic syndrome and cardiovascular diseases, and reducing visceral adipose tissue also decreases the risks of these obesity-related disorders. Our study found a significant relationship between visceral adipose tissue thickness obtained from the retro-renal area called RR-ATT and gynecomastia ($p<0.05$). As far as we know, there is no other study investigating retrorenal fat tissue thickness and gynecomastia in the literature. Therefore, we believe that clinical evaluation regarding visceral obesity and obesity-related disorders would be beneficial in cases with incidental

gynecomastia detected on CT or primary gynecomastia diagnosed clinically.

Hepatic steatosis, also known as fatty liver disease, is associated with low testosterone and high estrogen levels, similar to gynecomastia (15). However, it is not possible to reach a definite conclusion in the literature to support a significant relationship between hepatosteatosis and gynecomastia. While a positive correlation was found between gynecomastia and subcutaneous fatty tissue thickness and body mass index (BMI) in literature performed with CT, no significant correlation was found with hepatosteatosis (6,16). Besides, CT scan has been reported to be more effective in diagnosing moderate to severe hepatic steatosis (17,18). Although our study did not specifically focus on cases diagnosed with fatty liver disease, no significant association was found with gynecomastia, similar to the literature ($p>0.05$) (6,19).

There may be several reasons for the result of our study to be insignificant with hepatosteatosis and significant but weakly related to adipose tissue thickness. The most critical reason may be the exclusion of patients with comorbidities known to cause gynecomastia. It is predictable that these comorbid factors are associated with gynecomastia or adipose tissue accumulation. In contrast, we focused on incidental gynecomastia detected without these etiological factors.

This study has some limitations. First, although our original sample was not small, a limited number of patients belonging to a particular time were studied. However, we did not analyze our sample by distributing it the age groups. Although some studies in the literature accept a cut-off of 1.5 cm for gynecomastia, we have studied 2 cm above, similar to the most accepted studies (7-9). It was not possible to correlate the gynecomastia findings observed on CT with MG or USG in all cases. BMI and physical examination findings could not be studied because of the retrospective design of the study. Despite the hospital records being examined in detail, it is not possible to know all the drugs used by the patients. Finally, liver size was not studied because it did not fully enter the cross-sectional area in any patients.

CONCLUSION

In conclusion, the resulting data of our study support that gynecomastia may be a precursor to more severe problems such as visceral obesity and obesity-related disorders rather than innocent hypertrophy. In clinical practice, a multidisciplinary management can be adopted in patients detected gynecomastia secondary to the CT scan.

ETHICS

Ethics Committee Approval: Our study was approved by İstanbul University-Cerrahpaşa, Non-Interventional Clinical Research Ethics Committee on 13/06/2022 with the decision number E-83045809-604.01.01-406516.

Informed Consent: Informed consent was waived because of the retrospective nature of the study.

Authorship Contributions

Surgical and Medical Practices: P.Ç.K., Concept: S.A.K., Y.K., A.K.U., Design: S.A.K., Y.K., A.K.U., Data Collection or Processing: E.M.Ö., E.Y.Ö., E.Ş.D., Analysis or Interpretation: S.A.K., Y.K., E.M.Ö., E.Y.Ö., Literature Search: S.A.K., A.K.U., E.Ş.D., Writing: S.A.K., Y.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Research

The Role of the Chemokine CXCL12 on the Pathogenesis of Several Diseases

Kemokin CXCL12'nin Bazı Hastalıkların Patogenezindeki Rolü

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ABSTRACT

Objective: The aim of this study was to evaluate the possible role of the chemokine CXCL12 in the pathogenesis of some diseases. It also aims to research diseases and connect with each other with bioinformatic tools.

Methods: STRING/GeneMANIA/KEGG PATHWAY/GSEA/MSigDB for gene set enrichment analysis for gene protein and pathway interaction, TargetScan/miRDB for miRNAs targeting CXCL12, Blood eQTL Browser/ to target CXCL12 BIOS/QTLdb, GRASP and GWAS CXCL12 and miRNA region SNPs were used for disease associations.

Results: Gene set enrichment analysis of the gene set co-expressed in the GSEA/MSigDB tool revealed the association of genes related to allergic disease, arthritis, autoimmune disease of the musculoskeletal system, osteomyelitis (FDR<5E-06). Five hundred and ninety-three miRNAs were identified. As a result of examining the disease associations of SNPs from each miRNA gene region in GWAS databases, it was determined that P<7E-40 for B lymphoblastic leukemia/lymphoma. SNPs in CXCL12 did not show any GWAS associations, but blood eQTL/meQTL for CXCL12 showed associations with respiratory system disease, intestinal disease, combined immunodeficiency, multiple sclerosis, hepatitis (P<8E-06) and GWAS.

Conclusion: It is thought that CXCL12 may play a strong role in autoimmunity, inflammation, cancer and other diseases.

Keywords: CXCL12, pathways, SNP, diseases

ÖZ

Amaç: Bu çalışmanın amacı kemokin CXCL12'nin bazı hastalıkların patogenezindeki olası rolünü değerlendirmektir. Ayrıca biyoinformatik araçlarla hastalıkları araştırmayı ve birbirleriyle bağlantı kurmayı amaçlamaktadır.

Gereç ve Yöntem: Gen-protein ve yolak etkileşimi için STRING/GeneMANIA/KEGG PATHWAY/GeneCards gen seti zenginleştirme analizi için GSEA/MSigDB, CXCL12'yi hedefleyen miRNA'lar için TargetScan/miRDB, CXCL12'yi hedeflemek için Blood eQTL Browser/BIOS/mQTLdb, hastalık ilişkileri için GRASP ve GWAS CXCL12 ve miRNA bölgesi SNP'leri kullanıldı.

Bulgular: GSEA/MSigDB aracında birlikte eksprese edilen gen setinin gen seti zenginleştirme analizi, alerjik hastalık, artrit, kas-iskelet sisteminin otoimmün hastalığı, osteomyelit (FDR<5E-06) ile ilgili genlerin ilişkisini ortaya koydu. Beş yüz doksan üç miRNA tanımlandı. GWAS veri tabanlarında her bir miRNA gen bölgesinden SNP'lerin hastalık ilişkilerinin incelenmesi sonucunda, B-lenfoblastik lösemi/lenfoma için P<7E-40 olduğu saptandı. CXCL12 içindeki SNP'ler herhangi bir GWAS ilişkisi göstermedi, ancak CXCL12 için kanda eQTL/meQTL olarak, solunum sistemi hastalığı, bağırsak hastalığı, kombine immün yetmezlik, multipl skleroz, hepatit (P<8E-06) ile GWAS ilişkileri gösterildi.

Sonuç: CXCL12 otoimmünite, enflamasyon, kanser ve diğer hastalıklarda güçlü bir rol oynayabileceği düşünülmektedir.

Anahtar Kelimeler: CXCL12, yolaklar, SNP, hastalıklar

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INTRODUCTION

Approximately 50 chemokines have been identified in humans (1). Chemokines are protein molecules with a molecular weight of 8-12 kD and multiple domains. *Chemokine* genes 17q11.2-12 and *C-X-C chemokine* genes are also located at 4q13 locus. Stromal cell-derived factor 1 is a chemokine coded by the *CXCL12* gene and this gene on chromosome 10 in human (2). It has been shown in multiple tissues and cells. CXCL12 has a ligand relationship with the CXCR4 receptor. In adulthood, endothelial progenitor cells in the bone marrow (BM) are involved in angiogenesis by the CXCL12-CXCR4 mechanism (3).

CXCL12 is expressed in multiple sites, including the thymus, kidney, heart, spleen, liver, lung, brain, and BM. The *CXCL12* gene has chemotactic properties for lymphocytes (4). In embryogenesis, it regulates the formation of large blood vessels and the migration of hematopoietic cells from the fetal liver to the BM. Additionally, CD20 expression in B cells is regulated by CXCL12 signaling (5). CXCL12, which is also chemotactic for mesenchymal stem cells (MSCs), has a suppressive effect on osteoclastogenesis. It does this by controlling the inflammatory process during bone destruction (6).

SDF-1, an intercrine alpha family member, is composed of two forms. These are "SDF-1α/CXCL12a" and "SDF-1β/CXCL12b" formed by an alternative splicing mechanism. Chemokines are defined by four conserved cysteines forming two disulfide bonds and two disulfide bonds they form. The proteins belonging to the *CXCL12* gene are in the CXC chemokine group and are separated by the insertion of an amino acid between the first cysteine pair. Also, the first eight regions of the CXCL12 N-terminal function as a kind of receptor binding site, but only "Lys-1" and "Pro-2" are even directly involved in the activation of the receptor. The RFFESH motif is located at the loop site (residues 12-17) and acts as a CXCL12 insertion site for receptor binding (7).

It plays a role in functional states such as embryogenesis, angiogenesis, development of the immune system, development of infection, tissue homeostasis, tumor growth, metastasis. All chemokine receptors are membrane-bound molecules and contain 7-transmembrane domains in their structures and form pairs with G-proteins. Chemokine receptors are "G-protein-coupled proteins" and are expressed on leukocytes. The chemokines communicate with specific G-protein-coupled cell surface receptors on target cells, thereby initiating intracellular signaling. It induces activation and cell migration. Up to 20 chemokine receptors have been identified to date (8).

Substances such as platelet-derived growth factor, vascular endothelial growth factor-A released from platelets after the stimulus that initiates the inflammatory process in the tissue outside the vessel (bacteria, surgery, ag-ab complex, etc.), chemokines such as CXCL1, CXCL5, CXCL7, CXCL4 in the CXC type, act against the invading microorganism in the first stage. These are effective in creating barriers (9). Under the inhibitory effect of IL-1 and tumor necrosis factor-, the production of CXCL12, which is synthesized from fibroblasts and keratinocytes, gradually decreases until the 6th day. Then, from the 14th day onwards, many lymphocytes accumulate in the region under the effects of CXCL9 and CXCL10. A population of non-hematopoietic cells expresses intensely high degree levels of CXCL12 and stem cell factor (SCF), forked box C1 (FOXC1) and early B-cell factor 3 (EBF3) in human adult BM (10).

The CXC chemokine ligand has BM-specific MSCs called "reticular CXCL12 abundant reticular (CAR) cells" that strongly interact with leptin. Lep receptor (r) + stomal cells are the key component of hematopoietic stem cell (HSC) niches in murine BM (11). CAR T-cells that characterized MSCs by several distinctive features, including much higher expression of the LepR and HSC niche factors. Needed for the maintenance of HSCs, CXCL12, SCF and transcription factors are expressed relative to other cell types such as FOXC1 and EBF3 (12).

Multiple myelomas (MM) is known as a plasma cell malignancy characterized by the uncontrolled growth of malignant plasma cells starting in the BM. MM is the most common type of cancer seen in plasma cells. Targeting plasma cell precursors rather than HSCs is controlled by the chemokine CXCL12 (13,14). This chemokine also binds its receptor, CXCR4, on MM malignant cells, regulating their integration into the BM, transendothelial migration, and target identification (15-17). In the BM microenvironment, CXCL12 is mainly produced by specialized reticular BMSCs known as CAR cells.

This study aimed to evaluate the role of CXCL12 in MM and other diseases using Search Tool for Retrieval of Interacting Genes/Proteins (STRING), GeneMANIA, KEGG PATHWAY, the Molecular Signatures Database (MSigDB), TargetScan, miRDB Blood eQTL Browser/BIOS/mQTLdb, genome-wide repository of associations between SNPs and phenotypes (GRASP) and genome-wide association studies (GWAS).

METHODS

GeneCard

GeneCards is a searchable, integrative database of all annotated and found human genes providing extensive and detailed user-friendly information. The knowledge base

automatically presents gene-centered data to the user using ~150 web resources, involving genomic, proteomic, genetic, transcriptomic, clinical and functional information (https://www.genecards.org/cgi-bin/carddisp.pl?gene=CXCL12).

STRING

STRING is a biological database and comprehensive web resource of known and predicted protein-protein interactions (PPIs).

PPIs are the key components toward system-level understanding of cellular functions. It is a platform used for processing functional genomic data and distinguishing functional, structural and evolutionary features of the protein (https://string-db.org/).

GeneMANIA

GeneMANIA helps understand and reveal the function of the desired gene or gene clusters. GeneMANIA finds other genes associated with the selected gene using a countless set of functional data associations. Association data included co-expression, co-localization, protein, and genetic interactions, pathways, and protein domain similarity (https://genemania.org/).

KEGG Pathway

KEGG Pathway (https://www.genome.jp/kegg/pathway.html); provides information about the molecular reaction, interrelation and relationship networks for each substance listed below:

1. Metabolism
2. Genetic information processing
3. Environmental information processing
4. Cellular processes
5. Organismal systems
6. Human diseases
7. Drug development

GSEA/MSigDB

Gene set enrichment analysis (GSEA) is a computational method that determines whether an a priori defined set of genes. This method uses a statistical approach to show significant, concordant differences between two biological states.

MSigDB is a collection of descriptive gene set databases with using GSEA software (https://www.gsea-msigdb.org/gsea/msigdb/).

Target Scan Human

Target Scan is a web server in bioinformatics that predicts its biological targets by scanning sites [microRNAs (miRNAs)] that paired the seed region of each miRNA. For many species, 3'-compensating sites have been defined as other site types (http://www.targetscan.org/vert_80/).

Blood eQTL Browser/BIOS/mQTLdb

The eQTLGen consortium has been set up to identify the downstream consequences of trait-related genetic variants. The consortium incorporates 37 datasets, with a total of 31,684 individuals. These websites show the results of the cis-eQTL, trans-eQTL, eQTS, and replication: (Blood eQTL Browser: http://www.genenetwork.nl/bloodeqtlbrowser), (BIOS eQTL Browser: http://genenetwork.nl/biosqtlbrowser), (mQTLdb: http://mqtldb.org).

GRASP

GRASP is a web-based site. All information is obtained from the content and articles, and gives results by making genetic associations. All relationships with $p<0.05$ from GWAS defined as ≥25,000 markers tested for one or more than one features. (https://grasp.nhlbi.nih.gov/Search.aspx)

GWAS

GWAS attempt to identify genotypes and related genotypes. GWAS look for markers of the entire genomes of different individuals and offer statistical analysis at the population level. Thus, it reveals genotype-phenotype relationships (https://www.ebi.ac.uk/gwas/).

Data banks were used in this study and statistical analysis not done. This study includes bioinformatic analysis that does not require ethics committee approval. The study does not require financial support and this information is included in the method section.

RESULTS

STRING identified 32 genes interaction with CXCL12, including 8 additional CXCL genes in the vicinity of CXCL12 (Figure 1).

GeneMANIA showed 77.64% of CXCL12-related physical interactions (Figure 2). Also, it showed 8.01% CXCL12-related co-expression (Figure 3).

GeneCards showed 5,052 genes associated with CXCL12. We used Target Scan to select the miRNAs targeting CXCL12 and determined their other target genes. These results nearly significant as the co-expressed gene set, suggesting that miRNA's play a major role in the regulation of CXCL12 expression (Table 1).

The most meaningful co-expression pattern was noted in the BM followed by other organs (brain, heart, lung, kidney and skin). The KEGG Pathway Database found 35 pathways related to CXCL12 (Table 2). The GSEA of the co-expressed

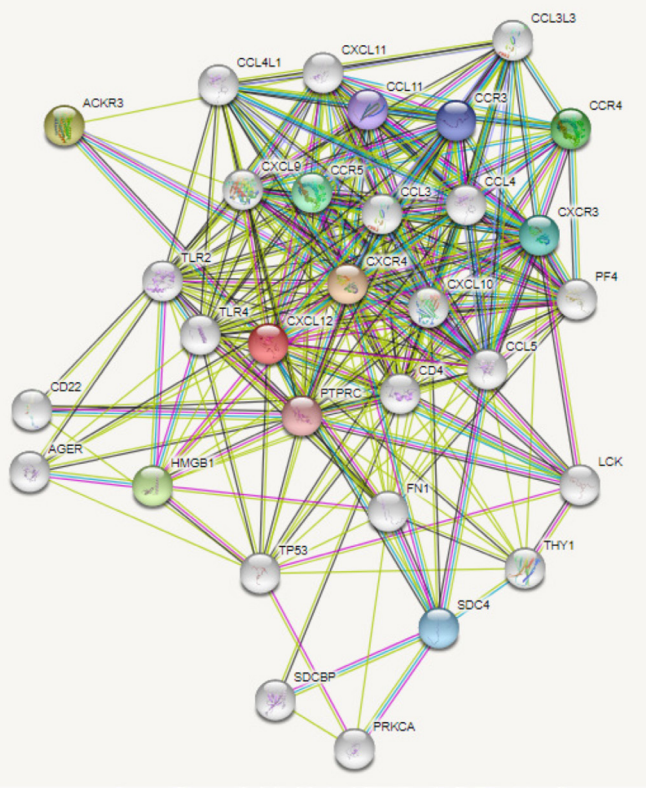

Figure 1. Gene interaction with CXCL12 (https://string-db.org/cgi/network?taskId=bjF8HZ0B6xjl&sessionId=bAKq4YR4db7U)

gene set in the GSEA/MSigDB tool suggested the enrichment of genes involved in allergic disease, arthritis, autoimmune diseases of the musculoskeletal system, bone inflammation disease (false discovery rates <5E-06). The investigation of disease associations of single nucleotide polymorphisms (SNPs) from each *miRNA* gene region in GWAS databases yielded results for B-lymphoblastic leukemia/lymphoma (p<7E-40). SNPs acting as eQTL/meQTL in blood for CXCL12 showed GWAS associations with; respiratory system disease, intestinal disease, combined immunodeficiency, multiple sclerosis, hepatic (p<8E-06).

DISCUSSION

Almost all *in vitro* studies of MM cell accumulation and MM cell migration have specifically selected the "CXCL12a isoform". Additionally, reported in vivo studies are lacking in conclusions regarding the specific functions of other isoforms of "CXCL12". This is because the mice used carry a complete excision of CXCL12 or a deletion of CXCR4, the cognate receptor for all isoforms (18-21). Additionally, the recently identified CXCL12 (CXCL12γ) gamma isoform showed much higher activity than the "canonical" CXCL12a isoform (15), inducing leukocyte recruitment and angiogenesis.

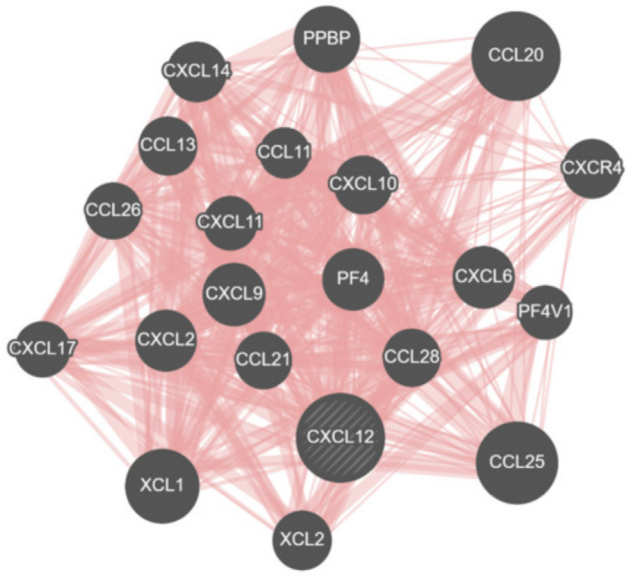

Figure 2. Physical interactions with CXCL12 (https://genemania.org/search/homo-sapiens/cxcl12)

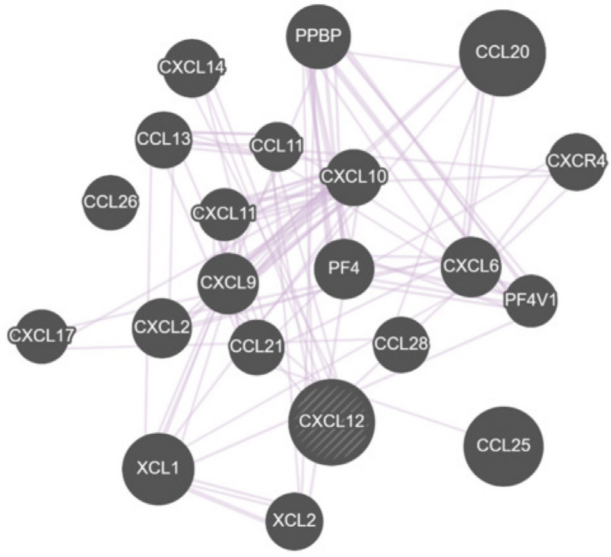

Figure 3. Co-expression with CXCL12 (https://genemania.org/search/homo-sapiens/cxcl12)

However, CXCL12γ plays similar roles during its interaction with the BM microenvironment in hematological malignancies is still open to debate and investigation (22).

Because of the induction of increased bone resorption by osteoclasts by activation of CXCL12 molecules, the release of factors such as IL-6 by osteoclasts can provide growth factors for the protection and expansion of multiple myeloma plasma cells in the bone environment (23).

Although CXCL12 signaling in osteoclast formation is highly complex, selective replacement of the hematopoietic

Table 1. miRNAs targeting CXCL12

miRNA	Position in the UTR	Seed match	Context ++ score	P_{CT}	Predicted relative K_D
Conserved sites					
hsa-miR- 135a-So	1306-1312	7mer-1A	-0.67	0.53	-2.353
hsa-miR-135b-5n	1306-1312	7mer-1A	-0.65	0.53	-2.353
bsa-miR-130a-So	1452-1458	7mer-m8	-0.59	0.18	-2.7
hsa-miR-23a-3n	1452-1458	7mer-m8	-0.55	0.18	-2 57
hsa-miR-23b-3o	1452-1458	7mer-m8	-0.53	0.18	-2.57
hsa-miR-23c	1452-1458	7mer-m8	-0.52	0.18	-2.57
hsa-miR-137	1472-1479	8mer	-0.52	0.77	-5.307

P_{CT}: Probability of preferentially conserved targeting, K_D: Relative K_D values are predicted using a convolutional neural network (CNN) that predicts binding affinity between any miRNA and any 12-nt sequence

population with CXCR4-deficient cells results in increased (rather than decreased) osteoclast number and bone resorption demonstrated in several studies (24).

As CXCR4/CXCL12 signaling during hematopoiesis is associated with the persistence of HSCs and the BM, whether CXCR4/CXCL12 antagonism can impair hematopoiesis should be further examined for more rational therapeutic strategies. Disruption of the CXCR4/CXCL12 relationship may affect the long-term activities of HSC, as this axis has been shown to be involved in the protection of HSCs against oxidative stress (25).

The relationship between CXCR4 and CXCL12 is crucial for targeting MM cells to the protective BM niche (26). However, CXCL12 is associated with human immunodeficiency virus (HIV), warts, hypogammaglobulinemia, infections and myelokathexis (WHIM) syndrome, cardiovascular disease, immunodeficiency, cancer types (27).

The CXCL12/CXCR4 communication mechanism maintains proliferation, tumor cell survival, and migration in cancer (28). Studies have identified CXCR4 expression as a prognostic marker in various human cancers, including ovarian, breast and pancreatic adenocarcinoma (29). CXCR4 plays a substantial role in the metastatic process and is a promising CXCL gene receptor for developing new therapeutic treatments against cancer (30).

In HIV disease, CXCR4 is the main co-receptor facilitating viral pathogen entry (31). This allows the viral molecules to fuse with the host cell membrane, thereby inducing the entry of the HIV into the target cell. It has been explained to block HIV-1 and HIV-2 infections early in the viral cell cycle through selective inhibition of CXCR4 (32).

CXCL12 has been widely studied in the context of atherosclerosis. It has also been observed that it is expressed at a significant level by various cell types in coronary artery disease, such as smooth muscle and endothelial cells of atherosclerotic plaques (33,34).

However, the results of experiments that use the mouse as a model are not sufficient to understand the biological effect of CXCL12 and its ligand (CXCR4) in atherosclerosis because the intercellular mechanism is very complex (35). Genetic studies have identified the CXCL12 locus as a novel site in coronary artery disease, and alleles that increase the risk have also been associated with increased CXCL12 gene expression (36).

Increasing knowledge of the mechanisms explaining the CXCL12/CXCR4-mediated aberrant responses is beginning to provide insight into the pathogenesis of the disorder (37).

Most of the 50 the syndrome of WHIM patients reported so far have one of four mutations that result in a 10-19 amino acid truncation in the C-terminal domain of the ligand-induced and receptor crucial CXCR4. McDermott and colleagues demonstrated a case of *de novo* WHIM syndrome where the deletion of the 5 base pair CXCR4 open reading frame nucleotide 986-990 resulted in a frameshift. This mutation results in impaired CXCL12-induced receptor desensitization and enhanced ligand-induced receptor signaling (38,39).

Observations obtained because of studies have shown the role of the CXCL12/CXCR4 axis in inflammatory responses. In one study, serum levels of CXCL12 were shown to be significantly elevated in patients with Parkinson's disease (PD) compared with healthy controls. Additionally, it was observed that CXCR4 expression increased in patients with PD (40). CXCL12 is involved in leukocyte migration across the blood brain barrier. For this reason, it is considered a key chemokine in neuroinflammation (41).

