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VOLUME 36 • ISSUE 2 • MAY • 2023

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ii. Walker M, Hull A. Preterm labor and birth. In: Taeusch HW, Ballard RA, eds. Avery's Diseases of the Newborn. Philadelphia: WB Saunders, 1998: 144,153.

iii. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 2017; 67: 1265-73. doi: 10.1016/j.jhep.2017.07.027.

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VOLUME 36 • ISSUE 2 • MAY • 2023

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Marmara Med J

MARMARA
MEDICAL JOURNAL

VOLUME 36 • ISSUE 2 • MAY • 2023

Contents

Original Articles

149 An effective and practical tool to assess physical frailty in older adults: Turkish validation of the FRAIL Scale

Ben Azir Begum HYMABACCUS, Rana TUNA DOGRUL, Cafer BALCI, Cemile OZSUREKCI, Hatice CALISKAN, Erdem KARABULUT, Meltem HALIL, Mustafa CANKURTARAN, Burcu Balam DOGU

157 Blood pressure and heart rate in aripiprazole once – monthly and paliperidone 1 and 3-month long-acting preparations

Gokce Elif SARIDOGAN, Mehmet Zafer GOREN

162 Morphological and biochemical evaluation of effects of *Myrtus communis* L. extract on heart and aorta in high fat-diet-induced obese rats

Nagehan OZYILMAZ YAY, Nurdan BULBUL AYCI, Rumeysa KELES KAYA, Ali SEN, Goksel SENER, Feriha ERCAN

171 The effect of serum activated ghrelin hormone on glycemic control in the diabetic patients with excessive body mass index

Yilmaz FAKI, Semih KALYON

175 Increased D-dimer is associated with disease progression and increased mortality in Turkish COVID-19 patients

Zeynep MERCANCI, Can ILGIN, Sehnaz OLGUN YILDIZELI, Derya KOCAKAYA, Baran BALCAN, Buket ERTURK SENGEL, Sait KARAKURT, Emel ERYUKSEL

182 The factors affecting the quality of life among women during the postpartum period

Gulsum Seyma KOCA, Yusuf Celik, Huseyin Levent KESKIN, Pinar YALCIN BALCIK

192 Is neurofibromatosis type 1 diagnosed in every patient who presents with café au lait macules? A single-center experience

Nursah EKER, Ayse Gulnur TOKUC, Burcu TAS TUFAN, Emel SENAY

197 T1 relaxation time in the evaluation of liver fibrosis; with native MR relaxometry

Firathan SARIALTIN, Hasan YIGIT, Elif ERGUN, Pinar Nercis KOSAR

203 Endogenous maternal serum preimplantation factor levels in early-onset preeclamptic pregnancies

Muhammet Atay OZTEN, Habibe AYVACI TASAN, Ece KARACA

210 Incidental findings detected on magnetic resonance imaging scans of the cervical, thoracic and lumbar spine of patients prediagnosed with discopathy

Samet Sancar KAYA, Hakan HATIRLI, Muhammed Azad SAHIN, Samet GENEZ, Mehmet OKCU

215 Healing effects of L-carnitine on experimental colon anastomosis wound

Emel KANDAS, Mustafa EDREMITLIOGLU, Ufuk DEMIR, Guven ERBIL, Muserref Hilal SEHITOGLU

223 Formation and branching patterns of deep palmar arch

Rasim HAMUTOGLU, Sukru Turan PESTEMALCI, Mehmet YILDIRIM

230 Is subclinical hypothyroidism a risk factor for gestational diabetes mellitus?

Halime SEN SELIM, Mustafa SENGUL

235 Assessments of energy, macro and micronutrient intakes in children and adolescents with type 1 diabetes mellitus

Volkan OZKAYA, Sebnem OZGEN OZKAYA

242 How does an additional insulin dose for a high-fat, high-protein breakfast affect glycemic response in adolescents with type 1 diabetes?

Aylin BAYINDIR GUMUS, Alev KESER, Zeynep SIKLAR, Merih BERBEROGLU

School of Medicine
Marmara Med J

MARMARA
MEDICAL JOURNAL

Review Articles

249 Crohn's disease: Etiology, pathogenesis and treatment strategies
Izel Aycan BASOGLU, Berna KARAKOYUN

255 A growing problem in childhood and adolescence: Metabolic syndrome and its relationship with physical activity and fitness
Adnan BARUTCU, Ceren ORNEK, Erkan KOZANOGLU

VOLUME 36 • ISSUE 2 • MAY • 2023

MARMARA
MEDICAL JOURNAL

Original Article

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An effective and practical tool to assess physical frailty in older adults: Turkish validation of the FRAIL Scale

Ben Azir Begum HYMABACCUS[1], Rana TUNA DOGRUL[1], Cafer BALCI[1], Cemile OZSUREKCI[1], Hatice CALISKAN[1], Erdem KARABULUT[2], Meltem HALIL[1], Mustafa CANKURTARAN[1], Burcu Balam DOGU[1]

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ABSTRACT

Objective: Practical scales with tested validity and reliability are needed to clinically determine frailty.The aim of this study is to find out whether the Fatigue, Resistance, Ambulation, Illnesses, and Loss of weight (FRAIL) Scale is an effective screening scale to show frailty.

Patients and Methods: The Frail non-Disabled (FIND) scale validated in the Turkish population was applied for FRAIL Scale validation. Comprehensive geriatric assessment and Fried Index were performed on 85 outpatients who were 65 years and older. The patients were examined in terms of comorbidity, number of falls, living environment, number of drugs used, and hospitalization in the last year.