Table 2. Pathways related to CXCL12

Pathway	Description	False discovery rate
hsa04061	Viral protein interaction with cytokine and cytokine receptor	2.05e-27
hsa04062	Chemokine signaling pathway	2.16e-06
hsa04620	Toll-like receptor signaling pathway	3.26e-34
hsa05340	Primary immunodeficiency	3.97e-14
hsa04060	Cytokine-cytokine receptor interaction	5.01e-06
hsa05323	Rheumatoid arthritis	5.63e-29
hsa04623	Cytosolic DNA-sensing pathway	6.45e-07
hsa04064	NF-kappa B signaling pathway	9.14e-11
hsa05163	Human cytomegalovirus infection	0.00012
hsa05142	Chagas disease	0.00013
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	0.00019
hsa05205	Proteoglycans in cancer	0.00041
hsa05146	Amoebiasss	0.0014
hsa05145	Toxoplasmosis	0.0021
hsa04670	Leukocyte transendothelial migration	0.0024
hsa05167	Kaposi sarcoma-associated herpesvirus infection	0.0024
hsa04657	IL-17 signaling pathway	0.0024
hsa05135	Yersinia infection	0.0024
hsa04933	AGE-RAGE signaling pathway in diabetic complications	0.0024
hsa05170	Human immunodeficiency virus 1 infection	0.0034
hsa04660	T-cell receptor signaling pathway	0.0046
hsa04514	Cell adhesion molecules	0.0076
hsa05161	Hepatitis B	0.0083
hsa05164	Influenza A	0.0114
hsa05203	Viral carcinogenesis	0.0182
hsa05162	Measles	0.0182
hsa05226	Gastric cancer	0.0182
hsa05224	Breast cancer	0.0189
hsa05132	Salmonella infection	0.0192
hsa04810	Regulation of actin cytoskeleton	0.0202
hsa05131	Shigellosis	0.0463
hsa04151	PI3K-Akt signaling pathway	0.0463
hsa04010	MAPK signaling pathway	0.0492
hsa05200	Pathways in cancer	0.0492

PD: Parkinson's disease, MAPK: Mitogen-activated protein kinase, AGE-RAGE: Advanced glycation end product-receptor, NF: Nuclear factor

In addition to all these, studies on the epigenetic regulation of CXCR4 are increasing. The most common use of epigenetic regulation is to control how a gene is expressed. This arrangement defines the function of the cell. Generally, epigenetic, non-coding RNAs such as miRNAs (42), long non-coding RNAs (43) and circular RNA gene expression can be silenced using. Epigenetic regulation of gene expression can occur through processes such as methylation (generally

associated with gene silencing) and acetylation (generally associated with gene activation). Many studies have been reported supporting the epigenetic regulation of CXCR4 via non-coding RNAs, methylation, or acetylation.

As is known, some molecules, such as acetyl-11 keto-β-boswellic acid, cause decreased CXCR4 gene expression, resulting in changes in cancer behavior, including reduced invasion and migration (44). Thus, there are many direct and indirect ways in which the expression of CXCR4 can be epigenetically controlled.

CONCLUSION

We found strong evidence for miRNA-mediated CXCL12 expression, the variants near miRNA's showed stronger genetic associations with lymphoblastic leukemia/lymphoma, respiratory system disease, intestinal disease, combined immunodeficiency, multiple sclerosis, hepatitis, and MM. We conclude that the role of CXCL12 is stronger in autoimmunity, inflammation and possibly MM. This study demonstrates the feasibility of preliminary dry laboratory projects before starting wet laboratory experiments.

Acknowledgementdge:

Süleyman Rüştü Oğuz contributed as equal author.

ETHICS

Ethics Committee Approval: This study includes bioinformatic analysis that does not require ethics committee approval.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Authorship Contributions

Concept: E.E.G., Design: E.E.G., S.R.O., S.K.B., Data Collection or Processing: E.E.G., S.K.B., Analysis or Interpretation: E.E.G., H.Ş.Ç., Literature Search: E.E.G., D.K., Writing: E.E.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Research

A Novel Technique in Comminuted Patella Fractures: Minimally Invasive Pericerclage Osteosynthesis Using Drainage Trocar

Parçalı Patella Kırıklarında Yeni Bir Teknik: Dren Trokarı Kullanılarak Minimal İnvaziv Periserklaj Osteosentez

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ABSTRACT

Objective: This study aims to describe a new technique for pericerclage application for comminuted patella fractures and to evaluate the clinical outcomes of patients who underwent this technique.

Methods: The clinical outcomes of 9 patients with AO/OTA C2 and C3 fractures and surgically applied percutaneous pericerclage using a drainage trocar were evaluated.

Results: Three of the nine patients included in the study were female, and 6 were male. Union was achieved for all patients. The mean Böstman score was 27.8 (24-30). The excellent clinical outcome was achieved in 7 patients.

Conclusion: In the surgical treatment of comminuted patella fractures, a drainage trocar can be safely used for percutaneous pericerclage, and satisfactory clinical results with stable fixation can be achieved.

Keywords: Patellar fractures, pericerclage, minimally invasive

ÖZ

Amaç: Bu çalışma, parçalı patella kırıklarında periserklaj uygulaması için yeni bir tekniği tanımlamayı ve bu teknik uygulanan hastaların klinik sonuçlarını değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: AO/OTA C2 ve C3 kırığı olan ve drenaj trokarı ile cerrahi olarak perkütan periserklaj uygulanan 9 hastanın klinik sonuçları değerlendirildi.

Bulgular: Çalışmaya dahil edilen dokuz hastanın üçü kadın, 6'sı erkekti. Tüm hastalarda kaynama sağlandı. Ortalama Böstman skoru 27,8 (24-30) idi. Yedi hastada mükemmel klinik sonuç elde edildi.

Sonuç: Parçalı patella kırıklarının cerrahi tedavisinde perkütan periserklaj için drenaj trokarı güvenle kullanılabilir ve stabil fiksasyon ile tatmin edici klinik sonuçlar alınabilir.

Anahtar Kelimeler: Parçalı patella kırıkları, periserklaj, minimal invaziv

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INTRODUCTION

Patellar fractures account for approximately 1% of all fractures and commonly occur between 20 and 50 years (1-3). Although both direct and indirect trauma cause patella fractures, they usually occur because of direct trauma to the anterior aspect of the knee (1,4). The importance of patellar fractures is because the patella is an essential component of the extensor mechanism and increases the extensor force of the quadriceps tendon by acting as a lever arm during knee extension (1).

Except for fractures with no displacement and the extensor mechanism is preserved, patellar fractures are usually treated surgically (1). The goal of surgical treatment is to achieve reduction and initiate early motion. Numerous surgical techniques have been described for patella fractures, such as pericerclage, osteosynthesis with plates and screws, tension band, and partial patellectomy (1,2). Regardless of the technique used, the soft tissues around the patella also play an essential role in instability (1,5). Repairing these tissues is as essential as the technique used (1). With the knowledge of the importance of soft tissue repair, different techniques for percutaneous fixation of comminuted patellar fractures have been defined (5,6).

This study aims to describe a new technique for pericerclage fixation in percutaneous fixation of patellar fractures and to evaluate the clinical results of the cases in which we used this technique.

METHODS

Patients who were surgically treated for patellar fracture between 2015 and 2018 and who underwent the percutaneous pericerclage method were included in the study. Additionally, patient records were reviewed after approval by the Ağrı Institutional Ethics Committee (no: 24, date: 08.12.2020). After the patients had been informed by the physicians about the operations, informed consent forms were given to read and sign.

Nine patients were included in the study. The patients' age, gender, mechanism of injury, and surgical time were obtained from the medical records. Anterior-posterior and lateral radiographs of the injured knee of the patients at the first admission were used, and the fractures were classified according to the AO/OTA classification. Percutaneous pericerclage was performed in all patients using the technique described below without a tourniquet, and osteosynthesis was achieved. All patients were allowed to press in full extension with a splint during the postoperative period. The active extension was allowed after six weeks. Healing of the fracture was assessed by radiographs taken at routine follow-up visits. Additionally, patients were clinically assessed at the last follow-up visit using knee range of motion and the Böstman scoring system (2).

Surgical Technique

After spinal anesthesia, patients were prepared supine on the radiolucent table. Thirty minutes before the incision, 2-g cephalosporin was administered to all patients. Tourniquet was not applied. After sterile dressing, the hematoma was aspirated from the suprapatellar region using a 50 cc injector. Then closed reduction was applied by extending the knee and using a weber clamp. First, an incision of about 1 cm was made from the superolateral corner of the patella. Next, the drainage trocar was inserted from the superolateral corner (Figure 1). To the superomedial corner inside the quadriceps tendon toward the superior border of the patella with a cerclage wire inserted into the tube (Figure 2) and exited from the superomedial corner. Then the trocar re-entered the superomedial exit point, advanced along the patella's medial border, and removed from the inferomedial corner. Next, the trocar was advanced along the inferior border of the patella through the patellar tendon by the same approach and removed from the inferolateral corner of the patella. Then the same procedure was performed along the lateral edge of the patella (Figure 3), and the trocar was removed from the superolateral corner, the first entry site with the cerclage wire inside the tube (Figure 4). Next, the

Figure 1. Application of a cerclage-loaded drain trocar with a mini-incision from the superolateral corner of the patella

Figure 2. Percutaneous transfer of the cerclage to the medial side with the help of a drain trocar and tube

Figure 3. After the pattela is passed from its medial and inferior edge, the cerclage is moved back to the superolateral corner

cerclage was tightened to compress the patella and knot in the superolateral corner (Figure 5). Next, the reduction was checked fluoroscopically (Figure 6a, b). The skin was closed primarily (Figure 7). A demonstration of the pericerclage method with the closed technique is shown (Figure 8).

Statistical Analysis

Statistical analysis was performed using the IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data are expressed as mean and median (minimum-maximum) for continuous variables and in number and frequency for categorical variables.

Figure 4. Moving the cerclage from the superolateral corner to the starting point

Figure 5. Knot tying by ensuring the tension of the cerclage wire

RESULTS

Nine patients (three women, six men) comminuted patellar fractures underwent closed reduction and fixation using the surgical technique we described. The mean age of the patients was 47.4 (29-67) years. The mechanism of injury was direct trauma in all patients. There were no accompanying fractures. All the patients had closed fractures. According to the AO classification, five patients had 34C2 fractures, and four had 34C3 fractures. Surgical treatment was applied to the patients an average of 2.4 days (1-6) after the injury (Figure 9 a-d).

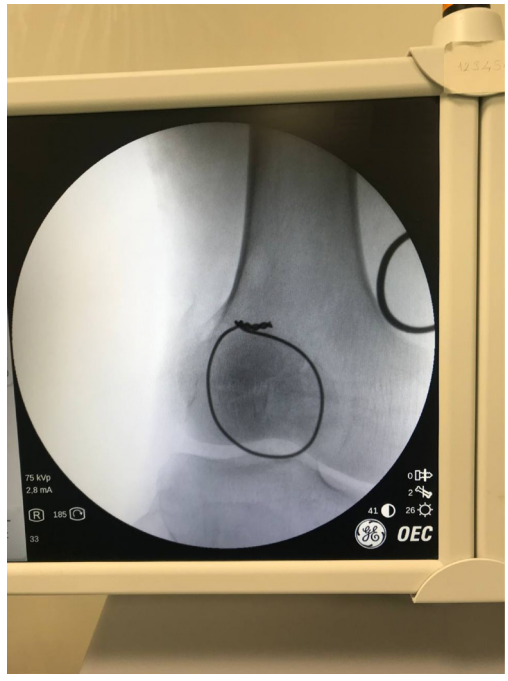

Figure 6. a, b. Fluoroscopic control after the procedure

Figure 7. Primary closure of incisions

The mean operative time was 50 (35-90) minutes. The mean follow-up time was 16 (12-27) months. None of the patients experienced early soft tissue problems or infections. No loss of reduction or implant failure was observed. Union was achieved for all patients. In 2 patients, the implant was removed due to the development of implant irritation. In these two patients, no complications occurred after implant removal.

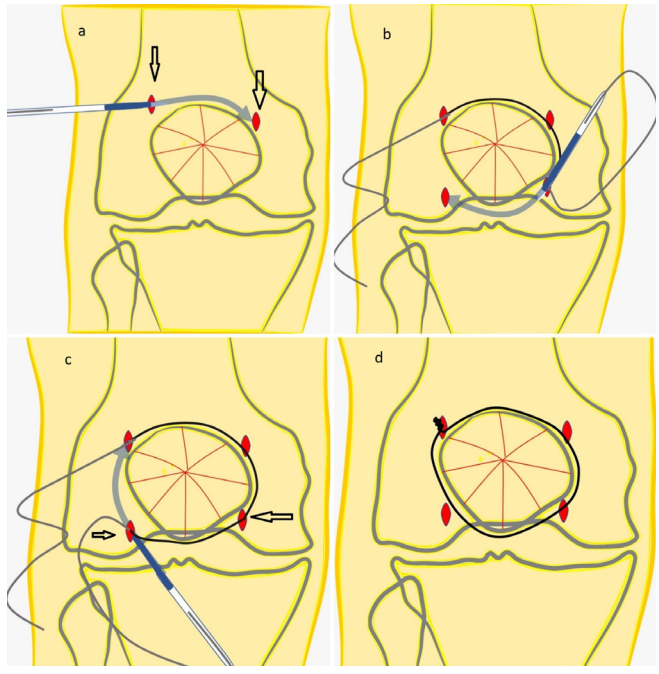

Figure 8. A schematic view of the technique. a. Cerclage-loaded drain trocar is advanced from the first incision in the superolateral corner from the superior edge of the patella to the medial. Arrows show the incision sites. b. Passing the cerclage from the medial side to the inferolateral side, close to the inferior edge of the patella, with the incision made from the inferiomedial side. c. Transfer of the cerclage wire to the superolateral with an incision from the inferolateral corner. d. Schematic view after knotting in the superolateral corner

The mean Böstman score was 27.8 (24-30). Excellent results were obtained in 7 patients and good results in 2 patients. According to the Böstman score, two patients with good results were patients who developed implant irritation.

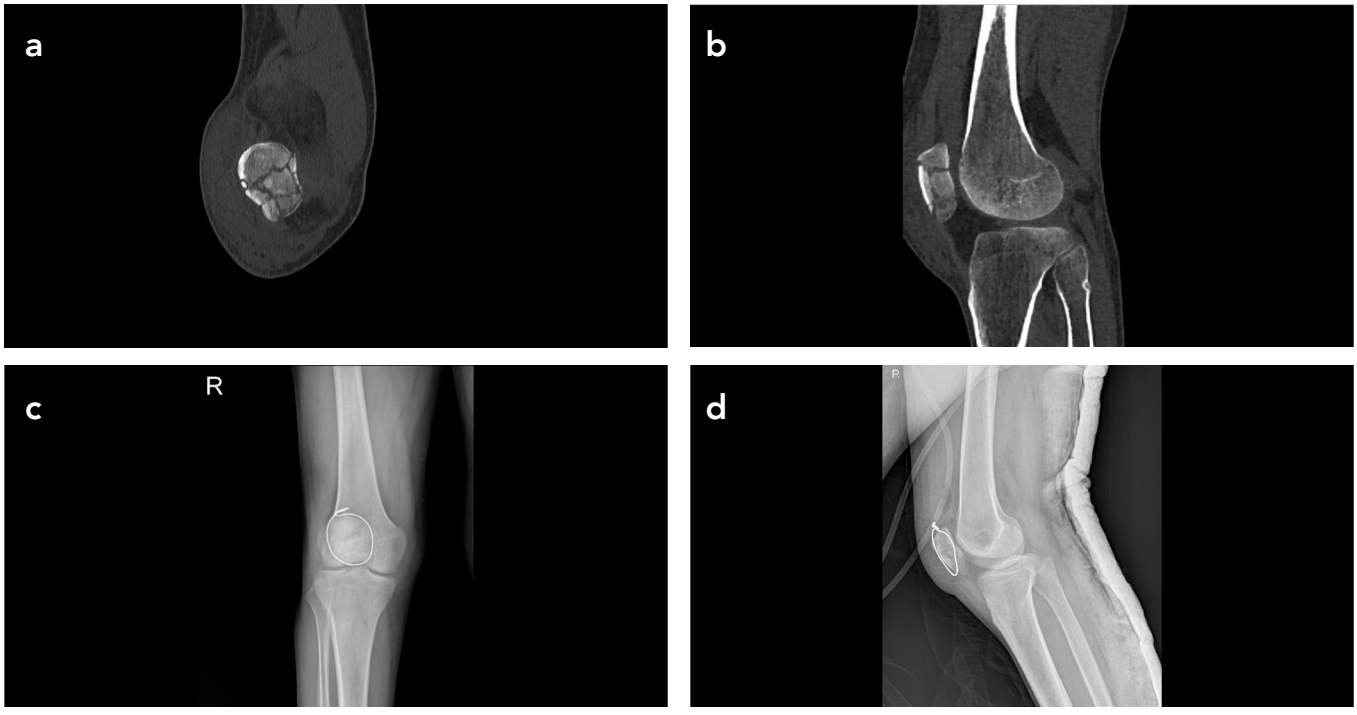

Figure 9. a-d. Preoperative and postoperative radiological images of a 47-year-old male patient

DISCUSSION

The goal of treating patellar fractures is to reduce the patellofemoral joint and maintain continuity of the extensor mechanism (1). Therefore, surgical treatment is recommended except for fractures with minimal displacement and preservation of the extensor mechanism. Many methods have been described for surgical treatment (1,5-11). Inserting a cerclage wire around the fractured patella is a commonly used and effective method (1,5). The cerclage wire can be used in both open and closed techniques (5,6). This technique shows that percutaneous osteosynthesis can be achieved by passing the cerclage wire, drainage trocar, and tube around the patella. All patients achieved the union, and excellent functional results were observed except for two patients.

The patella is a sesamoid bone. Its function is to provide mechanical advantage by prolonging the force generated by the quadriceps muscle and changing the direction of this force via the patellar tendon (12). The most crucial advantage of percutaneous osteosynthesis of patellar fractures is that the joint's range of motion can be more easily preserved in postoperative rehabilitation because the soft tissues around the patella are protected (1,5,6,11). During the extension, in addition to the patellar and quadriceps tendon, the iliotibial band vastus lateralis also contributes to the extension mechanism in the vastus medialis (12). It is also crucial for the extension that the patella moves stably in the femoral groove. It is also essential to protect the medial patellofemoral ligament, medial patellomeniscal ligament, medial patellotibial ligament responsible for patella stability, vastus lateralis, lateral fibrous tissue, and iliotibial ligament responsible for lateral stability (12). Since soft tissue dissection is not performed, the surrounding soft tissues are protected, and their damage is prevented. According to the result, excellent results were obtained. This technique, which we used with the closed method, helps preserve the range of motion in the postoperative period since no soft tissue dissection is performed.

Although using cerclage alone is unthought to provide adequate stability in patellar fractures (13), circumferential cerclage wire fixation is suitable for treating a comminuted patellar fracture (14). Additionally, it has been reported that it can be used alone in comminuted patellar fractures (15). In our study, there were no stability problems in any patient.

Implant irritation is a common complication after patellar fracture (1). Lazaro et al. (16) reported that 37% of implants had to be removed after symptomatic irritation. In cases where K-wires were used, implant irritation was reported twice more frequently than cannulated screws (17). The implant was removed in 2 cases due to irritation in our series. In 3 cases, implant irritation occurred at the superolateral corner where the knotted cerclage wire. We believe that the superficial incision may have increased the irritation. In 2 cases, the cerclage wire was removed through

two superolateral and inferiomedial incisions. No additional incision was required, and no complications occurred.

The limitations of our study: the small number of cases and the lack of a comparison group.

CONCLUSION

We think better results can be obtained if more extensive case series are compared with this procedure and other techniques. Additionally, controlling the reduction with only fluoroscopy creates a limitation, and combining the technique with arthroscopy will provide a better reduction opportunity.

ETHICS

Ethics Committee Approval: Additionally, patient records were reviewed after approval by the Ağrı Institutional Ethics Committee (no: 24, date: 08.12.2020).

Informed Consent: After the patients had been informed by the physicians about the operations, informed consent forms were given to read and sign.

Authorship Contributions

Surgical and Medical Practices: F.F., A.P., Concept: F.F., A.P., C.K., E.B., Design: F.F., A.P., C.K., Data Collection or Processing: F.F., A.P., C.K., E.B., Analysis or Interpretation: F.F., A.P., E.B., Literature Search: F.F., A.P., C.K., E.B., Writing: F.F., A.P., C.K., E.B.

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Research

Analysis of *Endotheal Nitric Oxide Synthase* Gene VNTR Variant in Turkish FMF Patients

Türk FMF Hastalarında *Endotelyal Nitrik Oksit Sentaz* Geni VNTR Varyantının Analizi

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ABSTRACT

Objective: Familial Mediterranean fever (FMF), caused by the *MEFV* gene encoding pyrin, is a prevalent monogenic autoinflammatory disease. Nitric oxide (NO), synthesized by nitric oxide synthase (NOS) is a gaseous free radical that modulates the immune response. Endothelial NOS *(eNOS)* gene variants may affect NO formation. Therefore, we investigated whether the variable *eNOS* variable number of tandem repeats (VNTR) is involved in the development of FMF. We also examined the association of this variant with clinical findings.

Methods: Three hundred seven subjects, including 147 controls and 160 FMF patients, were genotyped for the *eNOS* VNTR variant using polymerase chain reaction analysis. The patients and controls were compared regarding allele and genotype distribution using the $\chi 2$ test. The results were evaluated statistically.

Results: 51.9% of the patients had two or more MEFV mutations. The most common mutation in the patients was the homozygous M694V/M694V mutation (25%). The genotype and allele frequencies of the *eNOS* gene VNTR variant in FMF patients were all compared with those in the healthy controls. A significant difference was found between the patient and control samples for *eNOS* VNTR genotype distribution. *eNOS* VNTR homozygous 4a/4a and 4b/4b genotypes were higher in patients than those in the controls ($p>0.05$). The patients carrying the 4b/4b genotype had higher colchicine usage and responses to colchicine ($p<0.05$). There was no statistically significant difference between MEFV mutations and *eNOS* VNTR genotype distribution in the patients ($p>0.05$).

Conclusion: This study suggests that the VNTR variant of the *eNOS* gene is associated with FMF formation and some clinical findings in the Turkish population.

Keywords: Familial Mediterranean fever, endothelial nitric oxide synthase, VNTR, polymerase chain reaction

ÖZ

Amaç: Pirini kodlayan *MEFV* geninin neden olduğu Ailesel Akdeniz ateşi (FMF), yaygın tek genli otoenflamatuvar hastalıktır. Nitrik oksit sentaz (NOS) tarafından sentezlenen nitrik oksit (NO) gaz halindeki bir serbest radikaldir ve bağışıklık tepkisini düzenler. Endotelyal NOS *(eNOS)* gen varyantları, NO oluşumunu etkileyebilir. Bu nedenle, *eNOS* değişkeni ardışık tekrar sayısı (VNTR) varyantının FMF gelişiminde yer alıp almadığını araştırmayı amaçladık. Ayrıca bu varyantın klinik bulgularla ilişkisini inceledik.

Gereç ve Yöntem: Yüz altmış FMF hastası ve 147 kontrol dahil olmak üzere 307 kişi, polimeraz zincir reaksiyonu analizi kullanılarak *eNOS* VNTR varyantı için genotiplendirildi. Hastalar ve kontroller, $\chi 2$ testi kullanılarak alel ve genotip dağılımı açısından karşılaştırıldı. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Hastaların %51,9'unda iki veya daha fazla MEFV mutasyonu vardı. Hastalarda en sık görülen mutasyon homozigot M694V/M694V mutasyonuydu (%25). FMF hastalarında *eNOS* geni VNTR varyantının genotip ve alel frekanslarının tümü, sağlıklı kontrollerdekilerle karşılaştırıldı. *eNOS* VNTR genotip dağılımı için hasta ve kontrol örnekleri arasında anlamlı bir fark bulundu. *eNOS* VNTR homozigot 4a/4a ve 4b/4b genotipleri

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hastalarda kontrollere göre daha yüksekti (p>0,05). 4b/4b genotipini taşıyan hastaların kolşisin kullanımı ve kolşisine yanıtları daha yüksekti (p<0,05). Hastalarda MEFV mutasyonları ile *eNOS* VNTR genotip dağılımı arasında istatistiksel olarak anlamlı fark yoktu (p>0,05).

Sonuç: Bu çalışma, *eNOS* gen VNTR varyantının Türk popülasyonunda FMF oluşumu ve bazı klinik bulguları ile ilişkili olduğunu düşündürmektedir.

Anahtar Kelimeler: Ailesel Akdeniz ateşi, endotelyal nitrik oksit sentaz, VNTR, polimeraz zincir reaksiyonu

INTRODUCTION

Familial Mediterranean fever (FMF) is a recessively inherited autosomal inflammatory disease. It particularly affects people in the Mediterranean region, such as Turks, Armenians, Arabs, and non-Ashkenazi Jews. Its characteristics include recurrent episodes of fever, peritonitis, arthritis, rashes, and other serosal inflammations (1). This disease is caused by MEFV mutations, located on the chromosome 16 short arm (2). V726A, M694I, E148Q, M680I, and M694V, mostly located on exon 10, are the mutations of the *MEFV* gene with the highest frequency (3). Mutation of the pyrin protein encoded by the *MEFV* gene has been suggested to cause uncontrolled inflammation.

Nitric oxide (NO), as a gaseous free radical, is synthesized from oxygen and l-arginine and by four main isoforms of NO synthase (NOS), including endothelial NOS *(eNOS)*, neuronal NOS, inducible NOS, and more recently, mitochondrial NOS (4). NO powerfully regulates the immune response, activating a cascade of signal transduction pathways involved in autoimmune and inflammatory responses (5). The *eNOS* gene is located on chromosome 7q35-36. It consists of 26 exons spanning a 21 kb genomic region. Its primary expression is in the low-level endothelial cells in platelets (6). The *eNOS* can stimulate cyclooxygenase 2, KB nuclear factor, and pro-inflammatory cytokines (7). The plasma NO level in healthy people is linked to the variations in the *eNOS* gene. There are different polymorphic sites in the *eNOS*, one of which is VNTR (27 bp repeat, intron 4 VNTR a/b), which causes it to produce basal NO. Due to the possible bonding of the endothelial nuclear proteins to this region, the *eNOS* gene promoter efficiency and then the levels of *eNOS* protein and enzyme activity may be influenced by the VNTR (8). There has been a report of the VNTR five alleles, with 2-6 tandem 27-bp repeats (alleles 2-6) published up to date, among which are alleles 4 and 5 in all populations under study (9). It is shown that the concentration and activity of *eNOS* are lower in the heterozygotes of the 4a/4b variant than those in the homozygotes of 4b/4b (9).