Results: The FRAIL Scale had a high correlation with the FIND scale and Fried Index (correlation coefficients are 0.956 and 0.934, respectively). In addition, it was found to be associated with Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL) scales, the Mini-Mental State Examination (MMSE), Yesavage Geriatric Depression Scale (GDS), Mini Nutritional Assessment short-form (MNA-sf),Clock Drawing Test (CDT), handgrip strength, and timed up and go test ($p<0.05$). The compliance between independent practitioners and test-retest compliance were found to be 100% (full compliance,Cronbach's alpha coefficient is 1.00).

Conclusion: In the Turkish geriatric population, the FRAIL scale was found to be a reliable and valid scale in showing frailty.

Keywords: The FRAIL Scale, Frailty, Comprehensive geriatric assessment

1. INTRODUCTION

Frailty is defined as the state of weakness arising from the decrease in physiological reserves caused by physiological changes, diseases, and/or inadequate nutrition, etc. with advancing age [1]. Frailty is characterized by the impairment in adaptation to stress conditions such as acute disease and trauma depending on the decrease in the reserve in neuromuscular, metabolic, and immune systems [2]. This topic is gaining more and more important since frail older patients go through mortality, morbidity, and health expenditures when they are exposed to stress factors [3, 4].

As frailty is a dynamic process, it is of great importance to determine the frail population and provide appropriate treatment. According to a consensus involving international communities, all patients 70 years of age and above, those with chronic diseases, and individuals who have lost more than 5% weight over the last year should be screened for frailty [5]. There are many risk factors for frailty in older people [6]. Since frailty is a multifactorial clinical condition, it is quite difficult to measure [7]. Many easily applicable and reliable methods have been developed to determine frailty in geriatric clinics. "The Fatigue, Resistance, Ambulation, Illnesses, and Loss of weight (FRAIL) Scale developed by Morley et al., consists of five items [8]. In many countries, the FRAIL Scale has been validated and proved to be an effective method in determining frailty [9-13]. It allows for evaluation via the investigation of the patient's state of fatigue, resistance, weight loss, and other diseases. When it was first created, the FRAIL Scale was rather for measuring frailty in middle-aged Americans of African origin. However, it has been proved to be a reliable frailty scale in older patients living in many different societies. In the validity and reliability study of the FRAIL Scale on a Mexican population aged 60 and above in 2016, it was found to be a reliable scale in Mexican society, and

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Marmara Med J 2023;36(2): 149-156

associated with mortality, duration of hospital stay, dependency, and falls [10]. In the validation study of the FRAIL scale in Australia in 2015, it was proved to be a reliable frailty scale [12]. In the study conducted on 1,235 older people in China in 2017, the validity and reliability of the FRAIL Scale was proved [11].

There are no gold standard tests to measure frailty today, and the number of frailty measurement tests is quite high as well. In this study, it was aimed to reveal whether the FRAIL scale was an effective screening scale to show frailty. Frail non-Disabled (FIND) scale was used for FRAIL Scale validation in the Turkish population.

2. PATIENTS and METHODS

Participants

Eighty-five individuals, who presented to the Outpatient Clinic of Geriatric Medicine between March and July 2017, were 65 years old and older, agreed to participate in the study, and had the capability of understanding and answering the questions, were included in the study. Criteria of exclusion from the study were determined as follows: (1) Presence of active malignancy, (2) Patients with physical disabilities (extremity amputations, sequelae due to stroke, problems with speaking and hearing), (3) Patients with acute infections, (4) Patients with acute diseases (decompensated congestive heart failure, recent myocardial infarction/stroke, chronic obstructive pulmonary disease exacerbation), (5) Patients who were hospitalized or had an operation in the last month, (6) Patients with dementia at an advanced stage, (7) Patients unable to tell their medical history and not cooperating, (8) Patients with organic psycho-affective disorder and organic degenerative disease.

The patients were examined in terms of education status, gender, height and weight, smoking, alcohol consumption, living environment, number of falls in the last year, hospitalization in the last year, presence and type of urine incontinence, the status of vaccination, the number of drugs used, and presence of comorbidity. Furthermore, gait speeds (4.57 meters) and handgrip strengths (hand-held dynamometer (Takei A5401, Japan) determined during the examination were recorded. Individuals were asked about the hand they use in daily life activities such as eating and writing and in activities requiring power, and the hand they use for these tasks was determined as the dominant hand. The handgrip strength was measured with a Handgrip Dynamometer (Takei A5401, Japan) (measured by grip strength with a hand dynamometer). Measurements were made when the patients were standing, with the elbow and wrist in full extension. Measurements were repeated three times with intervals of five seconds, recorded in kilograms, and then averaged. For the gait speed test, the person was asked to walk a distance of 4.57 meters at a normal speed as in his daily life, and the duration of walking the 4.57 meters distance was calculated in seconds. The test was run 2 times and the best score achieved was recorded. The walking speed was recorded in m / sec by dividing the distance into the recorded times.

The FRAIL Scale

The FRAIL Scale is a test consisting of 5 questions, and an evaluation is made by investigating the patient's state of fatigue, resistance, ambulation, weight loss, and illnesses. To evaluate the state of fatigue of the patient, "How much of the time during the past 4 weeks did you feel tired?" is asked as the first question. The patient chooses one of 1=All of the time, 2=Most of the time, 3=Some of the time, 4=A little of the time, and 5=None of the time; if the patient's answer is 1 or 2, 1 point is given whereas the others get 0. To measure the resistance of the patient, "By yourself and not using aids, do you have any difficulty walking up 10 steps without resting?" is asked as the second question; if the patient says yes, 1 point is given, if no, then 0. To evaluate the ambulation of the patient, "By yourself and not using aids, do you have any difficulty walking several hundred meters?" is asked as the third question; if the patient says yes, 1 point is given, if no, then 0. To evaluate the illnesses of the patient, "Did a doctor ever tell you that you have (illness)? " (Hypertension, diabetes, cancer (except for small skin cancer), chronic lung disease, heart attack, congestive heart failure, angina, asthma, arthritis, stroke, kidney disease). If the patient has 0-4 diseases, 0 point is given; if 5-11 diseases, then 1 point. To evaluate weight loss, "How much do you weigh with your clothes on but without shoes? (current weight)" "One year ago, how much did you weight without your shoes and with your clothes on?" (Weight one year ago) are asked, and the percentage of weight change is calculated. If the weight change is above 5%, 1 point is given. In the FRAIL Scale, which consists of 5 items, 0 point is considered non-frail, 1-2 points pre-frail, and >2 points frail [8]. Patients participating in the study were evaluated as blind in terms of comprehensive geriatric assessment (CGA) and FRAIL Scale.

Reference Tools

Various screening and assessment tests are used for an objective, comprehensive geriatric assessment. As part of the comprehensive geriatric assessment, history, physical examination, geriatric syndrome questioning, and tests related to geriatric syndromes were performed. These include the Katz Activities of Daily Living (ADL) scale [14, 15], Instrumental Activities of Daily Living (IADL) scale [16], the Mini-Mental State Examination (MMSE)[17, 18], Mini Nutritional Assessment short-form (MNA-sf) [19, 20], Yesavage Geriatric Depression Scale (GDS) [21], 4.57-meters walking test and clock-drawing test (CDT) [22]. The patient's age, demographic characteristics, comorbid diseases, social status, cognition, mood, functionality, nutritional status, and geriatric syndromes were evaluated and recorded. A researcher who participated in the study recorded whether the patients were clinically frail or not according to the comprehensive geriatric assessment. These tests were applied to the patients by a geriatrician in the geriatrics outpatient clinic. Verbal responses were obtained from the patients.

The FIND scale was developed by Cesari et al. in 2014 [23, 24]. The FIND scale consists of 5 questions: 2 questions are about disability (walking 400 meters and climbing up one floor) and 3 questions are about frailty assessment (weight loss, fatigue, and physical activity). It ranges between 0-5 points, and the state

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Marmara Med J 2023;36(2): 149-156

of disability and frailty is determined according to the score received. If the patient gets 1 point from the first 2 questions, it is considered to be a disability, and if the patient scores zero, the other 3 questions are asked. If the patient gets 1 point from these questions, it is considered as frail.

The patients were subjected to the Fried Index. In the Fried Index, involuntary weight loss, fatigue stated by the individual, weakness, slow gait speed, and low physical activity are assessed [25]. Weight loss (1): The patient has an unintentional weight loss of 4.5 kg or more compared to the previous year, or a weight loss of 5% or more in body weight at follow-up compared to the previous year. The state of exhaustion (2) was determined by 2 questions on the Center for Epidemiological Research – Depression (CES-D) scale: How often in the last week you felt that everything you did was an effort?' and 'How often in the last week you felt that you could not go out? Participants who answered these questions 3-4 days a week or more were accepted as meeting the exhaustion criterion. Low physical activity (3) was assessed using the Minnesota Leisure Physical Activity Questionnaire [26]. Weekly calories spent on activities are calculated using a standard algorithm. This variable is classified according to gender. Men: Those who show physical activity less than 383 Kcal per week are considered frail for this criterion. Women: Those who show physical activity less than 270 Kcal per week were considered frail for this criterion. Slow gait speed (4) was calculated by looking at the walking speed at 4.57 meters. Weakness (5): According to the measurement made with Jamar hand dynamometer (Takei A5401, Japan), it was interpreted according to body mass index. According to these criteria, patients with 3 or higher points are reported as 'frail', those with 1 or 2 points as 'pre-frail', and those with 0 point as 'non-frail'.

Translation

Firstly, the FRAIL Scale, consisting of 5 questions, was translated from English to Turkish. To ensure the language validity of the scale, a group of specialists checked the Turkish translation after it was completed. After the translation was checked, the Turkish version was translated to English, its original language, by a translator who did know the original form of the test. Specialists and translators came together and decided upon the final version of the Turkish FRAIL scale. Accordingly, language validation was provided with the method of "forward-backward translation". The compliance between and within the independent observers were tested. The FRAIL Scale was implemented on 21 patients by a second researcher who did not know the outcome of the scale on the same day to test the compliance between the independent practitioners. To examine the test-retest compliance, the FRAIL Scale was re-implemented on 27 patients between 7-15 days by the first researcher with similar outpatient clinic conditions to examine the test-retest compliance, and the received scores were recorded. FIND and Fried Index were performed by another researcher.

Ethics

After the permission of J. E. Morley who developed the FRAIL Scale was obtained [8], the study protocol was evaluated and approved by the local Ethics Committee (Hacettepe University, GO 17/91-37, 28.02.2017). Informed consent was obtained from all the patients.