Ongoing chronic inflammation causes endothelial dysfunction. There is a relationship between endothelial dysfunction and the defects in endothelium-based vasodilation mediated by NO (10). It has been reported that endothelial biomarkers are abnormal in patients with FMF.

As the frequency of variants varies between ethnic groups and races, we examined the genotypic and allelic distribution of the *eNOS* VNTR variant in FMF patients in this study. We also evaluated the association between the *eNOS* VNTR variant and MEFV gene mutations.

METHODS

Study Population

The study population included 160 unrelated patients with FMF (89 females and 71 males) who attended the Department of Medical Genetics, Samsun Research and Training Hospital, University of Health Sciences Türkiye, Samsun, Türkiye. Five mutations were examined in patients (M694V, M680I, V726A, E148Q, and A744S). FMF was diagnosed on the basis of the Tel Hashomer criteria. A group of 147 healthy Turkish volunteers (80 females and 67 males) matched by age, sex, and ethnicity with no history or signs of FMF or inflammatory diseases were included as the control group. All the subjects lived in the Central Black Sea region and were over 18 years old. Each patient had detailed clinical characteristics that were recorded. All participants were informed about the study and gave their written informed consent for a protocol. This study was conducted on the basis of the Declaration of Helsinki. The study protocol was approved by the University of Health Sciences Türkiye, Samsun Training and Research Hospital Clinical Research Ethics Committee (protocol no: KAEK 2020/5/13, date: 16.06.2020).

Genotyping

As instructed by the manufacturer, 2-mL blood was taken from each participant, including the controls and the FMF patients, and the commercial kit was used to extract DNA from all samples. *eNOS* VNTR variant was genotyped using the polymerase chain reaction method, which was described previously method (11).

String Analysis

The functional interactions among the proteins are annotated by the STRING database in a cell. In this study, we analyzed the VEGF protein with the STRING database.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows (SPSS, Inc., Chicago, IL) was used

for the analysis of the data. The results are presented as the mean standard deviation (SD). Fisher's Exact test, the χ^2 test, or ANOVA were used to analyze the relationships between the *eNOS* VNTR variant and the patients' demographic and clinical characteristics. Risk factors were assessed using 95% confidence intervals (CIs) and odds ratios (ORs). There were two-tailed p-values, and p-values below 0.05 were regarded as significant.

RESULTS

In this study, 307 subjects, including 147 adult controls and 160 FMF patients, were genotyped for the *eNOS* VNTR variant. The mean age ± SD was 27.23±10.90 in the patients and 37.39±13.39 in the control group. Eighty nine (55.63%) women and 71 (44.37%) men were included in the patient group, and 80 (54.42%) women and 67 (45.58%) men were in the control group. Table 1 shows the patient and control groups' demographic and baseline clinical features.

We evaluated MEFV mutation distributions in FMF patients. 51.9% of patients had two or more MEFV mutations. The most prevalent mutation was the homozygous M694V/M694V mutation (25%). The distribution of MEFV mutations in patients is presented in Table 2.

The genotype and allele frequencies of the *eNOS* gene VNTR variant in FMF patients and healthy controls are given in Table 3. A significant difference was found in the distribution of genotypes of the *eNOS* VNTR variant between patients and controls. *eNOS* VNTR 4a/4a and 4b/4b genotypes were higher in patients compared to controls, respectively [p ($p<0.05$, OR 2.755, 95% CI: 1.380-5.021; $p<0.05$, OR 1.167, 95% CI: 0.928-1.452)]. The patients and the controls did not show significantly different allele frequencies of the *eNOS* VNTR variant ($p>0.05$).

We then evaluated the relationship between the distribution of the MEFV mutations and the genotype distribution of *eNOS* VNTR in the FMF patients (Table 4). No significant difference was found between MEFV mutations and *eNOS* VNTR variants in the patients ($p>0.05$).

We then evaluated the association between the clinical features of FMF and the *eNOS* VNTR genotype distribution (Table 5). Patients with the 4b/4b genotype had higher colchicine usage and responses ($p>0.05$).

When we analyzed the *eNOS* protein with the STRING database, we predicted the protein's functional partners with high confidence (score: 0.7) as follows: ESR1, KDR, VGFA, HSP90AA1, AKT1, CALM1, CAV1, KNG1, NOSTRIN. The interaction network of these proteins is shown in Figure 1.

DISCUSSION

FMF has been classified as a systemic autoinflammatory disorder (12). This disease is common in the Turkish population. Despite the discovery of the responsible gene (*MEFV*), there is no clear link between genotype and phenotype. The *MEFV* gene has a predominant expression in neutrophils (13). Pyrin encoded by MEFV forms the NLRP3 inflammasome complex element and modulates the

Table 1. Baseline clinical and demographics features of the patients with FMF patients and controls

Characteristic	Control group (n=147)	Patient group (n=160)
Gender, male/female, n (%)	67/80 (45.58/54.42)	71/89 (44.37/55.63)
Age, mean ± SD, years	37.39±13.39	27.23±10.90
Diagnose age, mean ± SD, years	-	18.00±12.61
Family history, yes/no, n (%)	-	77/82 (49.4/51.6)
Colchicine usage, yes/no, n (%)	-	91/68 (57.2/42.8)
Response to colchicine, yes/no, n (%)	-	90/69 (56.6/43.4)
Fever status, yes/no, n (%)		143/16 (89.9/10.1)
Abdominal pain, yes/no, n (%)	-	139/20 (87.4/12.6)
Chest pain, yes/no, n (%)	-	49/110 (30.8/69.2)
Joint pain, yes/no, n (%)	-	126/33 (79.2/20.8)
Appendicitis operation, yes/no, n (%)	-	21/138 (13.2/86.8)
Erythema, yes/no, n (%)	-	32/127 (20.1/79.9)
Amyloidosis, yes/no, n (%)	-	13/146 (8.2/91.8)

SD: Standard deviation, FMF: Familial Mediterranean fever

Table 2. Distribution of MEFV gene mutations in FMF patients

MEFV mutations	n (%)
0 mutation	36 (22.5)
1 mutation	42 (26.25)
M694V	22 (13.7)
M680I	14 (8.7)
V726A	2 (1.2)
E148Q	2 (1.2)
A744S	2 (1.2)
≥2 mutations	82 (51.9)
M694V/M694V	40 (25)
M694V/M680I	13 (8.1)
M694V/V726A	3 (1.8)
M694V/E148Q	1 (0.6)
M694V/A744S	1 (0.6)
M680I/M680I	13 (8.1)
M680I/V726A	5 (3.1)
M680I/E148Q	1 (0.6)
V726A/F479L	1 (0.6)
V726A/E148Q	1 (0.6)
E148Q/P369S	2 (1.2)
E148Q/P369S/K695R	1 (0.6)

FMF: Familial Mediterranean fever

pro-inflammatory cytokine interleukin-1 (IL-1β) generation. Therefore, FMF may be regarded as inflammasompathy (14).

Initially, IL-1β is released as a proinflammatory cytokine during attacks, significantly increasing the serum levels of acute-phase reactants such as C-reactive protein (CRP), fibrinogen, erythrocyte sedimentation rates (ESRs), and serum amyloid A (15). The endothelial adhesion molecules, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, can facilitate the cellular interactions between B- and T-lymphocytes, monocytes/macrophages, and neutrophils. Endothelial cells can be facilitated by a rich source of proteolytic enzymes and oxygen reactive species, i.e., neutrophils, which are against junctions of the end. This endothelial dysfunction basic mechanism, which neutrophils initiated causes various inflammatory disorders, including FMF (16,17). The pathogenesis of the disease may be caused by several environmental factors and other modifying genes. Studies have found an association between different inflammatory gene variants and FMF (18,19).

Host defense and homeostasis are affected by NO when generated at a low level in the short term; but when it is generated at higher concentrations in the longer term, it becomes mutagenic and genotoxic. For this reason, the NO-mediated effects have complex biological outcomes depending on the external and internal environment of the cell's generation and target sites, as well as the generated

Table 3. Genotype and allele distribution of the eNOS VNTR variant in groups

eNOS VNTR	FMF patients n=160 (%)	Controls n=147 (%)	OR (CI 95%)*	p-value
Genotypes				
4a/4a	9 (5.62)	3 (2.04)	2.755 (1.380-5.021)	**<0.05**[&]
4a/4b	43 (26.87)	59 (40.13)	0.669 (0.463-0.919)	>0.05
4b/4b	108 (67.50)	85 (57.82)	1.167 (0.928-1.452)	**<0.05***
4a4a+4a4b:4b4b	52:108	62:85	0.661(0.41-1.05)	>0.05
4b4b+4a4b:4a4a	9:151	3:144	0.350 (0.07-1.26)	>0.05
Alleles				
4a	61 (19.06)	65	0.830 (0.55-1.23)	>0.05
4b	259 (80.93)	229 (77.89)		
HWE p-value	0.102	0.045		

OR: Odds ratio, CI: Confidence interval, FMF: Familial Mediterranean fever, eNOS: Endothelial nitric oxide synthase, VNTR: Variable number of tandem repeats
*OR (95%CI) corrected according to gender and age, [&]Fisher's Exact test. Statistically significant results were bolded.

NO concentration (20). Different inflammatory diseases such as rheumatoid arthritis (21), osteoarthritis (22), Sjogren's syndrome (23), and systemic lupus erythematosus (SLE) (24) show excessive NO production. Balat et al. (25) reported that total nitrite levels were significantly higher in children with FMF. The endothelial NO production is primarily affected by *eNOS* activity. *eNOS*, which is an enzyme dependent on Ca2+, was first defined in the vascular endothelial cells. *eNOS* function and activity largely affect endothelial function (26). Endothelial dysfunction is heavily affected by oxidative stress caused by *eNOS* dysfunction.

TNF-α, IL-17, cluster of differentiation 40 ligand, interferons, CRP, and SLE-specific circulatory factors have promoted endothelial dysfunction by promoting abnormal *eNOS* function and increasing oxidative stress in recent studies (27). Oxidative stress increased in patients with FMF during attack (28). Yel et al. (29) reported the presence of endothelial damage, particularly during the active disease period, among children with FMF. Additionally, there is little information on endothelial dysfunction and renal involvement in FMF (10). There are two common alleles in the *eNOS* gene 4a/b variant of the 27-bp VNTR in intron 4: 4b with 5 repeats and 4a with 4 repeats (30). The *eNOS* VNTR variant has been suggested to regulate *eNOS* expression by forming small RNAs (siRNAs). There are lower levels of

Table 4. Relationship between MEFV mutations and eNOS VNTR variants in patients

MEFV mutations	eNOS variants	n (%)	p-value
0 mutation	4b/4b	23 (14.4)	
1 mutation	4b/4b	28 (17.5)	
≥2 mutation	4b/4b	57 (35.6)	
0 mutation	4a/4b	8 (5.0)	
1 mutation	4a/4b	12 (7.5)	>0.05
≥2 mutation	4a/4b	23 (14.4)	
0 mutation	4a/4a	4 (2.5)	
1 mutation	4a/4a	2 (1.2)	
≥2 mutation	4a/4a	3 (1.9)	

eNOS: Endothelial nitric oxide synthase, VNTR: Variable number of tandem repeats

Table 5. Evaluation of clinical characteristics according to genotypes in FMF patients

Clinical characteristics		eNOS genotypes			
		4b/4b n (%)	4a/4b n (%)	4a/4a n (%)	p-value
Family history	Yes	51 (32.1)	21 (13.2)	5 (3.1)	>0.05
	No	57 (35.8)	21 (13.2)	4 (2.5)	
Colchicine usage	Yes	54 (34.0)	29 (18.2)	8 (5.0)	**<0.05**
	No	54 (34.0)	13 (8.2)	1 (0.6)	
Response to colchicine	Yes	53 (33.3)	29 (18.2)	8 (5.0)	**<0.05**
	No	55 (34.6)	13 (8.2)	1 (0.6)	
Fever status	Yes	96 (60.4)	38 (23.9)	9 (5.7)	>0.05
	No	12 (7.5)	4 (2.5)	0 (0.0)	
Abdominal pain	Yes	94 (67.9)	37 (23.3)	8 (5.0)	>0.05
	No	14 (8.8)	5 (3.1)	1 (0.6)	
Chest pain	Yes	32 (20.1)	13 (8.2)	4 (2.5)	>0.05
	No	76 (47.8)	29 (18.2)	5 (3.1)	
Joint pain	Yes	83 (52.2)	36 (22.6)	7 (4.4)	>0.05
	No	25 (15.7)	6 (3.8)	2 (1.3)	
Erythema	Yes	26 (16.4)	5 (3.1)	1 (0.6)	>0.05
	No	82 (51.6)	37 (23.3)	8 (5.0)	
Amyloidosis	Yes	9 (5.7)	4 (2.5)	0 (0.0)	>0.05
	No	99 (62.3)	38 (23.9)	9 (5.7)	

FMF: Familial Mediterranean fever, eNOS: Endothelial nitric oxide synthase
*Statistically significant results were bolded.

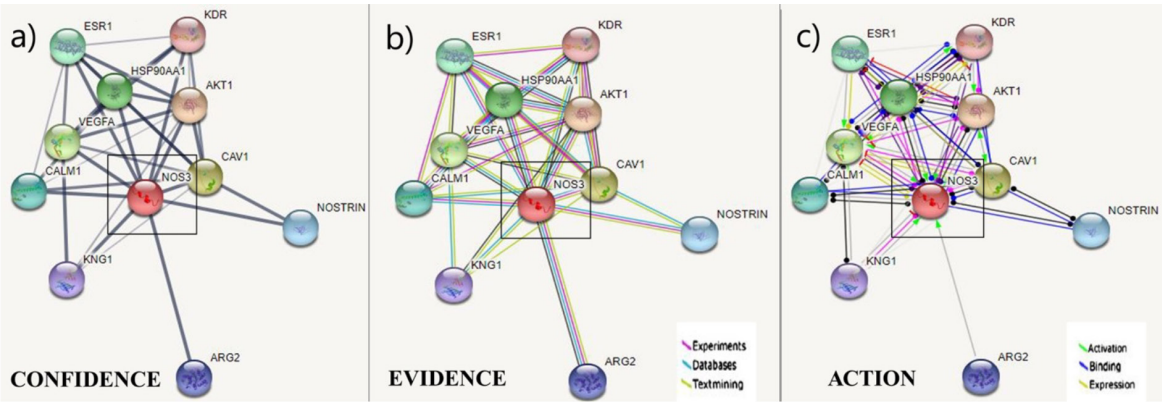

Figure 1. Interactions of Enos protein, according to STRING database predictions: a) confidence network: stronger associations are represented by thicker lines; b) this network represents the types of evidence for the association; c) presentation of the different modes of action, involved in the protein–protein interactions. eNOS protein is evidenced with a square

eNOS and higher quantities of siRNA in the endothelial cells containing five copies than in the cells containing four copies (31). The *eNOS* VNTR variant has been linked to many inflammatory diseases, including autoimmunity diseases such as SLE (32) and Behçet's disease (33).

In this study, we evaluated the distribution of the *eNOS* VNTR variant among Turkish FMF patients and whether this variant is a risk factor for developing FMF. As far as we know, this is the first study on the prevalence of the *eNOS* VNTR variant among Turkish patients with FMF. There was a statistical difference between FMF patients and controls in terms of *eNOS* VNTR variant genotype distribution (Table 2). *eNOS* VNTR homozygous genotypes (4a/4a and 4b/4b) were more common in patients than in controls. When we compared clinical findings with *eNOS* VNTR genotypes, we found that *eNOS* VNTR genotype distribution was associated with the response to colchicine and colchicine usage (Table 5). The responses to colchicine and colchicine usage were higher in patients with the 4b/4b genotype.

Our study had some limitations. First, we could not evaluate other acute complications of FMF. The other one was that our sample size was small.

CONCLUSION

NO's the ability to regulate immune responses has been remarkable over the last two decades. Every cell type in the body virtually produces NO, which is effective in regulating processes at a larger scale, such as the immune and nervous systems. The central issue in FMF is the dysfunction of the innate immune system as a self-reactive autoinflammatory disease. Despite intense research on the pathogenesis of FMF, there are still unknowns. Our results show that the *eNOS* VNTR variant is a risk factor for FMF susceptibility.

ETHICS

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Türkiye, Samsun Training and Research Hospital Clinical Research Ethics Committee (protocol no: KAEK 2020/5/13, date: 16.06.2020).

Informed Consent: All participants were informed about the study and gave their written informed consent for a protocol.

Authorship Contributions

Concept: Ö.S.T., S.Y., Design: A.F.N., A.T., Data Collection or Processing: Ö.S.T., Analysis or Interpretation: S.Y., A.T., Literature Search: A.F.N., Writing: A.F.N.

Conflict of Interest: No conflict of interest was declared by the authors.

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Research

Simplified Triangulation Technique for Renal Access During Percutaneous Nephrolithotomy: Description of a Novel Technique

Perkütan Nefrolitotomi Sırasında Renal Erişim için Basitleştirilmiş Triangülasyon Tekniği: Yeni Bir Tekniğin Tanımı

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ABSTRACT

Objective: Determination of the correct point and angle of puncture is the first and most important step in percutaneous nephrolithotomy. In this study, we presented our first clinical results of a new fluoroscopy-guided triangulation puncture technique.

Methods: A total of 50 patients who met the inclusion criteria were evaluated retrospectively between January 2015 and December 2017. Preoperative and postoperative features, data on percutaneous renal access, success, and complication rates were examined.

Results: Of 50 patients who underwent percutaneous nephrolithotomy, the mean age was 39.9±11.8 years and the mean body mass index was 26±4 kg/m^2. The mean stone burden was 587.7±198.5 mm^2. The mean operation time was 74.3±15.6 min and the mean fluoroscopy time was 3.6±1.1 min. Intraoperative or postoperative complications were detected in 9 patients. On the 1st postoperative day, the stone-free rate was 80% and clinically insignificant residual fragment was 12%.

Conclusion: This technique provided the correct point and angle of puncture with minimal complication rates. However, further investigations and comparisons with other techniques should be conducted to examine the efficacy and reliability of this method in detail.

Keywords: Percutaneous nephrolithotomy, simplified triangulation technique, renal access, kidney stones

ÖZ

Amaç: Perkütan nefrolitotomide ilk ve en önemli adım, ponksiyon noktasının ve açısının doğru belirlenmesidir. Bu çalışmada, yeni bir floroskopi yardımlı triangülasyon ponksiyon tekniğinin ilk klinik sonuçlarını sunmayı amaçladık.

Gereç ve Yöntem: Ocak 2015 ile Aralık 2017 arasında dahil edilme kriterlerini karşılayan toplam 50 hasta retrospektif olarak değerlendirildi. Ameliyat öncesi ve sonrası özellikler, perkütan renal erişim verileri, başarı ve komplikasyon oranları incelendi.

Bulgular: Perkütan nefrolitotomi uygulanan 50 hastanın yaş ortalaması 39,9±11,8 yıl ve ortalama vücut kitle indeksi 26±4 kg/m^2 idi. Ortalama taş yükü 587,7±198,5 mm^2 idi. Ortalama operasyon süresi 74,3±15,6 dk ve ortalama floroskopi süresi 3,6±1,1 dk idi. Toplam 9 hastada intraoperatif veya postoperatif komplikasyon görüldü. Postoperatif 1. günde taşsızlık oranı %80 ve klinik olarak önemsiz rezidüel fragman %12 idi.

Sonuç: Bu teknik, minimum komplikasyon oranları ile doğru ponksiyon noktası ve açısını sağladı. Bununla birlikte, bu yöntemin etkinliğini ve güvenilirliğini ayrıntılı olarak incelemek için daha fazla araştırma ve diğer tekniklerle karşılaştırmalar yapılmalıdır.

Anahtar Kelimeler: Perkütan nefrolitotomi, basitleştirilmiş triangülasyon tekniği, renal erişim, böbrek taşları

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INTRODUCTION

The idea of percutaneous nephrolithotomy (PCNL) was first introduced in 1950 when a percutaneous needle was used for renal puncture for treating hydronephrosis (1). Percutaneous removal of kidney stones was first introduced in 1976 by Fernström and Johansson (2). Today, it is the gold standard treatment for kidney stones greater than 2 cm (3).

Percutaneous renal access is the most important step that affects the success and complication rate of the surgery (4,5). Various radiological modalities such as ultrasonography (USG) and computed tomography (CT) are used in clinical practice to provide access to the collecting system, but the most commonly used imaging technique is C-arm fluoroscopy. Fluoroscopy-guided percutaneous renal access can be achieved by monoplanar or biplanar techniques (triangulation, eyes of the needle/bull's eye) (6). However, all fluoroscopic approaches face the same limitation, which is the difficulty of the two-dimensional approach to the three-dimensional renal anatomy (7). Performing percutaneous renal access more systematically, which is the most challenging aspect of PCNL, can overcome these limitations and help shorten the learning curve and radiation exposure, especially for surgeons at the beginning of the learning curve.

In this study, we present our first results of a new fluoroscopy-guided triangulation puncture technique that we used in our clinic, with the aim to demonstrate the feasibility, efficacy, and reliability of this technique.

METHODS

After the approval of the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (decision no: 2021-20-02, date: 18.10.2021), we analyzed patients with kidney stones greater than 2 cm or lower calyx stones smaller than 2 cm who underwent PCNL via modified triangulation technique between January 2015 and December 2017. Patients with non-opaque stones, horseshoe kidney, renal anomalies such as ectopic kidney, previous open or percutaneous surgery in the kidney scheduled for surgery, and those requiring multiple or intercostal/upper calyx access were excluded from the study. Written informed consent was obtained from all patients.

All patients underwent blood and urine laboratory tests before the surgical procedure. The stone burden was calculated by multiplying the longest diameter by the perpendicular diameter of the stone (mm^2) by plain radiography. The patients underwent preoperative CT and intravenous pyelography (IVP). The patients with sterile urine culture were administered cefuroxime axetil prophylaxis intravenously for 3 days, starting 1 h before the surgery. Those with proliferation in urine culture were given appropriate antibiotic treatment and underwent the process once the culture was negative. Treatment of patients who used anticoagulants was regulated.

Perioperative and postoperative variables, puncture time (PT), operative time (OT), fluoroscopy time (FT), hematocrit decrease, and stone-free rate (SFR) were determined. PT was defined as the time from the fluoroscopic imaging of the kidney until the time of urine discharge through the needle. Complications were classified according to the modified Clavien Grading System (8).

Plain radiography was performed on the first postoperative day. The nephrostomy tubes of the patients, who were evaluated as stone-free or having clinically insignificant residual fragment (CIRF), were sealed after a lighter urine color was obtained and were removed given that the patient did not feel any pain. An antegrade nephrostogram was performed on patients who were in pain to check that there were no blockages in the passage. CIRF was described as asymptomatic, non-obstructive, non-infectious stones smaller than 4 mm in diameter. All patients were evaluated with IVP 3 months after discharge.

Surgical Technique

The patients were placed in the lithotomy position under general anesthesia and standard 4-6 F ureteral catheters were inserted into the ipsilateral ureter with a 22 F cystoscope. Open-ended ureteral catheters were inserted in some cases, when necessary. 16 F Foley catheters were inserted. Then, the ureteral catheter was fixed to the urethral catheter and the patient was placed in the prone position. After the surgical site was cleaned with an antiseptic solution, a sterile surgical veil set was used to cover the patient, camera, and C-arm fluoroscopy.

Technique of Puncture

Fluoroscopy images were obtained by a C-arm. The C-arm was rotated to 0° from the standard vertical projection. The target stone was marked while nonopacified. The pelvicalyceal system was opacified by the administration of a diluted contrast agent through the ureter catheter. Air was injected in necessary cases to identify the lower pole posterior calyces. Once the lower pole posterior calyx was detected, the C-arm was used to mark the calyx as point A and a line parallel to the vertebra was drawn from this point (Figure 1). Then, the C-arm was rotated 30° toward the surgeon and the targeted calyx was marked as point B

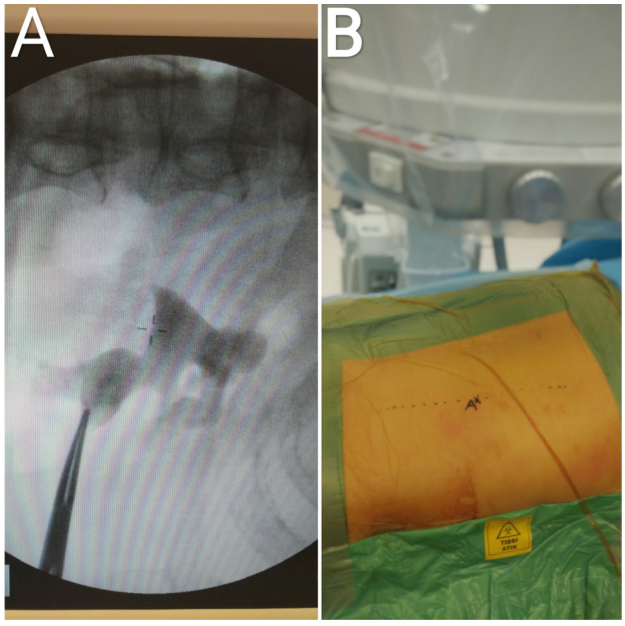

Figure 1. A) Fluoroscopic view of the renal calyceal system, opacified by contrast material, at an angle 0°. The targeted posterior calyx of the lower pole is marked using the tool. B) The targeted calyx is marked as point A, from which a line is drawn parallel to the vertebra

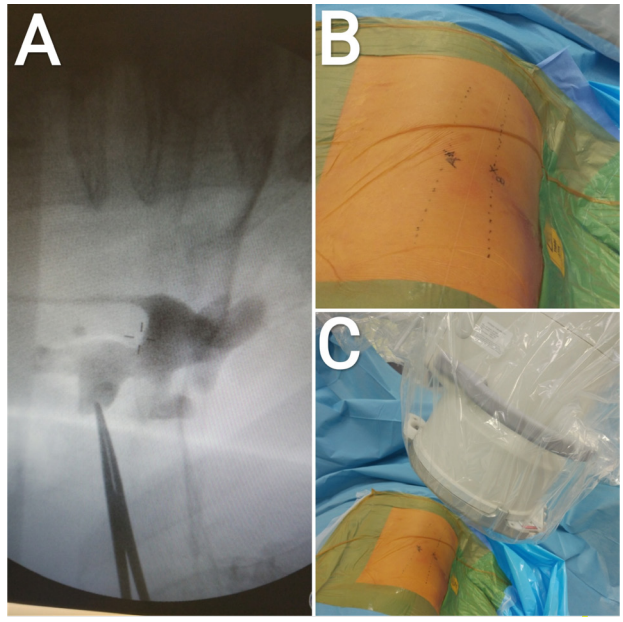

Figure 2. A) Fluoroscopic view of the renal calyceal system, opacified by contrast material, at an angle 30°. The targeted posterior calyx of the lower pole is marked using the tool. B) The targeted calyx is marked as point B, from which a line is drawn parallel to the vertebra. C) 30° fluoroscopic angle

and a line parallel to the vertebra was drawn from this point (Figure 2). The C-arm was rerotated to a position of 0°. The 18 G percutaneous needle was positioned at point A and from this point, the straight line was drawn according to the targeted caliceal direction until it intersected the B line. The point, where the line along the targeted calyx intersected the B line, was determined as the point of puncture (C) (Figure 3). While the C-arm was positioned at 0°, a 15-cm, 18 G two-part trocar needle (Percutaneous Access Needle, Boston Scientific, USA) was used to enter the target calyx. The C-arm was angled at 30° to confirm entry into the system when needed. A guidewire (Sensor TM Guide Wire, Boston Scientific, USA) was inserted into the pelvicalyceal system through the needle. After the tract was dilatated, a 30 F amplatz sheath (Boston Scientific, USA) was positioned. A 26 F rigid nephroscope was used. A pneumatic lithotripter (Vibrolith, Elmed, Türkiye) was used for stone fragmentation. The presence of residual stones was determined by fluoroscopy, endoscopy, and antegrade nephrostogram. The procedure was completed by placing a 14 F nephrostomy tube. Antegrade double J ureteral stents were placed in some cases, when necessary. The tubeless procedure was not performed in any cases.

Statistical Analysis

The demographic and operative data of the patients are presented as mean ± standard deviation. Statistical analysis was performed using the SPSS 13.0 software. The chi-square test was used to compare the ratios and a p-value less than 0.05 was accepted as statistically significant.

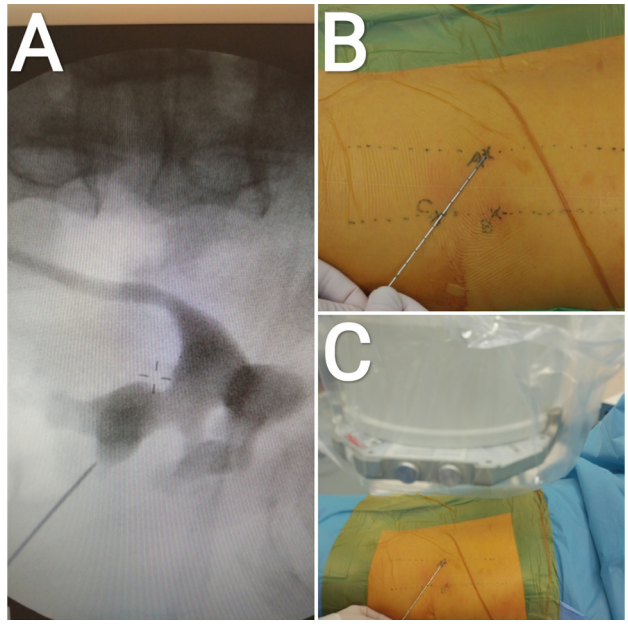

Figure 3. A) Fluoroscopic view of the renal calyceal system, opacified by contrast material, at an angle 0°. The needle is placed in accordance with the angle of the targeted lower pole of the posterior calyx. B) The point where the needle intersects the line drawn from point B is marked as point C (puncture point). C) 90° fluoroscopic angle

RESULTS

The mean age of the patients was 39.9±11.8 years. Twenty-nine patients (58%) were male and 21 (42%) were female.

The mean body mass index was 26±4 kg/m^2. Twenty-six of the stones were in the left kidney and 24 were in the right kidney. The mean stone burden was calculated as 587.7±198.5 mm^2.

All patients were operated using single access. The mean OT was 74.3±15.6 min. The mean PT and FT were determined as 1.6±0.7 and 3.6±1.1 min, respectively. The preoperative and postoperative hemoglobin, urea, and creatinine values were determined as 14.8±2.1 g/dL and 12.6±1.5 g/dL (p=0.0001), 28.1±4.3 mg/dL and 27.6±5.6 mg/dL (p=0.19), and 1±0.7 mg/dL and 1±0.5 mg/dL (p=0.442), respectively. The mean nephrostomy time and hospital stay were 2.2±0.9 and 2.4±0.8 days, respectively. On the 1st postoperative day, the SFR was 80% and CIRF was 12%. According to the modified Clavien classification, 6 (12%) patients had Clavien 1 (fever), 2 (4%) had Clavien 2 (blood transfusion) and 1 (2%) had Clavien 3 (urine leakage requiring double J stent) complications. The SFR was 90% and CIRF was 6% on the 3rd postoperative month. One patient underwent ureterorenoscopy and 1 patient received extracorporeal shock wave therapy as an additional treatment. The preoperative, intraoperative and postoperative data are shown in Table 1 and 2.

Table 1. Preoperative, intraoperative and postoperative data

Mean age (years; SD)	39.9±11.8
BMI (kg/m^2; SD)	26±4
Stone burden (mm^2 ; SD)	587.7±198.5
Hounsfield unit (mean; SD)	1000.9±260.9
Mean operation time (mn; SD)	74.3±15.6
Punction time (mn; SD)	1.6±0.7
Floroscopy time (mn; SD)	3.6±1.1
Stone-free rate (n; %)	
Postoperative day 1	40 (80%)
Postoperative 3rd month	45 (90%)
CIRF (n; %)	
Postoperative day 1	6 (12%)
Postoperative 3rd month	3 (6%)
Complications according to Clavien (n; %)	
Grade 1 (fever)	6 (12%)
Grade 2 (ERT)	2 (4%)
Grade 3 (urinary leakage)	1 (2%)
Total	9 (18%)
Nephrostomy time (day; SD)	2.2±0.9
Hospital stay (day; SD)	2.4±0.8

BMI: Body mass index, CIRF: Clinically insignificant residual fragment, ERT: Erythrocyte replacement therapy, SD: Standard deviation

Table 2. Preoperative and postoperative changes in laboratory parameters

	Preoperative	Postoperative	p
	Mean ± SD	Mean ± SD	
Hemoglobin (g/dL)	14.8±2.1	12.6±1.5	0.0001
BUN (mg/dL)	28.1±4.3	27.6±5.6	0.19
Creatinine (mg/dL)	1±0.7	1±0.5	0.442

BUN: Blood urea nitrogene, SD: Standard deviation

DISCUSSION

At present, many clinical approaches are used to perform renal puncture in PCNL. Appropriate access to the pelvicalyceal system is directly correlated with the success rate of the surgery and the occurrence of complications (6). Many of these techniques mostly include two-dimensional imaging techniques such as fluoroscopy and USG. Studies have shown that these two approaches have similar efficacy and complication rates (9-11). However, the USG approach has some additional advantages: it minimizes radiation exposure, allows imaging of structures between the skin and kidney, distinguishes the anterior and posterior calyxes (11). Despite these advantages of the USG approach, Clinical Research Office of the Endourological Society (CROES) data suggest that the most common fluoroscopic approach is used in PCNL (86.3%) (12).

The gold standard access technique has not yet been defined in PCNL. The ideal tract is the shortest, straight, the direct path along the axis of the calyx through the papilla into the desired calyx. The preferred approach is by way of a posterior calyx (13). The monoplanar and biplanar techniques are the two main techniques that are most frequently used. After detecting the appropriate calyx, Hatipoglu et al. (6) only used the technique of monoplanar access under fluoroscopy on a vertical plane only, where the needle was advanced at an angle of about 30° to the infundibula from a suitable puncture point. Monoplanar access has advantages such as reliability, shortening of PT, and minimizing direct exposure of the surgeon to radiation. However, it has some disadvantages, such as projecting the renal calyx on a vertical plane only and not giving accurate renal depth. However, biplanar access allows the determination of calyceal orientation and the selection of the optimal calyx of entry (14). Triangulation and bull's eye techniques are the major biplanar techniques for achieving appropriate percutaneous renal access (13,14). In the literature, alternative methods have also been defined

in addition to these frequently used access techniques. Cadeddu et al. (15) used a mechanical device they called "PAKY" to facilitate access. Lazarus and Williams (16) described a novel access technique using an apparatus called ''the locator". Li et al. (17) introduced the access technique of 'stereotactic localization'. Again; Bader et al. (18) introduced the access technique called 'all-seeing needle' using optical puncture; Basiri et al. (19,20) introduced the blind puncture and biplanar oblique access techniques and Shergill et al. (21) introduced the 3-finger technique to establish the correct depth. Mues et al. (4) reported that they successfully facilitated the access by rotating the C-arm approximately 30° towards the patient's head for the lower calyx access and rotating the C-arm approximately 20° towards the opposite side of the surgeon for the middle and upper calyceal access. In our study, we present the first results of our technique, which includes the combination of both monoplanar and biplanar techniques, thus providing the advantages of both punctures. The monoplanar access was primarily achieved; however, we used the 30° of angle to verify the depth and location in the calyceal system.

The primary purpose of PCNL is to achieve stone-free status and many studies have focused on SFR. SFR was determined as 68% in a study conducted by The British Association of Urological Surgeons, in which more than 1,000 PCNL cases were evaluated (22). In a prospective study of 1,338 patients conducted by Duvdevani et al. (23), SFR was found 89.1% at discharge, whereas it was found 75.7% in the latest CROES study of 5,803 patients (24). However, in general, the impact of the percutaneous renal access technique on SFR was not taken into considered. Moreover, Tepeler et al. (13) determined the SFR as 80% and 82.5%, respectively, in a study of 80 patients, in which the bull's eye and triangulation techniques were compared. Again, SFR was determined as 73.6% and 71.2%, respectively, in a study of 195 patients, in which Budak et al. (25) compared the bull's eye and triangulation techniques. In a study of 661 patients conducted by Dede et al. (5), patients undergoing monoplanar access were compared with those who underwent biplanar access. The SFR was determined as 79% and 82%, respectively. Again, in the study in which monoplanar access was defined, Hatipoglu et al. (6) determined the success rate on 1st postoperative day as 80.5%. In our study, SFR on 1st postoperative day was 80% and 90% on postoperative third month, which was consistent with the literature, and it was determined that the access technique we described was comparable with the other techniques.

In the literature, researchers have investigated PT and, more importantly, FT and factors that affect them in many studies. The major limitation of renal access under fluoroscopy is radiation exposure. Surgeons are exposed to direct radiation at 30° positions, especially in biplanar access (6). Li et al. (17) calculated the mean PT as 7 and 17 min, respectively, in a study comparing the access techniques of "stereotactic localization" and standard PCNL. In a study using the monoplanar technique, Hatipoglu et al. (6) determined PT as 0.83 min and FT as 4.36 min. Dede et al. (5) determined that monoplanar access leads to shorter PT and FT in a study, which they compared the monoplanar and biplanar access techniques. Tepeler et al. (13) calculated FT time as 3.9 and 3.7 min using the bull's eye and triangulation techniques, respectively. Again, in a study by Budak et al. (25), FT was determined as 2.5 and 2.4 min using the bull's eye and triangulation techniques, respectively. In our study, the mean PT was 1.6±0.7 min and the mean FT was 3.6±1.1 min. Although these values are consistent with the literature, the main component of our technique is the monoplanar access. We use the 30° angle in case of uncertainty for a very short time to verify the depth and location in the calyceal system. Therefore, we think that our method leads to relatively lower radiation exposure compared to biplanar techniques.

In the literature, complications and success rates in PCNL access techniques have been compared with each other in various studies. Tepeler et al. (13) demonstrated that the triangulation technique did not have any advantage except for lower blood loss compared to the bull's eye technique. Dede et al. (5) demonstrated that there was no significant difference in complications between the monoplanar and biplanar techniques. In our first experience with 50 patients, 9 patients (18%) presented with complications, only 1 (2%) of which were the Clavien 3 complication.

It has been proposed that PCNL, to fusion systems used in the prostate biopsy, should provide a 3D image of the kidney during access by combining preoperative CT or magnetic resonance imaging scans with the real-time USG. This technology, which is widely used in the prostate biopsy, can be very effective in PCNL. Additionally, a needle with an inexpensive camera attached will provide kidney access with projection in the future. However, considering the costs and accessibility of all these technological innovations, it is inevitable that fluoroscopy, USG, and their modifications will still be used as standard access techniques.

We believe that the method we have described is easy to understand and easily applicable, especially for surgeons at the beginning of the learning curve. Additionally, we believe that our technique is more systematic than the monoplanar access and the surgeon may be exposed to less radiation

than the biplanar access. Although our current results demonstrate the efficacy and reliability of the method, further studies are needed, including a greater patient population and the learning curve to verify these findings.

Our study has some limitations. The first limitation is the retrospective nature of this study. The second limitation is the limited number of patients due to our meticulousness in patient selection as it is a novel technique. The third one can be considered the absence of a control group. Another limitation is that this technique could not be used in more complex cases requiring multiple access, upper pole entry, or intercostal access.

CONCLUSION

This technique provided the correct point and angle of puncture with minimal complication rates. However, it is necessary that this technique be performed in a larger population and compared with other standard techniques such as the bull's eye, triangulation, and monoplanar techniques.

ETHICS

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (decision no: 2021-20-02, date: 18.10.2021).

Informed Consent: Written informed consent was obtained from all patients.

Authorship Contributions

Surgical and Medical Practices: İ.E., T.K., S.K., M.T., Concept: İ.E., K.G.Ş., S.K., M.T., Design: K.G.Ş., M.E., E.Ş., F.A., Data Collection or Processing: K.G.Ş., M.E., E.Ş., F.A., Analysis or Interpretation: İ.E., E.Ş., F.A., A.H., Literature Search: T.K., A.H., Writing: İ.E., M.E., E.Ş., T.K., A.H.

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Research

The Effect of Physical Activity During the COVID-19 Pandemic on the Metabolic Control in Children with Type 1 Diabetes Mellitus

COVİD-19 Pandemisi Sürecindeki Fiziksel Aktivitenin Tip 1 Diabetes Mellitus Tanılı Çocuklardaki Metabolik Kontrol Üzerine Etkisi

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ABSTRACT

Objective: Coronavirus disease-2019 (COVID-19) pandemic caused lifestyle changes for many people. In this study, we evaluated the effects of physical activity (PA) at COVID-19 pandemic on the metabolic control of children with type 1 diabetes mellitus (T1DM).

Methods: Age, sex, blood pressure, comorbidity, anthropometric and sociodemographic data of 6-18 years old 82 patients with T1DM were recorded. Averages of glycosylated hemoglobin (HbA1c), lipid profiles, and daily insulin dose during the pandemic and a year prior were also recorded. With "Physical Activity Questionnaire-A/C" PA and screen time during the pandemic and a year prior were questioned. Patients were divided into 2 subgroups: normal weighted (group 1) and overweighted/obese (group 2) and were compared.

Results: Of the 82 patients, 47 were girls and mean age was 12.2±3.2 years. Group 1 had 64, group 2 had 18 patients and no difference was observed in terms of age, sex, comorbidity and sociodemographic data between the groups. There was a significant increase in screen time, the number of parents staying at home with children and body mass index (BMI) standard devation (SD), and a significant decrease in PA, blood pressure SD and low-density lipoprotein levels, but there was no change in HbA1c levels at the pandemic in both groups. The insulin dose was increased in group 1 at pandemic. There was a negative correlation between PA and the insulin dose.

Conclusion: During the pandemic, PA of patients with T1DM decreased, screen time, the number of parents staying at home with children and BMI SD was increased, but their metabolic controls did not deteriorate. This result was attributed to the fact that the support of the family might be much more decisive for metabolic control during a pandemic.

Keywords: COVID-19 pandemic, children, physical activity, metabolic control, type 1 diabetes mellitus

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVİD-19) pandemisi birçok insanda yaşam tarzı değişikliklerine neden oldu. Bu çalışmada COVİD-19 pandemisinde fiziksel aktivitenin (FA) tip 1 diabetes mellituslu (T1DM) çocukların metabolik kontrolü üzerindeki etkilerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Altı-18 yaş arası 82 T1DM hastasının yaş, cinsiyet, tansiyon, komorbidite, antropometrik ve sosyodemografik verileri kaydedildi. Pandemi sırasında ve bir yıl öncesinden glikat hemoglobin (HbA1c), lipid profilleri ve günlük insülin dozu ortalamaları da ayrıca kaydedildi. "Fiziksel Aktivite Anketi-A/C" ile pandemi sırasında ve bir yıl öncesindeki FA ve ekran süresi sorgulandı. Hastalar normal ağırlıklı (grup 1) ve fazla tartılı/obez (grup 2) olmak üzere 2 alt gruba ayrıldı ve birbirleri ile karşılaştırıldı.

Bulgular: Seksen iki hastanın 47'si kızdı ve yaş ortalamaları 12,2±3,2 yıl idi. Grup 1'de 64, grup 2'de 18 hasta vardı ve gruplar arasında yaş, cinsiyet, komorbidite ve sosyodemografik veriler açısından fark izlenmedi. Her iki grupta da pandemide ekran süresi, evde çocuklarla kalan ebeveyn sayısında ve vücut kitle indeksi (VKİ) standart sapmada (SS) anlamlı artış, FA, kan basıncı SS ve düşük yoğunluklu lipoprotein düzeylerinde

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anlamlı düşüş oldu ancak HbA1c düzeylerinde değişiklik olmadı. Pandemide grup 1'de günlük insülin dozu arttı. FA ile insülin dozu arasında negatif bir ilişki vardı.

Sonuç: Pandemi sırasında T1DM'li hastaların FA'sı azaldı, ekran süresi, evde çocuklarla kalan ebeveyn sayısı ve VKİ SS arttı, ancak metabolik kontrolleri bozulmadı. Bu sonuç, aile desteğinin pandemi sırasında metabolik kontrol için çok daha belirleyici olabileceği sonucuna bağlandı.

Anahtar Kelimeler: COVİD-19 pandemisi, çocuklar, fiziksel aktivite, metabolik kontrol, tip 1 diabetes mellitus

INTRODUCTION

The coronavirus disease-2019 (COVID-19) pandemic has affected everyone's daily lives all around the world (1). Children and adolescents with type 1 diabetes mellitus (T1DM) had a disease patterns similar to the children lacking diabetes (2). But despite this, with the outbreak of the pandemic, elective and scheduled healthcare appointments were canceled or changed to telemedicine visits, so people having chronic diseases, such as children having T1DM, had some difficulties. However, some studies showed that telemedicine visits successfully manage the metabolic control of T1DM (3,4).

T1DM is a lifelong illness that requires multiple daily insulin injection, healthy diet, psychological support and physical activity (PA) (5). In our country, patients never had any problem of gaining insulin at the pandemic. Regular PA or exercise is an important part of T1DM management because it helps get better glucose regulation, weight control, decrease in comorbidity (hypertension, dyslipidemia, cardiovascular diseases) and improves well-being (6-8). The PA of the children having T1DM in our country had to change during the pandemic because schools were closed and a long-term lockdown was declared in March 2020 by the government to prevent the spread of the disease. Studies have been conducted to evaluate the role of PA in the metabolic management of T1DM during the pandemic. Some showed that pandemic did not affect glycemic control (9,10), as well as those who stated that pandemic did not affect it negatively (11).

Considering this knowledge, in this study, we evaluated the PA changes during the COVID-19 pandemic and the effect of these changes on the metabolic control in children with T1DM.

METHODS

In our study, a patient group of 82 children, aged 6-18 years, who had T1DM for at least 2 years and had regular visits every 3 months to the pediatric endocrinology outpatient clinic of our hospital were included. The age, sex, comorbidity and sociodemographic data (mother's level of education, father's level of education, mother's and father's working status before and at pandemic, type of house, presence of park or recreation areas suitable for PA nearby house, the way of transportation to school) were recorded. The frequency of going to school (days/week) during the pandemic was also asked. The measurement of height, weight and blood pressure were measured in the last 60 days before the pandemic and in the last 60 days of the first year of the pandemic. Height standard deviation (SD), weight SD, the body mass index (BMI) (ratio of the weight in kilograms to height in meters squared- kg/m^2) and SD values were calculated through the usage of reference values of Turkish children (12). Systolic and diastolic blood pressure SD scores were calculated with the reference of "Clinical practice guideline for screening and management of high blood pressure in children and adolescents" (13). The averages of glycosylated hemoglobin (HbA1c) values requested every 3 months, lipid profiles [cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL)] requested once a year, and the daily insulin dose (U/kg/day) during the pandemic period and a year prior were recorded from their files. The international Physical Activity Questionnaire-A (PAQ-A) or PAQ-C (14), which was validated for Turkish children (15), was completed, and the PA and screen time during the pandemic period and a year prior were assessed with a score between 8 and 40. In this PA evaluation questionnaire, 10 questions were asked about the different physical activities and their frequency done in the last 7 days. The frequency of weekly activities was scored 1 to 5. Score 1 shows that the activity has been seldom done, and score 5 shows that the activity has been done almost every day, and the total score increases as the frequency of physical activities increases. The patients were divided into 2 goups: normal weighted (group 1) and overweighted/obese (group 2). All parameters measured during the pandemic period and a year prior were compared both within the groups and between groups 1 and 2. The correlation between PA-screen time and HbA1c, lipid profile and BMI SD was evaluated.

The patients and the families of the participants were informed about the study and signed informed consent

forms were obtained according to the Helsinki Declaration before they have were included in the study. The study was conducted after approval was given by the Ethics Committee of University of Health Sciences Türkiye, İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital (approval no: 48670771-514.10, date: 21 June 2021).

Statistical Analysis

For the statistical analyses, in the descriptive statistics of the data, mean, SD, median, minimum, maximum, frequency and ratio values were used. The distribution of variables was measured with the Kolmogorov-Smirnov test. The Mann-Whitney U test was used for two independent groups that did not show a normal distribution, and the Wilcoxon test was used for the comparison of two dependent groups that did not show a normal distribution. Chi-square test was used in the analysis of qualitative independent data, and Fisher's Exact test was used when the chi-square test conditions were not met. Spearman correlation analysis was used in the correlation analysis. "p" value <0.05 was considered as statistically significant.

RESULTS

The mean age of the patients was 12.2±3.2 years (median 12.4 years), and 47 (57.3%) of them were female, 35 (42.7%) of them were male. Group 1 (normal weighted) had 64, group 2 (overweighted/obese) had 18 patients. When these 2 groups were compared, no difference was observed in terms of age, sex, comorbidity and sociodemographic data (mother's level of education, father's level of education, type of house, presence of park or recreation areas suitable for PA nearby house, the way of transportation to school) and the frequency of going to school at pandemic (Table 1).

The number of fathers who went out to work did not change significantly, but the number of mothers staying at home increased significantly at pandemic (p<0.001) (Table 2).

There was no difference within the groups and between the groups in terms of height SD, HbA1c levels, cholesterol, HDL and triglyceride levels when compared before the pandemic and at pandemic period. LDL levels and although the patients were normotensive, systolic blood pressure SD and diastolic blood pressure SD were significantly decreased at the pandemic in both groups, but the difference was not significant when the two groups were compared. Weight SD and BMI SD increased significantly in both groups at the pandemic. Eight patients switched from group 1 to 2 in terms of BMI, and this shift was significant (p=0.004). The daily insulin dose was significantly higher in group 1 at pandemic. A comparison of anthromopetric measurements, biochemical parameters and insulin requirements between the pandemic period and the year prior is shown in Table 3.

The PA index was significantly decreased whereas the screen time (h/day) was significantly increased at pandemic in both groups (Table 4). There was no correlation between PA-screen time and HbA1c levels, lipid profile and BMI SD. There was a negative correlation between the PA and daily insulin dose (p=0.037, r=-0.261) (Table 5).

DISCUSSION

This study showed that the PA decreased and screen time and the number of parents staying at home with children increased, but the glycemic control of the children having T1DM did not change at a pandemic.

The regular PA is an important component of effective treatment of T1DM (6-8), but it is also reported to be ineffective in glycemic control in some studies (16-19). The COVID-19 pandemic, most of the people's PA opportunities had were greatly reduced due to lock down. Sarıkaya et al. (20) and Al Agha et al. (9) found no change in HbA1c levels of children having T1DM during the COVID-19 pandemic, whereas glycemic control was reported to be better in some other studies (3,10,21). In our study, despite the significant decrease in PA and increase in screen time because of prolonged school closure and home confinement of the children during the pandemic period, no significant change in HbA1C levels and no correlation between these parameters were found. During at pandemic, the number of parents staying at home compared to the year prior, has increased. The parents and children had more chance to make decisions together about the management of the disease. The support of the family might be the reason of no significant change in glycemic control of children having T1DM.