Statistical Analyses

Statistical analysis was performed using the IBM SPSS Statistics 22.0 software. The number of patients included in the study was decided by power analysis. The conformity of the numeric variables, whose descriptive statistics was given first, to a normal distribution was examined by using visual (histograms and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics were given for the variables with a normal distribution by using mean and standard deviation (mean±SD) values, and for the variables without a normal distribution by using median and minimum-maximum values. Categorical variables were stated as numbers and percentages (%). Cronbach's alpha coefficient was used to test the internal consistency of the FRAIL Scale. The correlations between the FRAIL Scale, FIND Scale, Fried Index, MMSE, and other numeric variables were checked with the Spearman correlation coefficient. Test-retest and interrater reliability were examined with the intraclass correlation coefficient. The difference in quantitative variables (gender, education status, etc.) according to the FRAIL groups (robust-non-frail, pre-frail and frail) was investigated with the Chi-Square or Fisher's Exact tests. The variables with a normal distribution between the Frail groups were compared via the one-way analysis of variance, and those without a normal distribution via the Kruskal-Wallis test. The significance of the differences was tested by the Student T-test (for one-way analysis of variance) and Mann-Whitney U test (for Kruskal-Wallis) with a Bonferroni correction for multiple comparisons (significance level $\alpha = 0.05/m$, with m = number of multihypothesesesis tested). Since there were three different groups (robust, pre-frail, frail), three different pairwise comparisons were performed and the adjusted P-value was found to be $0.05 /3 = 0.016$.

Receiver operating characteristic (ROC) evaluation was made by grouping frail and non-frail and the Kappa coefficient was calculated. A Kappa coefficient of 0.80 and above was interpreted as a perfect fit. If the area under the ROC curve (AUC) was close to 1, it was considered to have excellent diagnostic accuracy, and sensitivity and specificity values were determined. A 5% type I error level was used to infer statistical significance.

3. RESULTS

The mean age of the patients participating in the study was 75.45±5.20, and 69.4% were women. According to the FRAIL Scale, 42.4% of the patients were robust (n:36), 24.7% were pre-frail (n:21), and 32.9% were frail (n:28). When the mean age of groups of the patients were reviewed, frailty was shown to increase as the age advanced, which was found statistically significant (p=0.008). Women were seen to be more frail than men (p=0.015). From the robust group to the frail group ADL, IADL, MMSE, MNA sf, CDT score, and influenza strength decreased and were found to be statistically significant ($p < 0.001$). As the degree of frailty increased, the score of the

http://doi.org/10.5472/marumj.1297696
Marmara Med J 2023;36(2): 149-156

Yesavage GDS was observed to increase (p=0.001). Patients' demographic characteristics and results of the comprehensive geriatric assessment are given in Table I.

Table I. *Demographic characteristics of the patients and results of the comprehensive geriatric assessment*

	Robust (n:36)	Pre-frail (n:21)	Frail (n:28)	p
Age, year, mean±SD	72.8±6.05	75.95±7.40	79.79±6.06	**<0.001**
Female gender, n (%)	18 (32.2)	19 (30.5)	22 (37.3)	**0.015**
Education level, n (%)				
Illiterate	5 (13.9)	7 (33.3)	7 (25.0)	0.856
Primary School	18 (46.2)	9 (42.9)	12 (42.9)	
Secondary school	4 (11.1)	2 (9.5)	12 (7.1)	
High school	5 (13.9)	1 (4.8)	3 (10.7)	
University	4 (11.1)	2 (9.5)	4 (14.3)	
Living environment, n (%)	6 (16.7)	2 (9.5)	2 (7.1)	0.293
Alone	17 (47.2)	9 (42.9)	9 (32.1)	
With Spouse	13 (36.1)	10 (47.6)	13 (46.4)	
Other	0 (0)	0 (0)	4 (14.3)	
BMI, kg/m², mean±SD	29.46±5.67	28.0±6.19	28.09±6.91	0.812
Smoking status, n (%)				0.99
Never used	26 (72.2)	16 (76.2)	20 (71.4)	
Ex-smoker	8 (22.2)	4 (19.0)	7 (25.0)	
Active smoker	2 (5.6)	1 (4.8)	1 (3.6)	
Drinking alcohol, n (%)	2 (5.6)	0 (0.00)	0 (0.00)	0.99
Number of drugs, mean±SD	4.17±2.69	5.05±2.62	7.07±4.03	**0.014**
Number of comorbidities (%)	3 (0-8)	3 (1-8)	6 (1-9)	**<0.001**
Number of hospitalizations in the last year, n (%)	4 (11.1)	3 (14.3)	10 (35.7)	**0.038**
Number of falls, n (%)	6 (16.7)	5 (23.8)	22 (76.8)	**<0.001**
Urinary incontinence, n (%)	8 (22.29)	10 (47.6)	23 (82.1)	**<0.001**
Vaccination, n (%)				
Influenza vaccine	10 (27.8)	6(28.6)	11(39.3)	0.803
Pneumococcal vaccine	4(11.1)	2(9.5)	8(28.6)	0.119
Katz ADL score, mean±SD	5.81±1.01	5.81±0.60	4.04±1.87	**<0.001**
Lawton-Brody IADL score, mean±SD	7.75±0.69	6.57±2.09	3.21±2.82	**<0.001**
MNA-SF score, mean±SD	13.22±1.78	11.62±3.01	8.50±3.01	**<0.001**
MMSE score, mean±SD	28.08±2.9	25.90±4.74	20.11±6.50	**<0.001**
CDT score, mean±SD	4.72±1.91	4.10±1.95	2.11±0.18	**<0.001**
Yesavage GDS score, mean±SD	2.50±3.08	4.24±3.39	6.33±4.19	**0.001**
Handgrip, kg, mean±SD	25.67±6.1	20.02±5.89	14.65±5.94	**<0.001**

** SD: Standard deviation, BMI: Body mass index, ADL: Activities of Daily Living, IADL: Instrumental Activities of Daily Living, MNA-sf: Mini Nutritional Assessment short-form, MMSE: Mini-Mental State Examination, CDT: clock-drawing test, GDS: Geriatric Depression Scale*

The FRAIL Scale used in our study was found to have an extremely high correlation with the FIND Scale (Spearman 0.956, p<0.001), Fried Index (Spearman 0.934, p0.001), and clinical frailty score (Spearman 0.877, p0.001) (Table II). A significant correlation was observed between the FRAIL Scale, age, and comprehensive geriatric assessment components (p<0.001) (Table III).