T1DM is associated with a high risk of microvascular and macrovascular complications and cardiovascular risk factors such as obesity, hypertension, hyperglycemia, dyslipidemia, and insulin resistance (22). Exercise is reported to increase aerobic fitness, improve lipid profile, but does not change BMI (7,17). Al Agha et al. (9) showed an increase in BMI

Table 1. Sociodemographic data of the patients

		Group 1*		Group 2†		p-value
		Mean ± SD		Mean ± SD		
Age (years)		12.4±3.1		11.5±3.6		0.274‡
		n	%	n	%	
Sex	Female	38	59.4	9	50.0	0.477§
	Male	26	40.6	9	50.0	
Comorbidity	No	47	73.4	12	66.7	0.572§
	Yes	17	26.6	6	33.3	
	Celiac disease	5	29.4	2	33.3	1.000§
	Hashimato thyroiditis	11	64.7	3	50.0	0.526§
	Other	2‖	11.8	1¶	16.6	0.270§
Mother's level of education	Primary school	3	4.7	3	16.7	
	Middle school	43	67.2	9	50.0	
	High school	15	23.4	3	16.7	0.891§
	University	3	4.7	3	16.7	
Father's level of education	Primary school	2	3.2	2	11.1	
	Middle school	33	51.6	8	44.4	0.839§
	High school	23	35.9	5	27.8	
	University	6	9.4	3	16.7	
Type of house	Apartment	58	90.6	17	94.4	
	Apartments with association	2	3.1	1	5.6	1.000
	Single family house	4	6.3	0	0.0	
Parks and recreation areas nearby house	No	37	57.8	11	61.1	0.802§
	Yes	27	42.2	7	38.9	
Way of transportation to school before the pandemic	Walking	42.0	65.6	11.0	61.1	
	Schoolbus	18.0	28.1	3.0	16.7	0.723§
	Family car	4.0	6.3	4.0	22.2	
Gone to school at the pandemic	No	31.0	48.4	5.0	27.8	0.119§
	Yes	33.0	51.6	13.0	72.2	
Days gone to school at pandemic (days/week)	2	33.0	100.0	12.0	92.3	0.283§
	5	0.0	0.0	1.0	7.7	

*Normal weighted patients, †Overweighted/obese patients, ‡Mann-Whitney U test, §Chi-square test, SDS: Standard deviation, ‖vitiligo, ¶Hydronephrosis

of children having T1DM during the COVID-19 pandemic and attributed this result to the decrease in rates of PA and following a healthy diet. However, Turan et al. (11) and Shah et al. (21) found that BMI of children having T1DM was not affected significantly during the COVID-19 pandemic although the weight was increased. In our study BMI SD was significantly increased during the pandemic in both groups, but the difference in increase between the groups was not significant. There was a significant shift in group 1 to 2. This might be due to decreased PA and increased screentime, which means an increase in sedentary behavior.

Table 2. The mothers and fathers working status before and after the pandemic

	BP	AP	p-value
Number of working mothers (n)	31	12	<0.001*
Number of mothers staying home (n)	51	70	
Number of working fathers (n)	62	59	0.59*
Number of fathers staying home (n)	20	23	

BP: Before the pandemic, AP: At pandemic, *Chi-square test

Lifestyle factors are determinants of children's blood pressure levels; increase in BMI, waist circumference and frequency of eating while watching television are found to elevate the blood pressure (23). No relationship was found between PA and blood pressure in healthy children and children having T1DM (7,18,24). Incompatible to the literature, although BMI SD was higher after the pandemic, we found a significant decrease in systolic and diastolic blood pressure SD in both groups, when compared the pandemic period and the year prior. As far as we know, there is no study in the literature, evaluating the blood pressure values of the children having T1DM during the pandemic. Although the children with T1DM and their caregivers are educated about managing T1DM, these children do not have enough support at school. There is "Diabetes at School Program" in our country for about 10 years, but strengthening diabetes care in schools in Türkiye for the children with T1DM is still a work in progress (25). At the pandemic, children managed the disease with the family support at home, instead of managing it alone at school. We thought that the reduction in blood pressure SD in our study may be due to the low anxiety about managing the disease. For this hypothesis to be supported, questionnaires assessing anxiety about managing T1DM are needed.

PA is an effective factor in decreasing the insulin requirements of children having T1DM (7,18). The weight and adipose tissue is another important factor in adjusting the insulin dose (6). Turan et al. (11) and Shah et al. (21) reported in their studies that the insulin requirement for treating T1DM did not change during the pandemic, and there was no significant difference in BMI values, too. In our study, we found a significant increase in the daily insulin dose of group 1 during the pandemic and a negative correlation between the PA and daily insulin dose. Eight patients in group 1 became overweighted/obese at pandemic and this shift was significant. Based on this result, it was thought that in addition to the decrease in PA, the increase in adipose tissue and the shift of BMI to the overweight/obese group may explain the increase in daily insulin requirement at the pandemic in group 1.

Many studies have reported that PA improves the lipid profile (7,8,17-19,24,26). Ludvigsson (4) evaluated the effect of a pandemic on lipid profile of children having T1DM and found no deterioration. In our study we found a significant decrease in LDL levels in both groups at the pandemic. Based on this finding, we thought that although the PA, which is an important factor in preventing the development of dyslipidemia, decreased, the LDL decrease might be due to the possibility of eating healthier meals in the family environment, rather than school canteens and due to limitations of snacks.

The American Academy of Pediatrics recommends that television watching among children should be limited to less than 2 h/day (27). Li et al. (28) showed in their longitudinal 5 year follow up study that, a decrease in screen time (3 h a day to 1 h a day) improves HbA1c levels in children having T1DM. The habit of snack consumption in front of the screen negatively affects metabolic control. In our study, we found a significant increase in screen time (h/day) at the pandemic in both groups, which was mostly for online school education. There was no correlation between screen time and HbA1c levels, lipid profile and BMI SD. We thought that most of the screen time increase was due to active class participation in front of the camera in the online education system, so it was impossible to snack during this time.

Our study had some limitations. Firstly, we didn't ask the daily dietary intake, snacks, junk food and fizzy drinks, so we couldn't evaluate the relationship between nutrition and BMI, HbA1c levels. Secondly, we couldn't measure PA with a device like a pedometer before and after the pandemic and compare activity levels. These would be more helpful in explaining the results of the study.

CONCLUSION

In conclusion, this study demonstrated that during the pandemic, the PA of patients with T1DM decreased, their screen time, the number of parents staying at home with children and BMI SD increased, but their glycemic and metabolic controls did not deteriorate. This result was attributed to the fact that although PA is a cornerstone in the management of T1DM, the support of the family might be much more decisive for metabolic control during the pandemic.

Table 3. Comparison of anthromopetric measurements, biochemical parameters and insulin requirements between the pandemic period and the year prior

	Group 1*		Group 2†		p-value‖
	Mean ± SD	Median	Mean ± SD	Median	
Systolic blood pressure SD					
BP	0.16±0.83	0.32	0.37±0.99	0.68	0.228‡
AP	0.12±0.83	0.40	0.27±0.95	0.63	0.316‡
BP/AP difference	-0.04±0.13	-0.05	-0.11±0.22	-0.04	0.702‡
p¶		0.001§		0.029§	
Diastolic blood pressure SD					
BP	0.34±0.81	0.37	0.41±0.70	0.40	0.831‡
AP	0.28±0.82	0.28	0.29±0.52	0.37	0.920‡
BP/AP difference	-0.06±0.08	-0.06	-0.12±0.29	-0.05	0.774‡
p¶		0.001§		0.005§	
Weight SD					
BP	-0.61±1.09	-0.40	0.70±1.38	0.74	0.001‡
AP	-0.27±1.17	-0.06	0.94±1.55	1.19	0.001‡
BP/AP difference	0.34±0.62	0.25	0.24±0.47	0.19	0.487‡
p¶		0.001§		0.043§	
Height SD					
BP	-0.38±1.24	-0.44	-0.58±1.60	-0.52	0.836‡
AP	-0.30±1.18	-0.40	-0.62±1.60	-0.55	0.924‡
BP/AP difference	0.08±0.51	-0.05	-0.04±0.33	-0.14	0.426‡
p¶		0.508§		0.433§	
BMI SD					
BP	-0.57±0.97	-0.50	1.21±0.79	1.26	0.001‡
AP	-0.15±1.04	0.05	1.44±1.96	1.38	0.001‡
BP/AP difference	0.42±0.80	0.35	0.23±0.40	0.22	0.398‡
p¶		0.001§		0.029§	
Daily insulin dose (U/kg/day)					
BP	0.97±0.32	1.00	0.87±0.33	0.85	0.339‡
AP	1.01±0.31	1.00	0.90±0.31	0.80	0.150‡
BP/AP difference	0.04±0.19	0.00	0.03±0.12	0.01	0.898‡
p¶		0.004§		0.203§	
Cholesterol (mg/dL)					
BP	166.3±39.7	161.5	179.5±56.0	173.5	0.361‡
AP	158.0±36.3	151.0	169.2±34.4	166.0	0.211‡
BP/AP difference	-7.2±27.2	6.0	-7.8±34.0	2.5	0.458‡
p¶		0.052§		0.349§	

Table 3. Continued

	Group 1*		Group 2†		p-value‖
	Mean ± SD	Median	Mean ± SD	Median	
Triglyceride (mg/dL)					
BP	93.1±65.2	71.0	132.6±142.1	77.5	0.175‡
AP	101.3±50.1	90.0	118.2±62.9	97.0	0.266‡
BP/AP difference	6.9±59.7	6.0	-1.9±89.8	10.5	0.737‡
p¶		0.069§		0.571§	
HDL (mg/dL)					
BP	57.6±13.5	58.0	56.6±12.5	56.5	0.758‡
AP	60.5±12.7	59.5	58.1±12.3	59.5	0.541‡
BP/AP difference	2.1±12.3	1.0	1.9±12.4	1.0	0.856‡
p¶		0.099§			0.619§
LDL (mg/dL)					
BP	91.7±28.0	87.0	127.0±86.2	97.0	0.129‡
AP	80.5±28.1	76.5	90.3±29.1	90.0	0.165‡
BP/AP difference	-9.2±19.1	-6.0	-22.8±52.3	-6.5	0.796‡
p¶		**0.001**§		**0.016**§	
HbA1c (%)					
BP	8.6±1.6	8.4	8.1±1.1	8.0	0.241‡
AP	8.5±1.9	8.2	8.1±1.4	7.9	0.473‡
BP/AP difference	-0.16±1.58	-0.10	-0.02±1.12	-0.20	0.711‡
p¶	0.100§			0.631§	

*Normal weighted patients, †overweighted/obese patients, ‡Mann-Whitney U test, §Wilcoxon test, ‖p-value for the comparison of the parameters between group 1 and 2, ¶p-value for the comparison of the parameters in each group within themselves, SD: Standard deviation, BP: Before pandemic, AP: At pandemic, BMI: Body mass index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HbA1c: Glycosylated hemoglobin

Table 4. Comparison of physical activity and screen time between the pandemic period and the year prior

	Group 1*		Group 2†		
	Medium ± SD	Median	Medium ± SD	Median	p‖
Physical activity index					
BP	25.9±7.2	26.0	25.9±5.4	25.5	0.951‡
AP	14.7±4.6	14.0	15.3±4.8	14.0	0.698‡
BP/AP difference	-11.2±6.6	-12.0	-10.7±5.1	-10.5	0.707‡
p¶	0.001§		0.001§		
Screen time (h/day)					
BP	4.2±1.7	-	4.3±2.3	4.0	0.927‡
AP	7.3±2.0	-	7.7±1.9	8.0	0.392‡
BP/AP difference	3.0±1.7	-	3.3±2.6	2.0	0.728‡
p¶	0.001§		0.001§		
For online school education AP	4.0±2.2	4.0	3.7±2.3	4.0	0.819‡

*Normal weighted patients, †Overweighted/obese patients, ‡Mann-Whitney U test, §Wilcoxon test, ‖p-value for the comparison of the parameters between group 1 and 2, ¶p-value for the comparison of the parameters in each group within themselves, SD: Standard deviation BP: Before the pandemic, AP: At pandemic

Table 5. Correlation between physical activity-screen time and lipid profile, HbA1c, BMI SD

		Triglyceride	HDL	LDL	HbA1c	Cholesterol	BMI SD	Daily insulin dose
Physical activity index	r*	-0.178	-0.073	0.010	-0.094	-0.114	0.118	-0.261
	p	0.160	0.566	0.936	0.461	0.370	0.355	0.037
Screen time	r*	0.254	-0.108	0.004	0.049	-0.120	0.198	-0.183
	p	0.051	0.394	0.972	0.698	0.343	0.117	0.148

*Spearman correlation, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, SDS: Standard deviation

ETHICS

Ethics Committee Approval: The study was conducted after approval was given by the Ethics Committee of University of Health Sciences Türkiye, İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital (approval no: 48670771-514.10, date: 21 June 2021).

Informed Consent: The patients and the families of the participants were informed about the study and signed informed consent forms were obtained according to the Helsinki Declaration before they have were included in the study.

Authorship Contributions

Concept: D.B., P.Y., A.K., Design: D.B., P.Y., A.K., Data Collection or Processing: D.B., P.Y., A.K., Analysis or Interpretation: D.B., P.Y., Literature Search: D.B., P.Y., Writing: D.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Research

The Comparison of Video Fiberscope and DCI Video Laryngoscope Performed by Two Practitioners in Patients with an EGRI Score of >4: A Single-blind, Prospective, Randomized Study

Video Fiberskop ile DCI Video Laringoskop Kullanımının EGRI skoru >4 Hastalarda İki Uygulayıcı Tarafından Karşılaştırılması: Tek kör, Prospektif, Randomize Çalışma

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ABSTRACT

Objective: Endotracheal intubation is central to the practice of general anesthesia. Complications can be prevented by using alternative airway devices in difficult intubation cases. In this study, we compared the results of endotracheal intubation with video fiberscope and direct-coupled interface (DCI) video laryngoscope devices performed by an experienced (E) and inexperienced (H) practitioner.

Methods: This single-blind, prospective, randomized study included 60 patients with an El-Ganzouri risk index score of >4 and American Society of Anesthesiologists score of <4 who were operated between October 1, 2018 and March 1, 2019, in the operating room of the Ondokuz Mayıs University Medical Faculty Hospital. Endotracheal intubation was performed by two practitioners using two different devices (video fiberscope and DCI video laryngoscope). Intubation times, a number of attempts, failed attempts, postoperative complications and haemodynamic responses were recorded.

Results: There were no significant differences between demographic data, the number of attempts, unsuccessful attempts, postoperative complications and haemodynamic data between the groups. In the DCI video laryngoscope group, time to intubation was significantly shorter by the E practitioner than that the H practitioner (p=0.047). The E practitioner performed intubation DCI video laryngoscope in a statistically significantly shorter time than using a video fiberscope (p=0.014).

Conclusion: In our study, unlike other studies in the literature, endotracheal intubation was performed with two different devices by two E and H practitioners in difficult intubation cases. We saw that the E practitioner provided endotracheal intubation in a shorter time with the DCI video laryngoscope compared to the video fiberscope and in a shorter time than the H practitioner. We believe that the comparison of two devices under different difficult intubation conditions by different practitioners may give a different perspective to the studies in the literature.

Keywords: DCI video laryngoscope, EGRI score, intubation, video fiberscope

ÖZ

Amaç: Endotrakeal entübasyon genel anestezi uygulamalarında önemli yer tutmaktadır. Önceden tespit edilmiş güç entübasyon olgularında alternatif hava yolu araç-gereçleri kullanılarak komplikasyonların önüne geçilebilmektedir. Çalışmamızda deneyimli (E) ve deneyimsiz (H) iki farklı hekimin video fiberskop ile direct-coupled interface (DCI) video laringoskop cihazları ile endotrakeal entübasyon uygulamalarının sonuçlarını karşılaştırmayı amaçladık.

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Gereç ve Yöntem: Randomize, prospektif ve tek kör nitelikteki çalışmamıza Ondokuz Mayıs Üniversitesi Tıp Fakültesi Hastanesi ameliyathanesinde, 1 Ekim 2018 ve 1 Mart 2019 tarihleri arasında opere edilen, 18-65 yaş arası, El-Ganzouri risk indeksi skoru >4, Amerikan Anestezi Derneği skoru <4 olan 60 hasta dahil edildi. İki uygulayıcı tarafından iki farklı cihazın (video fiberskop ile DCI video laringoskop) kullanımı ile endotrakeal entübasyon uygulamaları gerçekleştirildi. Entübasyon süreleri, girişim sayıları, başarısız girişimler, postoperatif komplikasyonlar ve hemodinamik yanıtlar kaydedildi.

Bulgular: Hastaların demografik verileri, uygulayıcıların girişim sayıları, başarısız girişimleri, postoperatif komplikasyonları ve hemodinamik veriler arasında anlamlı fark bulunamadı. DCI video laringoskop kullanımında E uygulayıcısının H uygulayıcısına göre istatistiksel olarak anlamlı şekilde kısa sürede entübasyonu gerçekleştirdiği görüldü (p=0,047). E uygulayıcısının DCI video laringoskop ile entübasyonu video fiberskop kullanımına göre istatistiksel olarak anlamlı şekilde daha kısa sürede gerçekleştirdiği görüldü (p=0,014).

Sonuç: Çalışmamızda literatürdeki diğer çalışmalardan farklı olarak güç entübasyon olgularında deneyimli ve deneyimsiz iki uygulayıcı tarafından iki farklı cihazla endotrakeal entübasyon gerçekleştirildi. E uygulayıcının DCI video laringoskopla hem video fiberskopla yapılanlara göre daha kısa sürede hem de H uygulayıcıdan daha kısa sürede endotrakeal entübasyonu sağladığını gördük. Farklı entübasyon güçlüğü koşullarında iki cihazın yine deneyimleri farklı uygulayıcılar tarafından karşılaştırılmasının literatürdeki çalışmalara farklı bakış açısı kazandırabileceğini düşünmekteyiz.

Anahtar Kelimeler: DCI video laringoskop, EGRI skoru, entübasyon, video fiberskop

INTRODUCTION

Endotracheal intubation is critical to maintain a patent airway, control airway and respiration, secure breathing effort and airway control during resuscitation, decrease dead space and aspiration risk, and surgical comfort for the surgeon by eliminating the need for an anesthesiologist and surgical equipment. However, it is a time-consuming procedure and requires experience and skills in difficult cases and is associated with certain complications (1). As the number of intubation attempts with classical laryngoscope increases, the complication rate increases (2). Therefore, the American Society of Anesthesiologists (ASA) recommends to avoiding repetitive attempts in difficult airway cases and to use alternative techniques (3). Recently, there has been a growing interest in developing alternative methods and devices.

In the literature, there are several studies compared the success rates, number of attempts, and time to successful endotracheal intubation of video laryngoscopes versus video fiberscopes (4,5). Many studies have shown that the results vary depending on the anesthesiologist's experience and skills (4).

In 1996, el-Ganzouri et al. (6) developed the El-Ganzouri risk index (EGRI), which is a multivariate model for stratifying the risk of difficult endotracheal intubation. Patients with an EGRI score of >4 should be considered difficult intubation cases and necessary precautions should be taken preoperatively.

In this study, we compared the results of endotracheal intubation with video fiberscope and direct-coupled interface (DCI) video laryngoscope devices performed by an experienced (E) and inexperienced (H) practitioner in patients with an EGRI score of >4.

METHODS

Study Design and Study Population

This single-center, single-blind, prospective, randomized study was approved by the Ondokuz Mayıs University Clinical Research Ethics Committee (decision no: OMÜ KAEK 2018/362, date: 27/07/2018). The study was conducted in accordance with the principles of the CONSORT guidelines and the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (NCT05243758).

The study included 114 patients aged between 18 and 65 years with an EGRI score of >4 and ASA score of <4 who were operated in the operating room of the Ondokuz Mayıs University Medical Faculty Hospital between October 1st, 2018, and March 1st, 2019. In the power analysis to determine the number of patients to be included in the study, when the article by Abdellatif and Ali (4) 'GlideScope® videolaryngoscope versus flexible fiberoptic bronchoscope for intubation of morbidly obese patient with predicted difficult intubation' was taken as reference, the number of samples for each group is at least 30 with 95% confidence and 99.9% test power. All patients were informed about the study and written informed consent was obtained. Exclusion criteria were as follows: being unwilling to give consent, having cerebrovascular event such as cerebral ischemia, hemorrhage or stroke, having carotid artery stenosis or a history of coronary artery disease, neurological disorders such as chronic head pain, epilepsy or previous head injury, alcohol or psychoactive drug abuse, severe heart and/or lung disease, hepatic and/or renal failure, uncontrolled diabetes and/or hypertension, dental abscess, <1.5 cm mouth opening, known bleeding disorder, pregnancy, mental retardation, and hypersensitivity to anesthetic agents. Finally, 60 patients were enrolled. The study flow chart is shown in Figure 1. EGRI is used to assess mouth opening, thyromental distance, Mallampati (oropharyngeal)

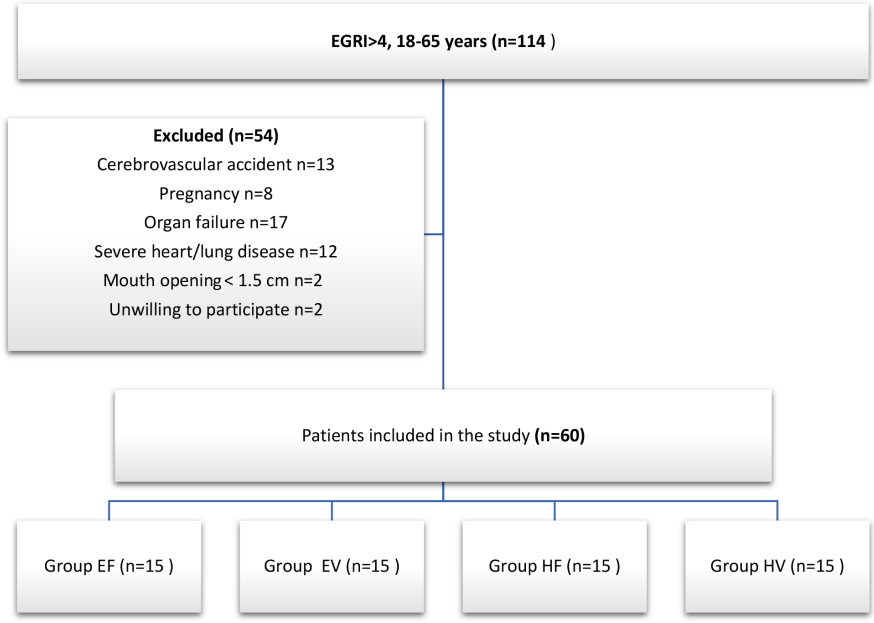

Figure 1. Study flowchart
EF: E practitioner using video fiberscope, HF: H practitioner using video fiberscope, EV: E practitioner using DCI video laryngoscope, HV: H practitioner using DCI video laryngoscope,EGRI: El-Ganzouri risk index

classification, neck movement, ability to prognath, body weight, and history of difficult tracheal intubation (Table 1) (6). We compared the success of both devices and practitioners with different experiences by using a DCI video laryngoscope and a video fiberscope in cases where difficult intubation is expected.

Endotracheal intubation was performed by an experienced anesthesiologist with a 10-year experience (E) or an inexperienced anesthesiologist who was in the last year (5th year) in Anesthesiology and Reanimation Residency Program (H).

Randomization

Randomization was performed using sealed envelopes. The randomization list was created in the electronic format and the groups were formed using the following initials: E practitioner using video fiberscope (EF), E practitioner using DCI video laryngoscope (EV), H practitioner using video fiberscope (HF), or H practitioner using DCI video laryngoscope (HV).

- Group EF (n=15): Patients undergoing endotracheal intubation using video fiberscope by the E practitioner.

- Group EV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the E practitioner.

- Group HF (n=15): Patients undergoing endotracheal intubation using video fiberscope by the H practitioner.

Table 1. El-Ganzouri risk index

Mouth opening		**Ability to prognathy**	
>4 cm	0	Yes	0
<4 cm	1	No	1
Thyromental distance		**Body weight**	
>6.5 cm	0	<90 kg	0
6-6.5 cm	1	90-110 kg	1
<6 cm	2	>110 kg	2
Mallampati classification		**History of difficult intubation**	
1	0	No	0
2	1		
3	2	Suspicious	1
4	2	Known	2
Neck movement			
>90°	0	**Total score**	
80-90°	1		
<80°	2		

Total score >4 indicates a difficult intubation risk

- Group HV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the H practitioner.

Before endotracheal intubation, the sealed envelopes were prepared by an independent individual who was excluded in the study and the practitioner and endotracheal intubation device were selected by another individual who was excluded in the study. Both practitioners and devices were kept prepared at the side of the patient.

Operation Technique

After a minimum 6-h fasting, the patients were placed in the rapid airway management position on the operating theater table and the intravenous route was created using a 22-gauge needle. Physiological saline (0.9%) was infused at a dose of 2 mL/kg/h. No premedication was administered. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) were measured. All patients were monitored using electrocardiography and peripheral oxygen saturation (SpO_2).

Before induction, preoxygenation was applied and the end-tidal oxygen was maintained at >80%. Following intravenous lidocaine administration (0.5 mg/kg), propofol was infused at a dose of 1.5 mg/kg. For intraoperative analgesia, remifentanil 0.05 to 0.2 µg/kg/min was infused via the intravenous route. Manual ventilation was applied via an anesthesia mask and neuromuscular block was maintained using intravenous rocuronium bromide at a dose of 0.6 mg/kg. Three minutes later, endotracheal intubation was performed using the DCI video laryngoscope or a video fiberscope.