Table II. *The correlation between the frailty parameters and the FRAIL Scale*

	The FRAIL Scale and the correlation coefficient	p
The FIND Scale	0.976	**<0.001**
Fried Frailty Index	0.934	**<0.001**
The Comprehensive Geriatric Assessment and the Clinical Frailty Score	0.877	**<0.001**

Table III. *The correlation between the FRAIL Scale, demographic characteristics and comprehensive geriatric assessment components*

	The FRAIL Scale and the correlation coefficient	p
Age	0.444	**<0.001**
BMI	-0.138	0.212
Number of comorbidities	0.50	**<0.001**
ADL	-0.615	**<0.001**
IADL	-0.753	**<0.001**
MMSE score	-0.644	**<0.001**
MNA-SF score	-0.722	**<0.001**
Yesavage GDS score	0.472	**<0.001**
CDT score	-0.49	**<0.001**
Handgrip	-0.650	**<0.001**
Number of falls	0.544	**<0.001**

**BMI: Body mass index, ADL: Activities of Daily Living, IADL: Instrumental Activities of Daily Living, MMSE: Mini-Mental State Examination, MNA-sf: Mini Nutritional Assessment short-form, GDS: Geriatric Depression Scale, CDT: clock-drawing test*

The FRAIL Scale consists of 5 sub-groups: fatigue, resistance, ambulation, weight loss, and illnesses. When all the sub-sections of the FRAIL Scale were evaluated separately and together, they were found to exhibit high reliability (Internal Consistency coefficient >0.60) (Table IV). The intraclass correlation coefficient was calculated for the reliability of the interrater test and found to be 1.00 (100% compliant). Test results performed by two independent practitioners were found to be similar. The intraclass correlation coefficient calculated for the test-retest was 1.00 (100% compliant). The test scores revealed by the same practitioner in two different time slots were completely similar. Thus, the scale was highly stable over time.

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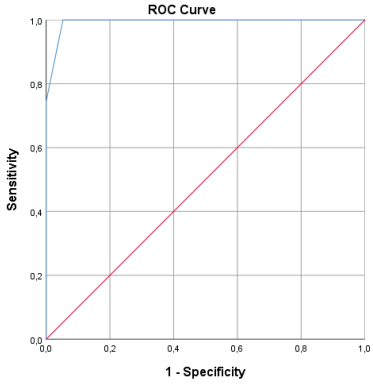
Marmara Med J 2023;36(2): 149-156

Table IV. *Cronbach's alpha internal consistency coefficients of the FRAIL Scale sub-headings*

The FRAIL Scale sub-headings	Internal Consistency coefficient
Fatigue	0.727
Resistance	0.682
Ambulation	0.677
Illnesses	0.787
Weight loss	0.822
Total	0.787

The result of the ROC analysis performed for the FRAIL scale is shown in Figure 1. The Kappa agreement coefficient was found to be 0.952. According to the FIND scale, the specificity of the FRAIL Scale was 94.7% and the specificity was 100%.



AUC: 0.993, p<0.001
AUC, Area under the curve; ROC, receiver operating characteristic curve
Figure 1. *ROC analyses of the FRAIL Scale*

4. DISCUSSION

In this study, the Turkish version of the FRAIL Scale was examined for its reliability and validity in the Turkish geriatric population. As a result of the study, the FRAIL Scale was found to have high internal consistency and reliability in test-retest and interrater administration. In our study, the FRAIL Scale was revealed to be valid and reliable in screening frailty in the geriatric age group in our country.

The FRAIL scale was developed by Morley, et al., in 2012 [8]. The validity and reliability of the FRAIL Scale, which is an effective and reliable scale to measure frailty, in Turkish society had not been tested before. There are many scales being used to measure frailty. None of these scales have been considered gold standard scales. Determination of frailty according to the results of the comprehensive geriatric assessment can be accepted as the most appropriate diagnostic method. Besides, using an easily applicable, effective, and reliable scale will facilitate determining frailty. For this reason, we planned to study the FRAIL Scale, which is a practical scale, for validity and reliability. In our practice in the outpatient clinic of geriatrics, patients are noted as non-frail, pre-frail, and frail by the doctor as a result of their general examinations and comprehensive geriatric assessments. In our study, the correlation between the result of the clinical frailty assessment and the FRAIL Scale was checked, and it was found extremely high. In our clinic, patients are followed up by experienced geriatricians, and it can be determined after anamnesis and examination whether patients are clinically frail or not. It is not possible to determine the level of the frailty of patients only clinically and with anamnesis in other centers where there are no geriatricians available; objective tests should be used. The FRAIL Scale can be used as a screening test due to its advantages such as being short and easily applicable characteristics.

Comprehensive geriatric assessment is considered a gold standard method for frailty screening in many sources [27]. In our study, the correlation between the FRAIL scale and the Comprehensive geriatric assessment tests was checked, and significant correlations were observed in the results. In its reliability and validity studies in Korea, Italy, and Mexico, the FRAIL scale was shown to be associated with IADL [9, 10, 28]. According to the FRAIL Scale, as the level of frailty increases, patients become more dependent, go into cognitive remission, and have poorer nutrition. The FRAIL scale and the Yesavage GDS were observed to be correlated at a moderate level. Our study supports the literature data, and a positive relationship was demonstrated between frailty and depression [29]. As the FRAIL Scale score increased, the score received from CDT and MMSE score decreased. In light of these results, it is seen that frailty does not only remain in the dimension of physical frailty but also interacts with all the other geriatric syndromes. In this respect, its determination via appropriate assessment and scales is of great importance.

In many studies conducted in different parts of the world, the female gender has been found to be associated with frailty. In our study, it was found that 23.1% of men were frail, while 37.3% of women were frail, and the difference between both genders was found to be significant. Similar results were obtained in other studies in which FRAIL Scale validation was performed [8-10]. Similar to the literature data, in our study, it was found that the degree of frailty increased with increasing age. In the validity and reliability study of the FRAIL Scale conducted in Mexico, the patients were grouped as 60-69 years old, 70-79 years old, and 80 years old and above, and it was found that the frailty increased as the age increased [10]. Italian and Korean studies observed that the relationship between frailty and age was not statistically significant [9, 28]. In our study, it was observed that the relationship between education level and frailty was not statistically significant, similar, in a study conducted in Italy. But it was shown in other studies that as the level of education increased, frailty decreased [8-10]. It was observed that the education levels of the patients participating in our study were generally low and it was thought that this factor might have affected the results.

The FIND scale and the Fried index have been proven in previous studies to be valid and reliable screening scales for showing vulnerability in Turkish society. In our study, its relations with

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Marmara Med J 2023;36(2): 149-156

the FRAIL Scale was examined. It was found that the FRAIL Scale correlated very highly with the FIND scale and the Fried index and was statistically significant. Its use is more practical because it can be evaluated in a short time in comparison with the Fried index. This correlation shows that the FRAIL scale can be used easily and reliably as a frailty test.

In our study, the internal consistency coefficient of the FRAIL Scale was found to be high; high homogeneity was observed when all its sub-sections were evaluated separately and together. In the reliability of the interrater test, the test results revealed by the two independent practitioners were found completely similar. The test scores obtained by the same practitioner in two different time slots were found completely similar; hence, the scale was observed to exhibit high consistency over time. In a previous study on validity and reliability in Korea, Italy, and Mexico, the internal consistency coefficient, interrater and test-retest were not calculated [9, 10, 28].

This study had some limitations. Firstly, the patient group included in the study may not represent the general geriatric population. Conducting the study in different centers and different settings, such as institutionalized older adults and inpatients will increase reliability and validity. Secondly, the reliability study on test-retest and interrater could be carried out with a low number of patients. The stability of the test can be ensured when it is repeated with more people over time. In the study, independent evaluators evaluated the FRAIL Scale in different environments, unaware of each other. Interrated reliability of 1.00 is one of the surprising results of the study. Evaluation of a small group may have been the reason for this situation.

Conclusion

Since frailty is a multifactorial clinical condition, it is quite difficult to measure. Many easily applicable and reliable methods have been developed to determine frailty in geriatric clinics. There is a need for scales tested for Turkish validity and reliability, which can be used practically to clinically determine frailty. The FRAIL scale developed by Morley et al. has been proved to be a valid and reliable scale to measure frailty in many countries. In this study, the Turkish version of the FRAIL Scale was examined for its reliability and validity in the Turkish older population. As a result of the study, the Turkish version of the FRAIL scale has been found to have high internal consistency, and test-retest and independent practitioner reliability.

Compliance with Ethical Standards

Ethical Approval: After the permission of J. E. Morley who developed the FRAIL Scale was obtained the study protocol was evaluated and approved by the local Ethics Committee (Hacettepe University, GO 17/91-37, 28.02.2017). Informed consent was obtained from all the patients.

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

Authors' Contribution: BABH, RTD and BBD: Study conception and design, BABH, RTD, CB, CO, HC, BBD, MC and MH: Data collection, BABH, RTD, BBD, and EK: Analysis and interpretation of results, RTD, BABH, and BBD: Draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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Supplementary file: Turkish version of the FRAIL Scale

FRAİL ÖLÇEĞİ	1	0
Yorgunluk: "Son 4 haftanın ne kadarında kendinizi yorgun hissettiniz?" 1=Her zaman 2=Çoğu zaman 3=Bazı zamanlarda 4=Çok az zaman 5=Hiçbir zaman *(cevap 1 veya 2 ise 1 puan verilir, diğerlerinin hepsine 0 puan verilir)*	1 veya 2	3 veya 4 veya 5
Direnç: "Kendi başınıza ve yardımcı cihaz kullanmadan, 10 basamak merdiveni dinlenmeden çıkmakta zorluk çeker misiniz?"	Evet	Hayır
Dolaşma: "Kendi başınıza ve yardımcı cihaz kullanmadan, birkaç yüz metreyi yürümekte zorluk çeker misiniz?"	Evet	Hayır
Hastalık: "Bir doktor size hiç şu hastalıklarınızın olduğunu söyledi mi?" (Hipertansiyon, diyabet, kanser (küçük cilt kanseri dışında), kronik akciğer hastalığı, kalp krizi, konjestif kalp yetmezliği, anjina, astım, artrit, inme, böbrek hastalığı) *(0-4 hastalık=0 puan, 5-11 hastalık=1 puan)*	5-11 hastalık	0-4 hastalık
Kilo kaybı: "Kıyafetleriniz üzerinizdeyken ama ayakkabısızken kaç kilosunuz? (şu andaki ağırlık)" "Bir yıl önce …yılının…ayında kıyafetleriniz üzerinizdeyken ama ayakkabısızken kaç kiloydunuz? (bir yıl önceki ağırlık)" *Ağırlık değişikliği yüzdesi şu formül ile hesaplanır:* *((bir yıl önceki ağırlık-şu andaki ağırlık)/bir yıl önceki ağırlık)x100* *Ağırlık değişikliği yüzdesi >5 ise (%5 kilo kaybını temsil eder) 1 puan verilir, <5 ise 0 puan verilir)*	≥%5 kilo kaybı	<%5 kilo kaybı
TOPLAM		

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Blood pressure and heart rate in aripiprazole once – monthly and paliperidone 1 and 3-month long-acting preparations

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ABSTRACT

Objective: This study aims to evaluate the blood pressure and heart rates of the patients treated with aripiprazole once-monthly, paliperidone 1-month, and paliperidone 3-month long-acting injections.