During endotracheal intubation, the average size of the tube for an adult female was 7.0 to 7.5 and an adult male was 8.0 to 8.5 which was made of polyvinyl chloride with a sharp-edged Murphy eye and rounded atraumatic edges and low cuff pressure. During video laryngoscopy, a soft distal-tip, atraumatic, plastic aluminum probe was used. Using video fiberscope, sterile lubricant gel was applied to ensure that the intubation tube could pass through the device. Using the DCI video laryngoscope, sterile lubricant gel was also used to retrieve the probe from the intubation tube.

Data Collection and Outcome Measures

Data including age and sex of the patient, body weight, height, body mass index, ASA score, previous surgeries, concomitant chronic diseases and drugs, EGRI score, time to reach the glottis (defined as the time from the device reaching the anterior incisors to reaching vocal cords in min), and duration of intubation (defined as the time elapse between the visualization of the vocal cords and advancing the intubation tube through the vocal cords in min) were recorded. Successful intubation was defined as passing the fiberscope camera through the vocal cords for a video fiberscope and passing of the intubation tube through the vocal cords for a DCI video laryngoscope.

Cormack-Lehane score: In the patients undergoing DCI video laryngoscope, endotracheal intubation tube was visualized before passing the vocal cords. The localization of the intubation tube was confirmed using capnography and a stethoscope by auscultation of the apex and base of both lungs from the mid-axillary line. If endotracheal intubation failed after three consecutive attempts and if the intubation duration was >3 min with a SpO_2 of <90%, endotracheal intubation was considered unsuccessful. In such cases, ventilation was applied using the anesthesia mask until a SpO_2 of 100% was achieved and alternative airway devices were used.

After the procedure, the patient was extubated and throat pain and/or aphonia was evaluated at 2 h. Pulse (bpm), SBP (mmHg), DBP (mmHg), MBP (mmHg), and SpO_2 were measured before induction (at baseline), during intubation, and at 1, 2, and 5 min. after intubation.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows version 22.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were presented in mean ± standard deviation or number and frequency, where applicable. The Shapiro-Wilk test was used for normality check. The Levene test was used for homogeneity assumption. Binary comparisons were performed using the independent t-test. A p-value of <0.05 was considered statistically significant at 95% confidence interval.

RESULTS

There was no statistically significant difference in the demographic characteristics of the patients (Table 2).

Table 2. Demographic characteristics of the patients

	Practitioner	n	Mean	Standard deviation	Standard error mean
Age	E	30	45.23	15.58	2.84
	H	30	53.56	12.84	2.34
BMI (kg/m^2)	E	30	33.70	10.16	1.85
	H	30	34.23	8.19	1.49

BMI: Body mass index

Table 3. EGRI scores of patients

	EGRI score 5	EGRI score 6
EF	12	3
HF	11	4
EV	11	4
HV	12	3

Group EF (n=15): Patients undergoing endotracheal intubation using video fiberscope by the E practitioner; group EV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the E practitioner; group HF (n=15): Patients undergoing endotracheal intubation using video fiberscope by the H practitioner; group HV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the H practitioner.

EGRI: El-Ganzouri risk index, EF: E practitioner using video fiberscope, HF: H practitioner using video fiberscope, EV: E practitioner using DCI video laryngoscope, HV: H practitioner using DCI video laryngoscope, DCI: Direct-coupled interface

According to the EGRI scores, 12 patients in the EF group had a score of 5, while 3 patients in the EF group had a score of 6. In the HF group, 11 patients had a score of 5 and 4 patients had a score of 6. In the EV group, 11 patients had a score of 5 and 4 patients had a score of 6. In the HV group, 12 patients had a score of 5 and 3 patients had a score of 6 (Table 3).

There was no statistically significant difference in the mean time to reach the glottis using the DCI video laryngoscope between the two practitioners; however, the E practitioner performed the endotracheal intubation using the video laryngoscope in a statistically significantly shorter time than the H practitioner (p=0.047) (Table 4).

Furthermore, there was no statistically significant difference in the mean time to reach the glottis using the video fiberscope or DCI video laryngoscope between the EF and EV groups. However, endotracheal intubation was performed in a statistically significantly shorter time using DCI video laryngoscope by the E practitioner (p=0.014) (Table 5).

DISCUSSION

Endotracheal intubation is a common procedure used in the anesthesiology practice to secure the airway and respiration during surgical interventions. Difficult mask ventilation and difficult endotracheal intubation cases should be identified before anesthesia induction and necessary precautions should be taken. Therefore, various complications ranging from a simple dental infection to devastating conditions can be prevented. There are several methods to identify difficult mask ventilation and/or difficult endotracheal intubation cases (7). The EGRI score, which was developed in 1996, is one of these methods. An EGRI score of >4 indicates difficult intubation, while an EGRI score of >7 indicates severe intubation difficulty (8,9).

In difficult endotracheal intubation cases, video laryngoscope and video fiberscope can be used rather than a classical laryngoscope. Using these devices, laryngeal and tracheal structures can be visualized on a wide screen before and during intubation, which provides comfort for the practitioner. It also provides comfort for the patient, as no

Table 4. Time to reach glottis and duration of intubation using DCI video laryngoscope according to the practitioners

	Group	Mean	SD	t	p-value
Time to reach glottis (sec)	EV	9.53	2.53	1.988	0.57
	HV	7.33	3.45		
Duration of intubation (sec)	EV	13.66	2.25	-2.179	**0.047**
	HV	28.60	26.44		

t: Two independent samples t-test. Group EV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the E practitioner; group HV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the H practitioner.EV: E practitioner using DCI video laryngoscope, HV: H practitioner using DCI video laryngoscope, DCI: Direct-coupled interface, SD: Standard deviation

Table 5. Time to reach glottis and duration of intubation using different device by the E practitioner

	Group	Mean	SD	t	p-value
Time to reach glottis (sec)	EF	9.66	7.27	0.67	0.947
	EV	9.53	2.53		
Duration of intubation (sec)	EF	26.80	19.26	2.62	**0.014**
	EV	13.66	2.25		

t: Two independent samples t-test. Group EF (n=15): Patients undergoing endotracheal intubation using video fiberscope by the E practitioner; group EV (n=15): Patients undergoing endotracheal intubation using DCI video laryngoscope by the E practitioner. EF: E practitioner using video fiberscope, EV: E practitioner using DCI video laryngoscope, DCI: Direct-coupled interface, SD: Standard deviation

head extension is required in difficult intubation candidates or patients with cervical spine instability. Additionally, these devices facilitate learning during training.

Although alternative methods have been widely used for anesthesia and airway management recently, endotracheal intubation is still the cornerstone of daily practice in anesthesiology and other medical fields. General anesthesia preparations should be performed for both cases, which require general and regional anesthesia. In difficult intubation cases, alternative airway devices and plans should be readily available. In the literature, there are several studies comparing the classical laryngoscope and video laryngoscope and a fiberoptic bronchoscope.

Many studies have shown that the results vary depending on the practitioner's experience and skills. In our study, we compared the DCI video laryngoscope and video fiberscope performed by an E and H practitioner in difficult intubation cases (EGRI score >4). We found no statistically significant difference in the mean time to reach glottis between both devices; however, intubation was performed in a statistically significantly shorter time by the E practitioner than by the H practitioner (p=0.047). In their study including 64 difficult intubation patients, Abdellatif and Ali (4) performed a fiberoptic bronchoscope and video laryngoscope for intubation in an awaken state and reported that intubation was maintained in a shorter time using a video laryngoscope, although it did not reach statistical significance. The lack of statistical significance can be attributed to the fact that the practitioners were not blinded to the intubation devices and all operations were performed by experienced anesthesiologists. Platts-Mills et al. (10) also compared the Glidescope® video laryngoscope and direct laryngoscope in the emergency setting in 233 patients. In both groups, the success rate of the first attempt was similar (81% vs. 84%, respectively). However, the success rate of the first attempt of the third- and fourth-grade residents was statistically significantly higher than the second-grade residents using direct laryngoscope. This can be explained by the higher number of endotracheal intubations using a classical laryngoscope than the video laryngoscope and the increased experience during residency training. Considering these data, our study showed that the success rate was associated with the experience of the practitioner (less attempts and/or shorter time).

In this study, there was no statistically significant difference in the mean time to reach the glottis using the DCI video laryngoscope in the EF and EV groups; however, the E practitioner performed endotracheal intubation using the video laryngoscope in a statistically significantly shorter time than the H practitioner (p=0.014). In a study, Aziz et al. (11) compared the C-MAC video laryngoscope and direct laryngoscope in difficult intubation cases. The success rate of the first attempt was significantly higher with the C-MAC video laryngoscope. However, direct laryngoscope provided endotracheal intubation in a significantly shorter time (33 sec vs. 46 sec, respectively). Additionally, the C-MAC group required less Gum-elastic bougie and/or external laryngeal manipulation (24% vs. 37%, respectively). There was no significant difference in the complication rate between the groups. In a meta-analysis including eight studies with 429 difficult intubation cases, Alhomary et al. (7) compared five video laryngoscope devices (Glidescope, Bullard, McGrath, C-MAC D Blade, Pentax AWS) and two fiberoptic bronchoscopy devices (Karl Storz and Olympus) in an awaken state. Despite heterogeneity among the studies, video laryngoscope ensured a significantly shorter time for intubation than fiberoptic bronchoscope. In another study, Moore et al. (12) compared the Glidescope® video laryngoscope and fiberoptic bronchoscope in 36 patients undergoing bariatric surgery under sedation by two experienced practitioners (both practitioners experienced more than 40 cases with both devices). In the aforementioned study, endotracheal intubation was performed in a significantly shorter time via video laryngoscope, consistent with our study. This can be attributed to the fact that video laryngoscopes have a design similar to conventional laryngoscopes with a relatively easy-to-use system. The video laryngoscope is a rigid system, that provides certain advantages such as visualization of the oral soft tissues and capability of the removal of secretion and blood from the camera.

In a study including 75 patients with obesity, Abdelmalak et al. (13) compared the Glidescope® video laryngoscope and flexible fiberoptic bronchoscope with the assumption that Glidescope® provided intubation in a shorter time. The authors found no significant difference in the intubation duration, number of attempts, and complications between the two devices after general anesthesia induction. In this study, endotracheal intubation was associated with the experience of the practitioner. Additionally, endotracheal intubation failed with both devices and alternative devices were used. The authors recommended that anesthesiologists to be skilled in more than one device. Similary, in our study, the E practitioner performed endotracheal intubation using a DCI video laryngoscope in a significantly shorter time than video fiberscope, which can be attributed to the fact that the video laryngoscope can be inserted into the mouth similar to the classical laryngoscope with a high level of practice in

using these devices by the practitioners; however, the video fiberscope is placed through a mouthpiece while observing an oral airway with a relatively slow attempt with a careful hand, eye, and body cooperation.

The current study provides an additional contribution to the literature, as it compares the DCI video laryngoscope and video fiberscope by two practitioners. As in all fields of medicine, it is a life-saving strategy to recognize case and device diversity in the field of anesthesiology, which is critical for human life. However, there are some limitations to our study. Difficult intubation is a stressful situation for anesthesiologists. In patients with an EGRI score >4, identifying patients in this life-threatening process, determining an intubation plan and recording patients data are among the limitations of our study.

CONCLUSION

In conclusion, the lack of significant differences in the hemodynamic parameters before and during endotracheal intubation between the DCI video laryngoscope and video fiberscope suggests that both devices can be promising alternatives to the classical laryngoscope in patients with hemodynamic instability. Both devices facilitate endotracheal intubation in patients with an EGRI score of >4. Although we included difficult intubation cases (EGRI score of >4) and adult patients in our study, we believe that our attempt to compare E and H practitioners in using these devices will provide a better understanding and insight into the literature on this subject.

*The work entitled "Video Fiberskop ile DCI Video Laringoskop Kullanımının EGRI Skoru >4 Hastalarda İki Uygulayıcı Tarafından Karşılaştırılması: Tek Kör, Prospektif, Randomize Çalışma" is being produced by one of the authors Dr. Halil Cebeci's dissertation.

ETHICS

Ethics Committee Approval: This study was approved by the Ondokuz Mayıs University Clinical Research Ethics Committee (decision no: OMÜ KAEK 2018/362, date: 27/07/2018). The study was registered at ClinicalTrials.gov (NCT05243758).

Informed Consent: All patients were informed about the study and written informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: H.C., E.K., Concept: H.C., Design: H.C., G.C.C., Data Collection or Processing: H.C., G.C.C., Analysis or Interpretation: H.C., Literature Search: H.C., Writing: H.C., G.C.C., E.K.

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Research

Associations of XRCC4, eNOS, and PER3 VNTR variants with Childhood Acute Lymphoblastic Leukemia in Turkish Patients

Türk Hastalarında XRCC4, eNOS ve PER3 VNTR Varyantlarının Çocukluk Çağı Akut Lenfoblastik Lösemi ile İlişkisi

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ABSTRACT

Objective: The genetic factors responsible for the etiopathogenesis of childhood acute leukemia have been extensively investigated. High-resolution expression analysis of the whole genome, and results of gene studies including whole genome sequencing, copy number changes of DNA, loss of heterozygosity and epigenetic changes revealed the classification of acute lymphoblastic leukemia (ALL). A variable number of tandem repeats (VNTRs) can regulate many biological processes, including gene transcription, protein function, morphological development, and cancer formation. They may also play a role in many disorders in humans such as labile repeat expansions. In this paper, our aim was to compare the genotype and allele frequencies in VNTR variants of XRCC4, eNOS, and PER3 between pediatric ALL patients and healthy controls.

Methods: Seventy-four high-risk pediatric ALL patients (82.4% B-ALL, 17.6% T-ALL) who were consecutively admitted to the Pediatric Hematology Units of İstanbul Medical Faculty and Yeni Yuzyıl Medical Faculty and 100 healthy volunteers were included in this case-control study. VNTRs of three genes were analyzed using the polymerase chain reaction method.

Results: The frequency of the eNOS VNTR 4a/4a genotype was found to be higher in the pediatric patients with ALL compared to the healthy controls (p=0.044) and the risk factor for childhood ALL was found to be 8.382 (95% confidence interval =0.985-71.262). The frequency of eNOS 4/a allele was found to be higher in the childhood ALL group compared to the controls (p=0.013). The frequencies of the 5R/5R genotype and 5R allele of the PER3 VNTR were found to be significantly lower in the childhood ALL patients (p=0.039 and p=0.015, respectively).

Conclusion: Our results show that functional variants of the *eNOS* and *PER3* genes may have an important relationship with the etiopathogenesis of childhood ALL. Further studies including larger groups and different ethnic populations are needed to determine the effect of VNTR variants on the risk of developing childhood ALL.

Keywords: VNTR, eNOS, XRCC4, PER3, childhood ALL

ÖZ

Amaç: Çocukluk çağı akut lösemi etiyopatogenezinden sorumlu olan genetik faktörler kapsamlı bir şekilde araştırılmıştır. Tüm genomun yüksek çözünürlüklü ekspresyon analizi, ve tüm genom dizilimi, DNA'nın kopya sayısı değişiklikleri, heterozigotluk kaybı, epigenetik değişiklikler gibi gen çalışmalarının sonuçları, tüm akut lenfoblastik lösemi (ALL) hastalarının sınıflandırılabilmesini sağladı. Değişken sayıda tandem tekrarları (VNTR) gen transkripsiyonu, protein fonksiyonu, morfolojik gelişim, kanser davranışı ve fizyoloji gibi birçok biyolojik süreci modüle edebilirler. İnsanlarda, kararsız tekrar açılımlarını da içeren birçok bozukluktan da sorumlu olabilirler. Bu çalışmada çocukluk çağı ALL'li hastalarda XRCC4, eNOS ve PER3'ün VNTR varyantlarındaki genotip ve alel frekanslarını sağlıklı kontrollerle karşılaştırmayı amaçladık.

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Gereç ve Yöntem: İstanbul Tıp Fakültesi Pediatrik Hematoloji Ünitelerine ve Yeni Yüzyıl Tıp Fakültesi'ne ardışık olarak başvuran toplam 74 yüksek riskli çocukluk çağı ALL hastası (%82,4 B-ALL, %17,6 T-ALL) ve 100 sağlıklı gönüllü bu olgu-kontrol çalışmasına dahil edildi. Polimeraz zincir reaksiyonu yöntemi kullanılarak üç gen VNTR bölgesi analiz edildi.

Bulgular: Çocukluk çağı ALL'li olgular sağlıklı kontrollere göre daha yüksek eNOS VNTR 4a/4a genotipine sahipti (p=0,044) ve çocukluk çağı ALL'si için (Olasılık oranı: 8.382 %95 güven aralığı =0.985-71.262) risk faktörü olarak gösterildi. eNOS 4a alelinin sıklığı, çocukluk çağı ALL grubunda kontrollere göre daha yüksekti (p=0,013). Çocukluk çağı ALL hastalarında sırasıyla PER3 VNTR'nin 5R/5R genotipi ve 5R alelinin frekansları önemli ölçüde daha düşük saptandı (p=0,039, p=0,015).

Sonuç: Sonuçlarımız, *eNOS* ve *PER3* genlerinin fonksiyonel varyantlarının çocukluk çağı ALL etiyopatogenezi ile önemli bir ilişkisi olabileceğini düşündürmektedir. VNTR varyantlarının çocukluk çağı ALL gelişme riski üzerindeki etkisini belirlemek için daha büyük gruplar ve farklı etnik kökenler ile daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: VNTR, eNOS, XRCC4, PER3, çocukluk çağı ALL

INTRODUCTION

The genetic factors responsible for the etiopathogenesis of childhood acute leukemia have been extensively studied. Results of gene studies, high-resolution whole genome expression analysis, copy number changes of DNA, loss heterozygosity epigenetic changes and whole genome sequencing enabled the recognition of new genetic changes so that all acute lymphoblastic leukemia (ALL) patients could be classified.

Nitric oxide (NO) is synthesized from L-arginine by the nitric oxide synthase (NOS) enzyme and is a dual molecule that can have a tumor protective or stimulating effect depending on its local concentration. There are three main isoforms of the NOS enzyme: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Over the past decade, clinical trials have shown that NOS2 expression is associated with many cancers. Overexpression of NOS2 is present in >50% of patients with glioma, melanoma, breast, prostate, pancreatic, liver, cervical, ovarian, nasopharyngeal, lung, stomach, colon, and esophageal cancers (1). These studies drew attention to the increased angiogenic and metastatic potential of NOS2 (2). High NO flux causes genotoxicity and protein modification. It has been shown that high NO levels may lead to deamination, leading to a transition from C to T in DNA (3,4). Further research has shown that high NO levels can inhibit specific DNA repair systems, particularly thiol-dependent ones such as alkyl transferase and zinc finger proteins. Oxidation of carcinogenic nitrosamines via cytochrome P450 (CYP450) generate DNA alkylating metabolites that cause DNA damage (2).

DNA repair defects may induce further cancer progression by causing genetic instability in the genome (5). Three major DNA-based excision repair genes act interactively in DNA repair processes; X-ray repair cross-complement 1 and 4 (XRCC1 and XRCC4) and xeroderma pigmentosum complement group D (6). Polymorphisms of these genes can alter gene transcription rate, the stability of mRNA, or protein functions. It is thought that variations in these genes may cause cancer development by affecting an individual's capacity to repair damaged DNA (7).

Mechanisms related to the circadian clock are extremely important in terms of cell cycle, DNA damage and tumor suppression (8). At the molecular level, circadian clocks consist of the products of "clock genes" regulated in the transcription-translation regulatory system. Some clock genes encode transcriptional activators, while others encode proteins that can inhibit their expression. The circadian clock is associated with the clock genes of the circadian rhythm and consists of two transcription factors: CLOCK (circadian locomotor output loops caput) is a histone acetyl transferase that is activated when heterodimerized with 1 (brain and muscle hydrocarbon receptor nuclear receptor-antigen 1). It provides the transcription of Period (*PER 1, 2,* and *3*), and Cryptochrome (*Cry 1* and *Cry 2*) genes (9-14).

Per proteins contain two consecutive PAS domains and can interact with one another and with other proteins through these regions. PER3 is under clock control but is not required for rhythm production. However, PER1 and PER2 are the central components of the clock (8,13,14).

Disruption of the circadian clock is instrumental in the development of different human cancers. Disruption of the circadian rhythm causes modifications that change cell proliferation and lead to oncogenesis and cancer (15,16).

We hypothesized that genotype and allele frequencies in the variants of XRCC4 intron 3 variable number of tandem repeat (VNTR), eNOS intron 4b/a VNTR and PER3 exon 18 (54 bp repeats VNTR) are linked to childhood ALL.

METHODS

Study Population

Seventy-four high-risk pediatric ALL patients, who were consecutively admitted to the Pediatric Hematology Units of İstanbul Medical Faculty and Yeni Yüzyıl Medical Faculty, were included in this study. One hundred healthy volunteers were included as a control group.

Ethics statement: This study was approved by the Clinical Research Ethics Committee of İstanbul University, İstanbul Faculty of Medicine (no: 242064, date: 23.11.2020). The authors assert that all procedures contributing to this work comply with the ethical standards of Istanbul University and the Helsinki Decleration of 1975, as revised in 2008. Informed consent form was not obtained because the study was retrospective.

DNA Extraction and Genotyping

The peripheral blood samples of the patients were obtained at the time of diagnosis procedures before treatment was applied. Genomic DNA was extracted from whole blood using the Plus Blood Genomic DNA Purification test kit (GeneMark, USA).

The VNTRs of three genes, including *XRCC4*, *eNOS*, and *PER3*, were analyzed using the polymerase chain reaction method. Gene polymorphisms were detected with the polymerase chain reaction method (17-19) (Table 1).

Statistical Analysis

The data were analyzed using the SPSS software version 21. Descriptive statistics included the mean and standard deviation for the continuous variables. Nominal variables were summarized as frequency and percentage. Odds ratio (OR) and corresponding 95% confidence interval (CI) were used to determine the strength of the association. Consequently, we presented the ORs and 95% CIs for associating MBL genotypes with the clinical parameters. The association of the alleles and homozygosity was compared with the chi-square test (χ^2) or Fisher's Exact test, and Bonferroni correction was used. The two groups were in accordance with the Hardy-Weinberg equilibrium ($p>0.05$). A value of $p<0.05$ was accepted to be statistically significant.

RESULTS

A total of 74 childhood ALL patients and 100 controls were included in this study. Demographic and clinical characteristics of the patients are shown in Table 2. The statistical analysis showed no significant relationship for alleles and frequencies of XRCC4 genotype between the patients and controls ($p>0.05$) (Table 3).

The frequency of the eNOS VNTR 4a/4a genotype was found to be higher in the pediatric patients with ALL compared to the healthy controls ($p=0.044$) and the risk factor for childhood ALL was found to be 8.382 (95% CI=0.985-71.262). The frequency of eNOS 4a allele was found to be higher in the childhood ALL group compared to the controls ($p=0.013$) (Table 3). The frequencies of the 5R/5R genotype and 5R allele of the PER3 VNTR were found to be significantly lower in the childhood ALL patients ($p=0.039$ and $p=0.015$, respectively) (Table 3). Forty six percent of the male patients and 15.8% of the female patients carried the 4R/5R genotype of PER3 VNTR. The difference between the two groups was statistically significant ($p=0.026$, OR=4.543, 95% CI=1.174-17.579) (Table 4). No statistically significant correlation was found between the *XRCC4*, *eNOS*, *PER* genes, and disease relapse ($p>0.05$) (Table 5). The frequency of the eNOS 4a/4a genotype was found to be higher in the childhood T-ALL group (30.8%) compared with the childhood B-ALL group (3.3%) ($p=0.010$) (Table 6).

DISCUSSION

The pathophysiology of ALL is a very complex relationship with various factors (genetic, immune, environmental and drugs) at different levels. NO plays a crucial role in regulating cancer progression. Several studies have shown that the NO and NOS systems play important roles in carcinogenesis. Some studies are attempting to uncover the potential to modulate NO levels to increase the efficacy of currently available treatments against lymphoma, leukemia, and myeloma. It is thought that NO modulation could aid hematological cancer management, either by directly targeting tumor cells or by activating the immune system to eliminate cancer cells. *eNOS* gene polymorphisms significantly influence serum NO concentrations (20). Polymorphisms T786C and G894T affect eNOS regulation and have been associated with various diseases. Sickle

Table 1. XRCC4 (intron 3), eNOS (intron 4) and PER3 VNTR primer sequences, and amplification conditions

VNTR	Primer sequence	Annealing
XRCC4 (intron 3)	5′-TCCTGTTACCATTTCAGT GTTAT-3′ 5′-CACCTGTGTTCAATTCCAGCT T-3′	55 °C and 32 cycles
eNOS (intron 4)	5′-AGGCCCTATGGTAGTGCCTTT-3′ 5′-TCTCTTAGTGCTGTGGTCAC-3′	57 °C and 35 cycles
PER3	5 -TGTCTTTTCATGTGCCCTTACTT-3 5 -TGTCTGGCATTGGAGTTTGA-3	60 °C and 35 cycles

VNTR: Variable number of tandem repeat

cell disease, a clinically diverse chronic hemolytic anemia, involves impaired nitric oxide bioavailability (21). This study found the frequency of the eNOS 4a allele to be higher in the childhood ALL patient group. Simultaneously, the frequency of the eNOS VNTR 4a/4a genotype was found to be higher as a risk factor in the pediatric ALL group compared to the control group. The frequency of the eNOS 4a/4a genotype was higher in the childhood T-ALL group compared to the childhood B-ALL group. XRCC4 encodes a DNA repair protein that preserves genome stability by repairing a double strand breaks using the error-prone method. XRCC4 is generally expressed as a protein (334 amino acids) involved in DNA ligase IV and the enzyme DNA-dependent protein kinase in repairing DNA double strand breaks. Defects in the protein-coding gene cause disruption of the DNA repair process and accumulation of DNA damage in the cell that can cause cancer development (22,23).