Patients and Methods: This study was a non-invasive observational study. Subjects using the same long-acting injection preparation for at least four months without skipped injections were assigned to 3 groups according to their treatments. They were screened starting from routine injection day and monthly for four months. Heart rate, systolic blood pressure, and diastolic blood pressure were recorded for each subject.

Results: Systolic and diastolic blood pressure among the three treatment groups demonstrated no statistical significance. The heart rate of the paliperidone 3-month group was significantly higher than the aripiprazole once-monthly group. However, the mean heart rate was within the physiological limits. Thus, a clinical significance can hardly be attributed.

Conclusion: Aripiprazole once-monthly, paliperidone 1-month, and paliperidone 3-month long-acting injections are non-inferior regarding heart rate, systolic and diastolic blood pressure during the maintenance treatment.

Keywords: Long-acting antipsychotic, Hypertension tachycardia, Schizophrenia, Maintenance treatment

1. INTRODUCTION

Schizophrenia is a mental disorder with various manifestations, with a mean worldwide prevalence of 0.5% [1]. Second-generation antipsychotics are the mainstream of the treatment, as well as long-acting injections (LAI) of these medications. Long-term maintenance and relapses are tremendously affected by the discontinuation of the antipsychotics, thus emphasizing the importance of adherence throughout the illness. Paliperidone palmitate is one of the recent LAIs shown to be effective and safe in schizophrenia treatment [2]. Another widely used newer LAI, Aripiprazole Once-Monthly (AOM), is the first LAI to be a D_2 partial agonist. Since the obstacle of non-adherence to oral antipsychotics exert a severe issue during the treatment, LAIs decrease the rates of hospitalizations, relapses, and overall cost of the illness [3,4].

Aripiprazole Once-Monthly has been previously reported to be non-inferior compared to placebo [5] and its oral form [6]. It exerts its effects through a partial agonism on D2 and 5-HT_{1A} receptors and antagonism on 5-HT_{2A} receptors [7]. The partial agonism of AOM provides a distinct action on positive and negative symptoms. On the other hand, histaminergic and α-adrenergic affinity without cholinergic activity leads to favorable tolerability [8].

Paliperidone is the active metabolite of risperidone. Paliperidone acts as an antagonist at D2, 5HT2A, α1, α2 adrenergic, and H1 histaminergic receptors with no affinity to muscarinic or β1 – and β2 receptors. Its receptor profile allows the agent to alleviate the positive symptoms with less tendency to induce extrapyramidal symptoms than typical antipsychotics [9].

Relapse rates of different formulations are documented. The lowest relapse rates were observed in Paliperidone 1-Month (P1M), Paliperidone 3-Month (P3M), and oral paliperidone, respectively. Furthermore, P3M, which can provide longer extended sustained release capacity with its larger particle size, was demonstrated to be as effective and safe as P1M [10,11].

Compared to P1M, AOM was reported to be superior in life quality, especially under 35-year-old patients, reflecting the

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interaction of both effectivity and safety parameters [12]. However, as the studies mentioned above mainly constitute findings from randomized controlled trials (RCTs), they can not objectively represent clinical, real-world practice due to artificial settings [13].

A limited number of studies reported a direct evaluation of blood pressure and heart rate related parameters, even though there is growing evidence from the case reports that aripiprazole and paliperidone can have clinical effects on these vital parameters. To our knowledge, AOM, P1M and P3M have not been previously compared for efficacy or tolerability related measures or in the maintenance treatment. Thus in this study, we assessed the monthly screening data for four months in order to evaluate the systolic blood pressure (SBP) and diastolic blood pressure (DBP), and heart rate (HR) values and their comparative analysis outcomes.

2. PATIENTS and METHODS

This study is designed and carried out as an observational, noninterventional naturalistic study. Clinical Studies Ethics Committee approved the study protocol and amendments (09.2019.468, date:03.05.2019) accordingly with the clinical approval of from the hospital and the local health authorities. The study was carried out in compliance with the Declaration of Helsinki and Good Clinical Practices. Volunteers among the patients and next to kin were informed about the research study and possible adverse reactions. Informed consent was obtained from all subjects after detailed information was provided.

During the maintenance treatment, patients who continued their treatments in the mental health center and the patients who were using AOM, P1M, and P3M preparations for the treatment of schizophrenia for at least four months before the study were included in this study and they were assigned to 3 groups according to their treatments. Adult patients aged between 18 and 65 were included in this study. Patients already being treated with one of the LAIs without a skipped dose and were treated only with monotherapy for at least four months participated in this study. Patients were previously diagnosed and met the criteria of diagnosis of schizophrenia (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, DSM-V).

Exclusion criteria included patients who lack a capacity of judgment or literacy in order to understand the study and the written consent, patients with dementia, significant suicide risk or behavior, substance dependence history within the last year, history of tardive dyskinesia or neuroleptic malign neuroleptic syndrome, involuntary hospitalization during screening, morbid obesity (BMI >40 kg/m2) and severe systemic disease that can be a contraindication for antipsychotic treatment.