Table 2. Demographic details of the patients. Values are either mean ± SD or n (%)

Parameters	Mean ± SD or n (%)
Total patients (n=74)	
Age, years	8.07±5.09
Female/male	24 (27.9%)/50 (72.1%)
Type ALL (B-ALL/T-ALL)	61 (82.4%)/13 (17.6%)
WBC count	50,000 (20,000-978,000)
Follow-up period	7.4±4.86 (1-15) years

WBC: White blood cell, SD: Standard deviation, ALL: Acute lymphoblastic leukemia

This study is the first to report of XRCC4 gene polymorphism in cALL in our population. We did not find any significant difference between pediatric ALL patients and healthy control groups in terms of the distribution of genotypes and alleles in XRCC4 VNTR.

Wu et al. (24) found differences in the frequency of XRCC4 G-1394T and intron 3 genotype between childhood

Table 3. The distribution of genotypes and alleles of XRCC4, eNOS, and PER3 variants in patients with childhood ALL and controls

		Patients	Controls	OR	95% CI	p
XRCC4	**Genotypes**	n=74	n=100			
	DD	17 (23.0%)	28 (28.0%)	0.766	0.382-1.538	0.488[&]
	ID	37 (50.0%)	43 (43.0%)	1.326	0.724-2.425	0.441*
	II	20 (27.0%)	29 (29.0%)	0.906	0.463-1.773	0.865*
	Allele					
	D	71 (48.0%)	99 (49.5%)	0.940	0.614-1.439	0.828[&]
	I	77 (52.0%)	101 (50.5%)	-	-	-
eNOS	**Genotypes**	n=74	n=96			
	4a/4a	6 (8.1%)	1 (%1.1)	8.382	0.985-71.267	0.044*
	4a/4b	18 (24.2%)	18 (%18.7)	1.393	0.665-2.914	0.449*
	4b/4b	50 (67.7%)	77 (%80.2)	0.514	0.255-1.035	0.075[&]
	Allele					
	4a	30 (20.3%)	20 (10.4%)	2.186	1.185-4.034	0.013[&]
	4b	118 (79.7%)	172 (89.6%)	-	-	-
PER3	**Genotypes**	n=69	n=97			
	4R/4R	40 (58.0%)	41 (42.3%)	1.884	1.008-3.521	0.058[&]
	4R/5R	26 (37.7%)	42 (43.3%)	0.791	0.421-1.489	0.523*
	5R/5R	3 (4.3%)	14 (14.4%)	0.269	0.074-0.977	0.039*
	Allele					
	4R	106 (76.8%)	124 (64.0%)	1.870	1.143-3.059	0.015[&]
	5R	32 (23.2%)	70 (36.0%)	-	-	-

*OR (95% CI) was adjusted for age and sex, [&]Fisher's Exact test. CI: Confidence interval, OR: Odds ratio, ALL: Acute lymphoblastic leukemia
Data written in bold was found to be statistically significant (p<0.05).

leukemia and control groups. They noted that deletions of the G allele of G-1394T and intron 3 were clear risk factors for susceptibility to childhood leukemia. They suggested that the G allele of XRCC4 G-1394T and deletion of intron 3 might be responsible for pediatric leukemia and might be useful in the early detection of cALL (24).

Cancer research in human and animal models has shown that endogenous factors contributing to the development

Table 4. Distribution of genotypes and alleles of the PER3 variant in male and female patients with childhood ALL

PER3	Male patients n=50 (%)	Female patients n=19 (%)	OR	95% CI	p
Genotypes					
4R/4R	25 (50.0)	15 (79.0)	0.266	0.077-0.916	**0.033**[&]
4R/5R	23 (46.0)	3 (15.8)	4.543	1.174-17.579	0.026*
5R/5R	2 (4.0)	1 (5.3)	0.750	0.063-8.791	1.000*
Allele					
4R	73 (73.0)	33 (86.8)	0.409	0.144-3.059	1.158[&]
5R	27 (27.0)	5 (13.2)			

*OR (95% CI) was adjusted for age and sex, [&]Fisher's Exact test. CI: Confidence interval, OR: Odds ratio, ALL: Acute lymphoblastic leukemia
Data written in bold was found to be statistically significant (p<0.05).

Table 5. The distribution of genotypes and alleles of XRCC4, eNOS, PER3 variants in childhood ALL patients with and without relaps

		With relaps	Without relaps	OR	95% CI	p
XRCC4	**Genotypes**	n=11	n=63			
	DD	2 (18.2%)	15 (23.8%)	0.941	0.229-6.237	0.682[&]
	ID	5 (45.5%)	32 (50.8%)	0.721	0.493-1.045	0.743*
	II	4 (36.4%)	16 (25.4%)	0.763	0.217-0.834	0.449*
	Allele					
	D	9 (40.9%)	62 (49.2%)	0.738	0.247-0.874	0.472[&]
	I	13 (59.1%)	64 (50.8%)	-	-	-
eNOS	**Genotypes**	n=11	n=63			
	4a/4a	1 (9.1%)	5 (7.9%)	1.160	0.123-11.006	0.999*
	4a/4b	2 (18.2%)	16 (25.4%)	0.652	0.127-3.346	0.954*
	4b/4b	8 (72.7%)	42 (66.7%)	1.133	0.320-5.554	0.895[&]
	Allele					
	4a	4 (18.2%)	26 (20.6%)	0.854	0.266-2.774	0.791[&]
	4b	18 (81.8%)	100 (79.4%)	-	-	-
PER3	**Genotypes**	n=11	n=58			
	4R/4R	7 (63.6%)	33 (56.9%)	1.326	0.349-5.034	0.750[&]
	4R/5R	4 (36.4%)	22 (37.9%)	0.935	0.245-3.365	1.000*
	5R/5R	0 (0.0%)	3 (5.2%)	0.689	0.033-14.287	1.000*
	Allele					
	4R	18 (81.8%)	88 (75.9%)	1.432	0.447-4.587	0.783[&]
	5R	4 (18.2%)	28 (24.1%)			

*OR (95% CI) was adjusted for age and sex, [&]Fisher's Exact test. CI: Confidence interval, OR: Odds ratio, ALL: Acute lymphoblastic leukemia
Data written in bold was found to be statistically significant (p<0.05).

Table 6. The distribution of genotypes and alleles of XRCC4, eNOS, PER3 variant in patients with childhood B-ALL and childhood T-ALL

		B-ALL	T-ALL	OR	95% CI	p
XRCC4	**Genotypes**	n=61	n=13			
	DD	13 (21.3%)	4 (30.8%)	0.609	0.161-2.299	0.479[&]
	ID	32 (52.5%)	5 (38.4%)	1.766	0.518-6.013	0.542*
	II	16 (26.2%)	4 (30.8%)	0.800	0.216-2.962	0.739*
	Allele					
	D	58 (47.5%)	13 (50.0%)	0.906	0.388-2.114	0.832[&]
	I	64 (52.5%)	13 (50.0%)	-	-	-
eNOS	**Genotypes**	n=61	n=13			
	4a/4a	2 (3.3%)	4 (30.8%)	0.086	0.013-0.535	0.010*
	4a/4b	18 (29.5%)	1 (7.7%)	4.636	0.558-38.468	0.166*
	4b/4b	41 (67.2%)	8 (61.5%)	0.911	0.249-3.332	1.000[&]
	Allele					
	4a	22 (18.0%)	9 (34.6%)	0.415	0.163-1.154	0.068[&]
	4b	100 (82.0%)	17 (65.4%)	-	-	-
PER3	**Genotypes**	n=57	n=12			
	4R/4R	34 (59.6%)	6 (50.0%)	1.478	0.423-5.157	0.541[&]
	4R/5R	20 (35.1%)	6 (50.0%)	0.540	0.157-1.898	0.347*
	5R/5R	3 (5.3%)	0 (0%)	1.606	0.077-33.130	0.984*
	Allele					
	4R	88 (77.2%)	18 (75.0%)	1.128	0.408-3.138	0.794[&]
	5R	26 (22.8%)	6 (25.0%)	-	-	-

*OR (95% CI) was adjusted for age and sex, [&]Fisher's Exact test. CI: Confidence interval, OR: Odds ratio, ALL: Acute lymphoblastic leukemia
Data written in bold was found to be statistically significant (p<0.05).

of disruption of circadian rhythms contribute to the development of cancer in mammals (25). Previous studies, it has been reported that circadian expression is altered in chronic myeloid leukemia (CML). In two different studies, expression changes of clock genes were shown in acute leukemia, BMAL1 expression was shown to be down-regulated by methylation in patients with AML and ALL (26) and PER2 expression was shown to be down-regulated in patients with AML (27).

The CRY1, CRY2, PER1, PER2, PER3, brain and muscle aryl hydrocarbon receptor nuclear translocator (BMAL1) genes have previously been shown to be associated with CML (28).

In this study, the frequencies of the 5R/5R genotype and 5R allele of the PER3 VNTR were foundto significantly lower in the pediatric ALL patients.

We determined that 46% of the male patients and 15.8% of the female patients carried the 4R/5R genotype of PER3 VNTR. The differences between the two groups were statistically significant. The fact that PER3 is the most down-regulated gene and recovery of PER3 correlates with better clinical outcomes in patients with acute leukemia, raises the possibility that deregulation of multiple molecular pathways may play a role in the development of acute leukemia, and at least one of them is tissue-specific inactivation of the *PER3* gene (29). Yang et al. (30) analyzed the expression of nine core circadian clock genes in patients with acute leukemia, and their data showed that different genes were dysregulated in AML and ALL. In both diseases, PER3 was the most down-regulated gene and improved PER3 expression was associated with better clinical outcomes (30).

A limitation of this study was the small number of patients. We anticipate that it may be more meaningful to work with a higher number of patients.

CONCLUSION

This study is one of the first studies investigating the relationship between *XRCC4, eNOS,* and *PER3* gene variants and cALL in our country. Our findings show that the *eNOS* and *PER3* genes may have a significant association with the etiopathogenesis of childhood ALL in Turkish subjects. Downregulation of circadian clock genes, particularly PER3, may promote proliferation of blastic cells, resulting in deregulation of the cell cycle.

ETHICS

Ethics Committee Approval: This study was supported by the Clinical Research Ethics Committee of İstanbul University, İstanbul Faculty of Medicine (no: 242064, date: 23.11.2020). This study was approved by the ethical review boards of the İstanbul University and conducted in accordance with the standards of the Declaration of Helsinki.

Informed Consent: Informed consent form was not obtained because the study was retrospective.

Authorship Contributions

Surgical and Medical Practices: R.O., M.G., A.A., Concept: R.O., S.P., Y.O., H.Ş.Ç., Design: R.O., S.P., Y.O., H.Ş.Ç., Data Collection or Processing: R.O., M.G., S.P., Y.O., Z.K., F.A., Analysis or Interpretation: R.O., S.P., Y.O., H.Ş.Ç., Z.K., Literature Search: R.O., M.G., S.P., Y.O., H.Ş.Ç., A.A., Z.K., F.A., Writing: R.O., S.P., H.Ş.Ç., F.A.

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Research

Investigation of the Relationship Between Methylation of Circadian Rhythm Genes and Menopause

Sirkadiyen Ritm Genlerinin Metilasyonu ile Menopoz Arasındaki İlişkinin İncelenmesi

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ABSTRACT

Objective: The circadian system acts at whole levels of a woman's life as a cornerstone: from follicle generation to the arrangement of hormonal balance; and the process from embryo implantation to birth.

Methods: We compared the promoter methylation status of the circadian genes, *BMAL1* and *CLOCK*, between pre-menopausal and post-menopausal women to find an epigenetic explanation in women with menopause. In this perspective, 56 postmenopausal women and 48 premenopausal women were enrolled in this study.

Results: Menopause and methylation status of the *BMAL1* or *CLOCK* genes did not show any statistically significant correlations (p>0.05). Moreover, the correlation of the methylation pattern of the *BMAL1* and *CLOCK* genes with age could not be detected (p>0.05).

Conclusion: The methylation status of the *BMAL1* and *CLOCK* genes in menopause was characterized for the first time in our study. Further studies should shed light on this subject.

Keywords: Menopause, BMAL1, CLOCK, MS-HRM, methylation

ÖZ

Amaç: Sirkadiyen sistem, bir kadının yaşamının tüm seviyelerinde bir mihenk taşı olarak hareket etmektedir: Folikül oluşumundan hormonal dengenin düzenlenmesine kadar; ve embriyo implantasyonundan doğuma kadar olan süreçlerde aktif olmaktadır.

Gereç ve Yöntem: Sirkadiyen ritm genleri olan *BMAL1* ve *CLOCK*'nin promotör metilasyon durumunu menopoz öncesi ve menopoz sonrası kadınlar arasında karşılaştırılmıştır. Bu amaç ile 56 postmenopozal kadın ve 48 premenopozal kadın bu çalışmaya dahil edilmiştir. **Bulgular:** *BMAL1* ve *CLOCK* genlerinin DNA metilasyonu değerlendirilmiştir. *BMAL1* veya *CLOCK* genlerinin menopoz ve metilasyon durumu arasında istatistiksel olarak anlamlı bir ilişki saptanmamıştır (p>0,05). Ayrıca *BMAL1* ve *CLOCK* genlerinin metilasyon paterni ile yaş arasında da istatistiksel olarak anlamlı bir ilişki saptanmamıştır (p>0,05).

Sonuç: Literatürde postmenopozal dönemde *BMAL1* ve *CLOCK* genlerinin metilasyon durumu ilk kez çalışmamızda araştırılmış olup çalışmamız gelecekteki çalışmalara ışık tutacaktır.

Anahtar Kelimeler: Menopoz, BMAL1, CLOCK, MS-HRM, metilasyon

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INTRODUCTION

The menstruation cycle is a monthly-based rhythm and menopausal transition is an important factor that alters this rhythm. Menopausal condition is described as a lasting cessation of menses as a consequence of deficiency of ovarian follicular activity (1). The consequences of menopause include physical and psychological disturbances, hot flashes, jitters, depression, sleeplessness, and tiredness (2). Although, with the other problems, sleep problems also start during the postmenopausal term (3). Generally, the time of menopause may be affected by irregularities in the gonadal function due to the desynchronization of the signal of environmental circadian signs (4). The circadian rhythm is defined as all of the biological processing which is monitored between 24 h, and the rhythms that occur within 24 h are established by the circadian clock (5). The circadian system acts in whole levels of a woman's life as a cornerstone: from follicle generation to the arrangement of hormonal balance; and the process from embryo implantation to birth (6). In the transcriptional translation feedback loop of circadian rhythms, *BMAL1* (basic helix-loop-helix ARNT like 1) and *CLOCK* (Circadian locomotor output cycles protein kaput) genes play an important roles (7). In this study, we analyzed the DNA methylation level of the *BMAL1* and *CLOCK* genes in postmenopausal subjects.

METHODS

Subjects Characteristics

A total of 56 postmenopausal and 48 premenopausal women were enrolled in this study. To participate in the study, women should have been in the post-menopause phase for at least a year are requested. However, women in the period of menopause unnaturally, women on antidepressants, antianxiety, or exogenic hormones, or women with mental retardation or serious illnesses were excluded from the study. The research Scientific Research Ethics Committee of the Near East University approved this study protocol and conducted it according with the Declaration of Helsinki (no: YDU/2021/93-1830, date: 29.07.2021).

This study was supported by the Near East University Scientific Research Project Unit (Grant Number: SAG-2016-2-012).

Epigenetic Analyses

Blood samples were collected from menopause and control cases. We extracted DNA from all subjects using the Qiagen AllPrep DNA/RNA/Protein isolation kit (Qiagen, Manchester, UK). We measured the amount of DNA using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Sodium bisulfite treatment was performed using an Epitect Bisulfite Modification kit (Qiagen, Manchester, UK). The sequences of primers for the promotor regions were designed according to the EpiTect® HRM™ PCR Handbook (Qiagen, Manchester, UK). The BMAL1 and CLOCK promotor methylation were analyzed according to EpiTect® HRM™ PCR Handbook protocol (Rotor-Gene Q, Qiagen).

Statistical Analysis

The chi-square test and two-tailed Fisher's exact test were used for statistical analysis of participant characteristics and their relationships with statistical tests. Calculations were conducted using SPSS 15.0 software (SPSS, Chicago, IL, USA) with a statistical significance of $p<0.05$.

RESULTS

The mean age of 48 participants who are in the stage of premenopause was 33.4±6.8, and the mean age of 56 participants who are in the stage of menopause was 56.6±4.8.

DNA Promoter Methylation Status of BMAL1, CLOCK in Menopause and Non-menopause Subjects

The *BMAL1* gene promoter was methylated in 34 of 56 subjects in post-menopause (60.07%) and 22 of the 44 non-menopause subjects (50.0%). There was no statistically significant difference between methylation status and menopausal condition identified ($p>0.05$) (Table 1).

The *CLOCK* gene promoter was methylated in 22 of the 54 menopause subjects (40.7%) and 20 out of the 48 non-menopause subjects (41.7%). There was no significant difference between methylation status and menopausal conditions ($p>0.05$) (Table 2).

DISCUSSION

CLOCK and BMAL1 form a heterodimer and lead to the activation of transcription of *PER* and *CRY* genes, which create a heterodimer structure in the cytoplasm and then turn back into the nucleus to being able to repress their transcription activity by suppressing the CLOCK-BMAL1 complex. This process occurs in nearly 24 h (7).

The *CLOCK* gene is located on chromosome 4q12. The single-nucleotide polymorphisms (SNPs) in the *CLOCK* gene are associated with sleep diminution, the concentration of adipocytokine, body mass index, and uptake of energy (8). The location of the *BMAL1* gene is on chromosome 11p15.3. The SNPs of the *BMAL1* gene are responsible for the occurrence of high blood pressure, diabetes mellitus,

Table 1. Methylation status of the *BMAL1* gene in pre-menopause and post-menopause subjects

BMAL1		Menopause status			
		Pre-menopause	Post-menopause	Total	p-value
Unmethylated	Observed	22	22	44	p>0.05
	% within column	50.0%	39.3%	44.0%	
Methylated	Observed	22	34	56	
	% within column	50.0%	60.7%	56.0%	
Total	Observed	44	56	100	
	% within column	100.0%	100.0%	100.0%	

Table 2. Methylation status of the *CLOCK* gene in pre-menopause and post-menopause subjects

CLOCK		Menopause status			
		Pre-menopause	Post-menopause	Total	p-value
Unmethylated	Observed	28	32	60	p>0.05
	% within column	58.3%	59.3%	58.8%	
Methylated	Observed	20	22	42	
	% within column	41.7%	40.7%	41.2%	
Total	Observed	48	54	102	
	% within column	100.0%	100.0%	100.0%	

and metabolic diseases, which lead to a raised risk of myocardial infarction (9).

The function of methylation of DNA in gene regulation has been supported by different researchers. DNA methylation is the most known epigenetic mechanism which is related to gene expression (10). The involvement of DNA methylation in imprinting disorders and cancer has been demonstrated by several studies (11). Furthermore, according to recent studies, DNA methylation take a part in autoimmune disorders, metabolic diseases, psychological diseases, obesity and aging (12).

Epigenetic biomarkers of aging based on methylation levels have been reported in various articles, illustrating the reflectance of chronological age on DNA methylation levels. Levine et al. (3) concluded that the age of menopause was substantially related to epigenetic age acceleration. In other words, earlier menopause correlates with raised epigenetic age due to reason of higher level of DNA methylation rate than the expected rate (3). The relationship between the age of menopause and night shift workers was investigated by Stock et al. (4) night worker women are at higher risk of menopause at an earlier age was concluded by Stock et al. (4). The risk of menopause at an earlier age is more clear for females younger than 45 years old since they face exposure to night shift work at the current time and during their lives. A higher risk of menopause among night workers cannot be supported, as the circadian rhythm deficiency has a suppressing effect on ovulation due to the disturbed circadian rhythm (4).

Circadian rhythms are controlled by the *BMAL1*, *CLOCK*, *PER*, and *CRY* genes (7). The circadian clock system is regulated by both epigenetic and genetic factors (13). Due to the circadian rhythm's role in metabolism and physiological mechanisms, diseases such as cancer and metabolic syndrome can be detected when the system is compromised (5). The polymorphisms of the *BMAL1* and *CLOCK* genes and their actions on menopause have been analyzed by various researchers. Semenova et al. (14) analyzed (CLOCK) 3111T/C gene polymorphism in the participants who were in the stage menopause. They did not find any differences in CLOCK 3111T/C genotypes or allele frequency between the control group and the main group (14).

With age, the circadian clock-controlled genes that act in the regulation of the circadian system lose their responsiveness. Consequently, females in the menopausal transition stage experience impairment in homeostasis and this is exacerbated by the hormonal imbalance. Hernandez-Morante et al. (15) investigated the expression of circadian genes in adipose tissue and their relationship with circadian gene expression in metabolic syndrome. They found that, in the subcutaneous adipose tissue, the PER3 expression level

of women who are in menopause is 42% higher than their counterparts who are in the premenopausal stage (15).

DNA methylation is the most commonly studied epigenetic process and represents a potential biomarker of future health outcomes. However, to date, there have no DNA methylation studies in this field. Our study is the first study in which the association of the methylation status of the *BMAL1* and *CLOCK* genes in menopause was investigated.

In our study, the methylation status of the *CLOCK* and *BMAL1* genes in both pre- and postmenopausal women was identified. We detected the BMAL1 promoter methylation in 50.0% of premenopausal women, and 60.7% of postmenopausal women. *BMAL1* gene was unmethylated in 50.0% of premenopausal subjects, and 39.3% of postmenopausal subjects. We determined the CLOCK promoter methylation in 41.7% of women in the period of premenopause, and 40.7% of women in the period of postmenopause. However, the unmethylation of the *CLOCK* gene was detected in 58.3% of control samples, and 59.3% of menopause samples. Both the genes were unmethylated in 63.6% of participants, and methylated in 45.8% of participants. The *BMAL1* gene was unmethylated, whereas the *CLOCK* gene was methylated in 36.4% of subjects. However, the *BMAL1* gene was methylated and the *CLOCK* gene was unmethylated in 54.2% of subjects. The correlation between the methylation pattern of the analyzed two genes and menopause, and an important relationship between the methylation pattern of the analyzed two genes could not be found statistically significant ($p>0.05$).

CONCLUSION

Studies have demonstrated SNPs of circadian rhythm genes and their importance during menopause. There is no DNA methylation studies have been performed in this field. From this perspective, our study will shed light and provide critical information to further epigenetic studies.

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ETHICS

Ethics Committee Approval: The research Scientific Research Ethics Committee of the Near East University approved this study protocol and conducted it according with the Declaration of Helsinki (no: YDU/2021/93-1830, date: 29.07.2021).

Informed Consent: A written informed consent form was obtained from each subject.

Authorship Contributions

Surgical and Medical Practices: R.K., G.K., Concept: R.K., Design: R.K., Data Collection or Processing: R.K., G.K., Analysis or Interpretation: G.K., Ö.T., Literature Search: R.K., G.K., Writing: R.K., G.K., Ö.T.

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Research

The Effect of Different Respiratory Exercise on Spirometer Test Parameters in Stroke Patients: Randomized Controlled Trial

İnme Hastalarında Farklı Solunum Egzersizlerinin Spirometre Test Parametreleri Üzerine Etkisi: Randomize Kontrollü Çalışma

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ABSTRACT

Objective: Respiratory muscle weakness causes increased morbidity and mortality in stroke patients, negatively affects respiratory functions, and even causes the recurrence of previous stroke. This study aims to investigate the effect of respiratory exercise and aerobic exercise programs, which are used in addition to Todd-Davies exercises, which is one of the neurodevelopmental classical treatment techniques on pulmonary function test parameters in stroke patients.

Methods: Thirty-six cases were randomly divided into two groups as breathing exercises group (group-respiratory) and aerobic exercise group (group-aerobics) after doctor's examination. Todd-Davies neurophysiological treatment approach was applied to both groups. Breathing exercises were applied to group-respiratory. An arm ergometer was used in group-aerobics. Follow-up of the cases consisted of a total of thirty sessions of one hour a day, five days a week, over a six-week period. The respiratory system-related parameters of the participants were measured on the first and last day of the forced expiration air volume (FEV1), forced vital capacity (FVC), Tiffeneau index (FEV1/FVC) and chest anthropometric measurements.

Results: When the groups were compared in the post-test evaluation, FEV1/FVC parameter [$t(34)=-2.922$; $p<0.01$] group-respiratory value was found to be higher than group-aerobic value. When the difference in chest circumference measurement was compared [$t(34)=4.049$; $p<0.01$], group-aerobic value was found higher than group-respiratory value.

Conclusion: In cases where aerobic training is added, the increase in chest circumference flexibility facilitates both chest expansion and stroke rehabilitation, and affects spirometry test results more positively than breathing exercises.

Keywords: Aerobic exercise, breathing exercises, neurological rehabilitation, respiratory function tests, stroke

ÖZ

Amaç: İnmeli hastalarda solunum kas zayıflığının artmış morbidite ve mortaliteye neden olduğu ve solunum fonksiyonlarını negatif yönde etkilediği, hatta geçirilen inmenin tekrarlamasına neden olduğu kabul edilmektedir. Bu çalışmanın amacı inmeli hastalarda nörogelişimsel klasik tedavi tekniklerinden biri olan Todd-Davies egzersizlerine ek kullanılan solunum egzersizi ve aerobik egzersiz programının solunum fonksiyon testi parametrelerine etkisini araştırmaktır.

Gereç ve Yöntem: Otuz altı olgu doktor muayenesinden sonra rastlantısal olarak solunum egzersizleri grubu (grup-solunum) ve aerobik egzersiz grubu (grup-aerobik) olmak üzere iki gruba ayrıldı. Her iki gruba da Todd-Davies nörofizyolojik tedavi yaklaşımı uygulandı. Grup-solunuma solunum egzersizleri uygulandı. Grup-aerobiğe ise kol ergometresi çalıştırıldı. Olguların takibi altı haftalık bir süreçte, haftanın beş günü, günde bir saat toplam otuz seanstan oluşturuldu. Katılımcıların solunum sistemi ile ilişkili parametreleri zorlu ekspiryumun 1. saniyesinde çıkarılan hava hacmi (FEV1), zorlu vital kapasite (FVC), Tiffeneau indeksi (FEV1/FVC) ve göğüs antropometrik ölçümü ilk gün ve son gün yapıldı.

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Bulgular: Grup-aerobikte FEV1 (p<0,01), FVC (p<0,01) ve göğüs çevre ölçümündeki farkı (p<0,01) anlamlı bulunup, grup-solunumda yalnızca göğüs çevre ölçüm farkı (p<0,01) anlamlı bulundu. Son test değerlendirmesinde gruplar karşılaştırıldığında FEV1/FVC parametresinde [t(34)=-2,922; p<0,01] grup-solunum değeri, grup-aerobik değerinden yüksek bulundu. Göğüs çevre ölçümü farkı karşılaştırıldığında ise [t(34)=4,049; p<0,01] grup-aerobik değeri, grup-solunum değerinden yüksek bulundu.

Sonuç: Nörofizyolojik egzersizlere solunum veya aerobik egzersiz eğitimini eklemek spirometre test parametrelerini olumlu etkilemektedir. Özellikle aerobik eğitimin eklendiği olgularda göğüs çevre esnekliğindeki artış hem göğüs ekspansiyonunu hem de inme rehabilitasyonunu kolaylaştırmakta ve spirometre test sonuçlarını solunum egzersizlerine oranla daha olumlu etkilemektedir.

Anahtar Kelimeler: Aerobik egzersiz, solunum egzersizleri, nörolojik rehabilitasyon, solunum fonksiyon testi, inme

INTRODUCTION

Stroke is one of the leading causes of morbidity and long-term disability worldwide. The most common complications are fall and pressure sores. Infections occur most frequently in the entire system with 17% and the pulmonary system with 13.6% (1).

Disruption of respiratory rhythm in the hemiplegic patient populations tissue oxygenation and affects the energy distribution (2). Respiratory muscle weakness causes increased morbidity and mortality and negatively affects respiratory functions. Additionally, the central nervous system origin of the lesions in the stroke picture, the involvement of the respiratory muscles and the decrease in muscle strength affect the coordination of the patients with exercise and accordingly prevent stroke rehabilitation (2,3). During respiration after a stroke, the movement of the diaphragm on the hemiplegic side is reduced. The parasternal muscles and particularly the abdominal muscles that help coughing are affected. Additionally, small ischemic lesions in the respiratory control center also affect ventilation (4). The affected side internal and external intercostal muscles, diaphragm and abdominal muscles are partially or totally weak in stroke patients (5). After stroke, respiratory dysfunctions are observed due to decreased vital capacity, total lung capacity, maximum inspiratory pressure, and particularly low expiratory reserve volume (6).

The classical conventional stroke rehabilitation focuses on regaining lost motor control and correct posture. According to Todd Davies, one of a neurodevelopmental classical treatment technique the use of a bilateral rather than unilateral treatment approach for treating hemiplegia in accordance with the Bobath principle gives better results. The aim of bilateral treatment is to first provide symmetry with normal balance reactions and then to facilitate normal movement patterns. For this reason, we focus on the trunk first and then work on normal joint movement in the extremities. Progress in accordance with the development determined during the treatment is added and a holistic approach is followed (7).

In this study, the Todd-Davies neurophysiological approach was applied to stroke patients. While symmetrical improvements are seen in the Todd-Davies method, which is focused on trunk symmetry first, the contribution of respiratory studies is not found in the literature. Therefore, this study was planned to investigate the relationship between respiratory exercise and aerobic exercise programs in addition to the Todd-Davies technique in stroke patients.

METHODS

In this study, in the province of İstanbul, T.C. Ministry of Health forty-six patients included who could understand and follow simple verbal instructions in a medical center affiliated with, who were diagnosed with stroke by specialist physicians and referred to physiotherapy and rehabilitation. After the doctor's examination, the patients were randomly divided into two groups according to their protocol numbers as group-respiratory, those with odd numbers, group-aerobics. Patients with chronic pulmonary and/or cardiac disease (n=3), uncontrolled hypertension (n=2), non-stroke chronic obstructive pulmonary disease and asthma (n=1), mini mental test score of 24 ten stroke patients who were under (n=2) and uncooperative (n=2) were excluded from the study (6,8). The patients were divided into two groups using the block randomization method, 18 in the aerobic exercise group and 18 in the respiratory exercise group. A randomized controlled study was conducted with 36 stroke patients. While the block randomization method eliminates selection bias, it is also a method that provides balancing of the number of individuals between groups (Figure 1).

After recording the sociodemographic and disease characteristics of the participants, respiratory system-related parameters; the volume of air exhaled in the first second of forced expiration (FEV1), forced vital capacity (FVC), FEV1/FVC values were evaluated with computerized spirometry (Schiller Type SP-260; Schiller AG Altgasse 68 CH-6341 Baar Switzerland) device test protocol (9). Brunsstrom upper extremity, chest anthropometric measurements were recorded from the xiphoid region by an independent physiotherapist after evaluating the first test before the exercise and the last test before the thirtieth session.

Follow-up of the cases consisted of 30 exercise sessions applied for 1 h a day, 5 days a week, over a 6-week period

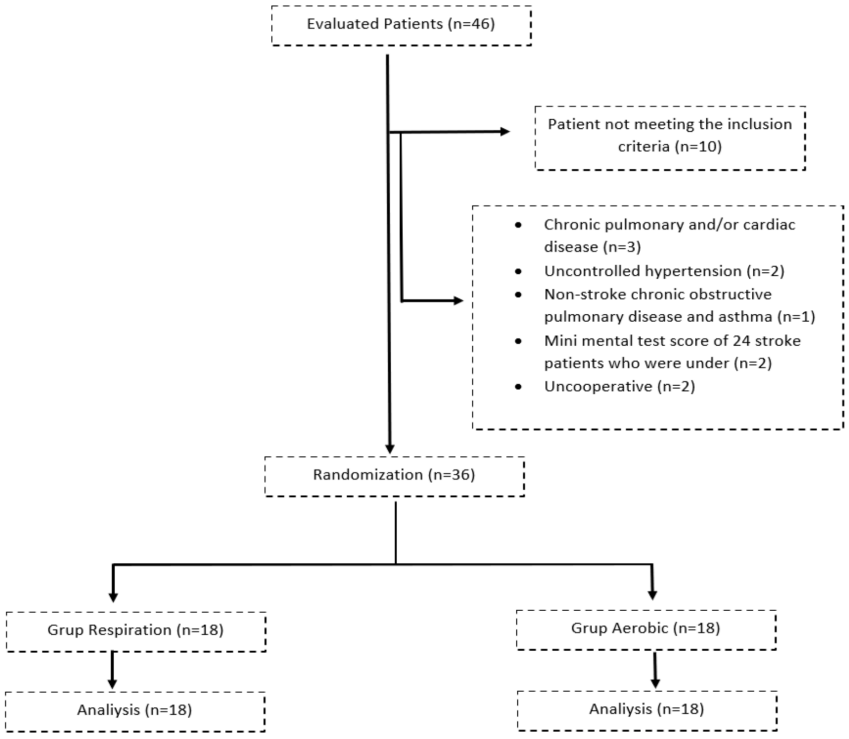

Figure 1. Flow chart

(10). In each session, the physiotherapist applied the 40-minute Todd-Davies approach to the participants. The patient was then directed to a quiet environment to perform the exercises for the respiratory group in which he was involved.

In addition to the Todd-Davies approach, diaphragmatic breathing and pursed lip breathing exercises were applied to group-respiratory cases at the end of the session. The patients were positioned in the supine position, with the knees semiflexed and head-shoulder supported by a pillow, with the hemiplegic hand at chest level and the intact hand in the abdominal region, and support was provided with a pillow for positioning when necessary (11). Breathing exercises consisted of diaphragmatic breathing for inspiration, pursed lip breathing for expiration, and spontaneous breathing, which we described as a silent breathing pattern. This cycle was applied to the patients for 3 sets of 6 min each, for 18 minutes (12). A maximum rest period of 5 min was allowed between each set (10). After the breathing exercise training, the patients showed the physiotherapist how to perform diaphragmatic and pursed lip breathing. The participant was questioned if he needed any question or repeated exercise demonstrations. Additional instructions were required during the exercise and he was asked to repeat the exercise (13).

In addition to the Todd-Davies approach to group-aerobic cases, arm ergometry was started at the end of the session. Aerobic training was performed on a motorized arm ergometer (Voit Dynamic® R-10 Exercise Bike). For exercise, the patients were seated in their own chair with the brakes off or on a fixed chair in such a way that the armrests did not hinder the patient. All participants started the exercise by turning the pedal forward. Each training session was created as a 15-minute forward and 15-minute reverse cycle. During the rest break, which lasted for a maximum of 5 min, between the two directions, the patient was asked to start turning in the opposite direction when he felt ready. Only verbal stimuli were given by the physiotherapist during the exercise (14). When necessary, the hemiplegic hand of the patient was properly fixed with a soft bandage.

Statistical Analysis

Sample size and power analysis were calculated with the program G*Power 3.1 (Franz Foul, Universitat Kiel, Germany). As in a similar study in the related literature, Joo et al. (9), the effect size was calculated as 0.848. To exceed the value of 95% in determining the power of the study; a total of 36 stroke patients in two groups, 18 in the aerobic exercise group and 18 in the respiratory exercise group, at a 5% significance level and an effect size of 0.848 were included in the study.

The data obtained in the study were analyzed using SPSS (Statistical Package for Social Sciences Inc, Chicago, IL, USA) for Windows 22.0 program. Number, percentage,

mean and standard deviation were used as descriptive statistical methods in the evaluating of the data. The relationship between grouped variables was tested by chi-square analysis. The t-test was used to compare quantitative continuous data between two independent groups. Repeated measurements within the group were analyzed with the paired group t-test.

Ethics

Ethics committee approval: This study was approved by the İstanbul Medipol University, Non-Invasive Clinical Research Ethics Committee (decision no: 653, date: 14.11.2018). Written informed consent was obtained from the patient.

RESULTS

There was no statistically significant difference between the groups in the patients' age, height, weight, body mass index, mini mental test scores, and the groups showed a homogeneous distribution in terms of these parameters (p>0.01) (Table 1).

There was no difference between the groups in the upper extremity Brunsstrom Staging in the first test (X2=6.804; p>0.01) and in the post-test Brunsstrom Staging (X2=5.222; p>0.01).

In group-aerobics; the increase in the FEV1 post-test value compared to the FEV1 first test value was significant (p<0.01). In group-respiration; the increase in the FEV1 post-test value compared to the FEV1 first test value was not significant (p>0.01) (Table 2). FEV1 first test and FEV1 post-test values of the patients did not differ significantly according to the group variable (p>0.01) (Figure 2).

Intra-group evaluations of both groups showed significant increases in FVC post-test value compared to FVC first test value (p<0.01) (Table 3). According to the group variable, the FVC first and posttest values of the patients did not differ significantly (p>0.01) (Figure 2).

Group-aerobics; The decrease in the FEV1/FVC post-test value compared to the FEV1/FVC first test value was not significant (p>0.01). Group-respiratory; the increase in the FEV1/FVC post-test value compared to the FEV1/FVC initial test value was not significant (p>0.01) (Table 4). When the FEV1/FVC post-test scores of the patients were examined, the group-respiratory FEV1/FVC post-test value was found to be higher than the group-aerobic FEV1/FVC post-test value [t(34)=-2.922; p<0.01] (Figure 2).

Group-aerobics; compared to the first test value of chest circumference difference ($\bar{x}$=0.639), the increase in the post-test value of the chest circumference difference ($\bar{x}$=1.417) was found to be significant (p<0.01). Group-respiratory; compared to the first test value of chest circumference difference ($\bar{x}$=0.528), the increase in the chest circumference difference post-test value ($\bar{x}$=0.822) was significant (p<0.01)

Table 1. Differences in age, height, weight, BMI, mini mental test scores by groups

	Aerobic (n=18)		Respiratory (n=18)		t	SD	p
	Mean	SD	Mean	SD			
Age	1965.944	10.315	1969.444	15.050	-0.814	34	0.422
Height	1.682	0.071	1.682	0.063	0.025	34	0.980
Weight	79.667	10.992	78.111	9.055	0.463	34	0.646
BMI	28.230	4.238	27.625	2.852	0.503	34	0.618
MMT	25.500	1.098	25.222	1.478	0.640	34	0.526

Independent groups t-test, BMI: Body mass index, MMT: Mini mental test, SD: Standard deviation

Table 2. Differentiation of FEV1 measurement scores by groups

	Aerobic (n=18)		Respiratory (n=18)		t	SD	p
	Mean	SD	Mean	SD			
FEV1 first test	2.923*	0.809	3.342	1.046	-1.344	34	0.188
FEV1 final test	3.590*	0.853	3.548	0.863	0.148	34	0.883
t	-4.177		-1.463				
p	0.001		0.162				

*Dependent groups t-test, FEV1: 1 second of forced expiratory volume, SD: Standard deviation

Table 3. Differentiation of FVC measurement scores by groups

	Aerobic (n=18)	Respirotary (n=18)	t	SD	p
	Mean SD	Mean SD			
FVC first test	3.431*	0.909	4.015	1.309	-1.554
FVC final test	4.438*	1.116	4.043	1.191	1.025
t	-3.928	-0.123			
p	0.001	0.903			

*Dependent groups t-test, FVC: Challenging vital capacity, SD: Standard deviation

(Table 5). When the chest circumference difference between the groups was examined, there was no significant difference between them in the first measurement (p>0.01), but the group-aerobic measurement difference in the last measurement and the chest circumference difference of the group-respiratory measurement were higher than the last measurement [t(34)=4.049; p<0.01] (Figure 2).

DISCUSSION

In our study, which was conducted to examine the effects of breathing exercises and aerobic exercise applied for 6 weeks on spirometry test parameters and chest expansion, group respiration was found to be significant in FEV1/FVC value, while group aerobic results were found to be significant in chest circumference measurement.

Pulmonary function tests are one of the most common measurement methods used in clinics. It is used to evaluate how efficiently the patient's pulmonary functions function (15). We preferred spirometric evaluation over pulmonary function tests in our study because it is cheap, portable and home-type. FEV1 and FVC values are used to determine the decrease in ventilation capacity in people with cardiopulmonary ventilation disorders. These findings are generally preferred to assess prognosis and monitor progress, as they show less variability than other indices (16). In our study, main respiratory indices such as FEV1, FVC, and FEV1/FVC were examined to evaluate pulmonary function.

There are quantitative results regarding the effect of repetitive aerobic training with arm ergometry on spasticity (17).

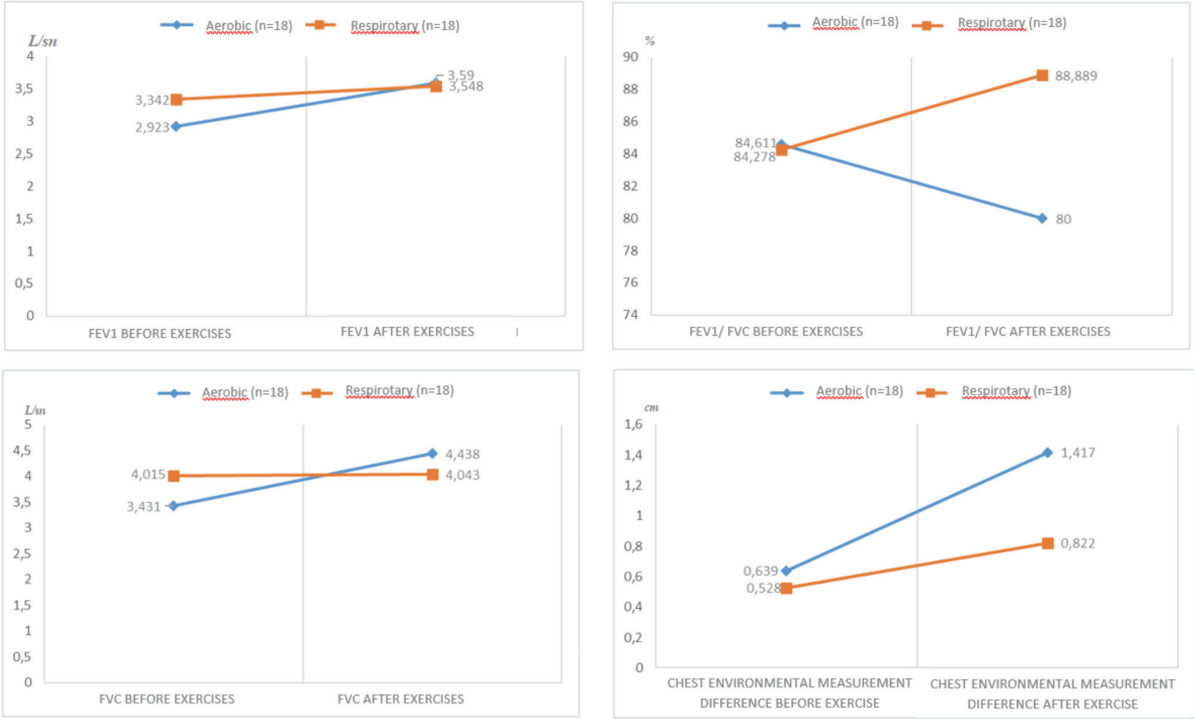

Figure 2. Differences in FEV1, FVC, FEV1/FVC, chest environmental measurement scores according to groups
FEV1: 1 second of forced expiratory volume, FVC: Challenging vital capacity, FEV1/FVC: Tiffeneau index

Table 4. Differentiation status of Tiffeneau index by groups

	Aerobic (n=18)		Respiratory (n=18)		t	SD	p
	Mean	SD	Mean	SD			
FEV1/FVC first test	84.611	10.308	84.278	13.190	0.084	34	0.933
FEV1/FVC final test	80.000*	10.053	88.889*	8.094	-2.922	34	0.006
t	1.766		-1.635				
p	0.095		0.120				

*Independent group t-test, FEV1: 1 second of forced expiratory volume, FVC: Forced vital capacity, FEV1/FVC: Tiffeneau index, SD: Standard deviation

Table 5. Differences in chest circumference measurements by groups

	Aerobic (n=18)		Respiratory (n=18)		t	SD	p
	Mean	SD	Mean	SD			
Chest circumference difference first test	0.639*	0.230	0.528*	0.118	1.821	34	0.080
Chest circumference difference final test	1.417*	0.493	0.822*	0.381	4.049	34	0.000
t	-7.714		-4.165				
p	0.000		0.001				

*Dependent and independent groups t-test, SD: Standard deviation

Aerobic exercise added to the neurodevelopmental approach increase the motivation of the patients to exercise, and the use of a bicycle ergometer as a repetitive activity may contribute to motor recovery. Bashir et al. (18) in their pilot study, they tested whether arm ergometry improves motor performance in stroke patients. Three patients received one-way cycling training on the arm ergometer for 20 min a day, 5 days a week, for 3 weeks. They stated that the grip strength of the lesioned hand increased after three weeks and that the arm ergometer was a useful device for motor training (18). In the data of our study, the difference between the first and last evaluations of aerobic exercise Brunnstrom upper extremity motor staging was not found significant. We think that the decrease in spasticity and the improvement of motor evaluation in aerobic exercise in a short period of 3 weeks may be due to the use of the arm ergometer in one direction throughout the exercise and the more repetition of the movement. In our study, the bicycle ergometer was applied forward for 15 min and backward for 15 min. Changing the direction of rotation during exercise may affect motor staging as it reduces the number of repetitions in the relevant muscle group. Longer unidirectional application of aerobic exercise may reduce spasticity in a shorter time and benefit motor evaluation. Further studies with more participants are needed to examine the effect of arm ergometer use on motor function in stroke patients.

In rehabilitation, verbal notification tone, use of imperative mood, emphasis and words used, or visual feedback may affect the measurement results. Kim et al. (19) divided 37 patients diagnosed with post-stroke hemiplegia into three groups. Among them, diaphragmatic training was given to the group, for 6 weeks, 5 days a week, 15 min, stimulating respiratory spirometry was applied with audible and visual back stimuli, and the changes in FEV1 were examined. Changes before and after exercise were found to be significant (19). In our study, the increase in the FEV1 post-test value was not found to be significant compared to the FEV1 first test value in group-respiratory cases. In our study, visual feedback was not given to the subjects in both groups during the exercises, only verbal feedback was used as an incentive. Visual feedback may be important for the FEV1 value in hemiplegic patients, and this notification may affect spirometry test results. It will be important to supplement exercise with visual feedback to improve respiratory test parameters. Feedback methods continue to be enriched with the development of technology, therefore, it is necessary to evaluate new methods and compare them with proven techniques and to increase the number of studies examining spirometry values, especially by using visual biofeedback in addition to audio feedback.

Also, Bang and Son (20) In another pilot study conducted, 12 stroke patients were included in aerobic exercise and traditional physical therapy training for 30 min a day, 5 days

a week, for 4 weeks. At the end of four weeks, it was found that FEV1 and FVC parameters were positively affected (20). In our study, the increase in the post-test values compared to the first test values of FEV1 and FVC was found to be significant in group-aerobic cases. This significant increase indicates that aerobic exercise positively affects respiratory parameters.

To obtain effective results in rehabilitation, the continuity of treatment is as important as patient motivation. The literature recommends at least 6 weeks of application for continuity. Seo et al. (21) In their study, 30 stroke patients were randomly and equally included in the study as the experimental group and the control group. The combined diaphragmatic breathing and pursed lip exercise was applied to the experimental group 5 times a week for 4 weeks. Significant differences were observed in the measurement of FEV1/FVC. No significant differences were found in the FEV1 and FVC values (21). In our study, the increase in FEV1 and FEV1/FVC post-test values was not found significant compared to the first test value in group-respiratory cases. Additionally, the increase in FVC post-test values was found to be statistically significant. We think that 4-6 weeks of respiratory training is not enough time for FEV1 value, and at least 6 weeks of combined breathing exercises should be performed for FVC.

There is a consensus in the literature that respiratory exercise programs shorter than 6-8 weeks have little effect.

Epigastric measurements are important to evaluate the chest expansion in breathing exercises. Aygün Keşim (22) performed, in a study in which 30 stroke patients participated, there was no significant difference between the two groups in axillary and subcostal measurements, while the difference in epigastric measurement was found to be significant (22). In our study, the difference in chest circumference measurement was made epigastric, and the increase observed in the post-test value was found to be significant both within and between groups and supports the literature. Thanks to the increased chest expansion, patients can adapt to exercise more easily and for a longer period.

One of our study limitations is that we did not create a control group to apply the optimum treatment in the clinic. As expected, the results in the respiratory or aerobic exercise studies conducted with the control group in the literature are in favor of the intervention group. The strength of our study is that we report which of the respiratory and aerobic exercises are more effective. Additionally, although we could not perform it because our laboratory conditions were not suitable, the relationship between aerobic exercise and blood pressure values was investigated in the literature and it was reported that systolic blood pressure increased during exercise, while diastolic blood pressure remained constant. Furthermore, in chronotropic incompetence is an issue that needs to be investigated in the relationship between exercise and blood pressure.

CONCLUSION

In conclusion, the addition of additional breathing exercises to conventional stroke rehabilitation had a positive effect on FEV1 and chest circumference measurement in the short term. Additionally, the effect of breathing exercises on the mental fitness and coordination of the patients can be investigated. Such exercises can be preferred in clinics because they do not require equipment. Including aerobic exercises in the rehabilitation program has significantly benefited the measurement of FEV1, FVC, and chest circumference. For this reason, adding aerobic training to exercise facilitates treatment, increases compliance with exercise, and facilitates fatigue management.

*This study entitled "İnme Hastalarında Farklı Egzersiz Yaklaşımlarının Solunum Fonksiyon Testi Parametrelerine Etkisi" is being produced by one of the authors Cansu Keskin's master of thesis.

ETHICS

Ethics Committee Approval: This study was approved by the İstanbul Medipol University, Non-Invasive Clinical Research Ethics Committee (decision no: 653, date: 14.11.2018).

Informed Consent: Written informed consent was obtained from the patient.

Authorship Contributions

Surgical and Medical Practices: C.K., Concept: C.K., S.Ç., Design: C.K., S.Ç., Data Collection or Processing: C.K., Analysis or Interpretation: C.K., S.Ç., Literature Search: C.K., S.Ç., Writing: C.K., S.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

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ERRATUM

Erratum: Musa Şahpolat, Mehmet Akif Karaman, Ebru Öztürk Çopur, Duygu Ayar, Cem Sesliokuyucu, Increased Neutrophil to Lymphocyte and Platelet to Lymphocyte Ratios in Patients with First Episode Psychosis, DOI: 10.4274/BMJ.galenos.2022.2021.11-9

The Ethics Committee Approval Form information of the article has been updated due to the inaccuracy of the existing information.

Revised Ethics Committee Approval Form Information:

METHODS

The relevant Kilis 7 Aralık University Ethics Committee approved the study protocol (decision no: 2020/09, date: 06.04.2020).

ETHICS

Ethics Committee Approval: We conducted the study following the principles of the Declaration of Helsinki. The relevant Kilis 7 Aralık University Ethics Committee approved the study protocol (decision no: 2020/09, date: 06.04.2020).

Kind regards,

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