Only short acting benzodiazepines, zopiclone, antiparkinsonian treatments, and all kinds of psychotherapy and psychosocial interventions were allowed during the study.

A sociodemographic data form was given to patients at day 0. The first observation was made right before their routine injection days. Screening days were determined as day 0, day 30, day 60, and day 90. On each screening day, SBP, DBP, and HR were recorded. SBP and DBP were recorded for four months monthly for each patient being present in the screening visit. SBP and DBP were recorded upon an independent investegator's (a trained health care professional) measuring blood pressure of the patients with a standard calibrated sphygmomanometer after they were at a seated position and relaxing on a chair for 10 min with a bare and stretched out upper arm supported and placed approximately at the heart level.

Patients were informed to avoid exposure to physical exertion, stress and caffeine intake at the day of the measurement. If they had significant pain or anxiety or if they were exposed to extreme heat or cold conditions this measurements were not taken into consideration in this study. At the same time heart rates of the patients were measured using a standard pulse oxymeter. All data were calculated as seperate monthly measures.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences software version 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

Mean and standard deviation or standard error of the mean, number, and percentage values are given while assessing the categorical variables. The sample distribution was evaluated through histogram, qq plots, Shapiro Wilk, and Kolmogorov Smirnoff tests. Chi-Square test statistics and Fisher Exact Test were applied to assess the relationship between categorical variables.

The One Way ANOVA (One Way ANOVA) test statistic was used to compare the means of more than two groups. Bonferonni statistics were applied as a Post Hoc test in case of a significance with ANOVA. If the sample did not demonstrate a normal distribution, the Kruskal-Wallis test was used to compare more than two groups. If a significant difference was found between the groups, Bonferonni correction of the Mann-Whitney U test was applied for each group. The statistical significance was considered as <0.05.

3. RESULTS

In this study, the number of participants in the AOM, P1M, and P3M groups was 8, 9, and 8, respectively.

According to the treatment groups, there were 6 (75%) male subjects in the A1M group, and there were 7 (78%) in the P1M group and 4 (50%) in the P3M group. The number of female subjects in the A1M, P1M and P3M groups was 2 (25%), 2 (22.2%), and 4 (50%), respectively. Statistical significance was observed between the P1M and P3M groups ($\chi 2 = 0.251$, $p < 0.05$). There were no other significant differences among the sociodemographic measures in this study.

Blood pressure and heart rate measurements according to treatment groups are shown in Table 1.

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Marmara Med J 2023;36(2): 157-161

Table 1. *Blood Pressure and Heart Rate Measurements According to Treatment Groups*

	AOM (N=28) M ± SEM MRI	P1M (N=35) M ± SEM MRI	P3M (N=26) M ± SEM MRI	Total	F Value/ H Value	P value
Systolic Blood Pressure (mmHg)	109.29 ± 1.62 (28)	111.71 ± 1.5 (35)	113.52 ± 2.26(27)	111.5 ± 1.03(90)	3.058H	0.22
Mean Rank	39.64	45.69	51.33			
Diastolic Blood Pressure (mmHg)	71.07 ± 0.94 (28)	72 ± 0.8 (35)	73.33 ± 1.31 (27)	72.11 ± 0.58 (90)	2.171H	0.34
Mean Rank	41.61	45.14	50			
Heart rate (bpm)	81.64 ± 1.7 *(28)	90.37 ±1.88 **(35)	95.07±3.07 b (26)	89 ±1.39 (89)	8.727	0.0001***

*AOM: Aripiprazole Once-Monthly Long Acting Injection Form, P1M: Paliperidone 1-Month Long-Acting Injection Form, P3M: Paliperidone 3-Month Long-Acting Injection Form, M:Mean, SEM: Standard Error of Mean, MR:Mean Rank, N: Number of Measurements, * Statistically significant at the p <0.05 level. ** Statistically significant at p<0.01 level. *** Statistically significant at p<0.001 level. Different superscripts indicate that there is a statistical significance between groups.*

4. DISCUSSION

Most antipsychotics are well-documented to cause orthostatic hypotension, which is possibly related to α1-receptor antagonism action, peripheral vasodilation, and reflex tachycardia [14]. In addition to α1-receptor antagonism, some antipsychotics exert significant effects on histamine 1 receptors, such as olanzapine or clozapine, which may frequently lead to acute hypotensive states [15].

Besides, changes in long-term resting blood pressure and fluctuations due to antipsychotics are not well documented in the literature. However, existing reports indicate considerable evidence for hypertension and hypotension [16].

Another study to monitor the pulse, SBP, and DBP weekly for 6 weeks comparing paliperidone and olanzapine reported that the only significant statistical difference was observed with the DBP in the olanzapine group (p=0.02). However, this finding was reported as clinically non-significant [17].

In a study by Parks et al., risperidone and olanzapine were reported to significantly increase the SBP (p=0.01), while clozapine decreased this parameter (OR = 18.02; CI, 3.42-29.49) during the first three days of the treatment. However, authors also reported in the same study in which they assessed antipsychotic (including olanzapine, haloperidol, risperidone, clozapine, quetiapine, and ziprasidone) effects among 60 patients, 30% (16 participants) of which were hypertensive before the onset of antipsychotic administration 17% of the patients continued to stay hypertensive. Only two remained to have sustained hypertension throughout the study period. At the same time, the hypertensive patients at the study onset demonstrated a decrease of 20mmHg and 12 mmHg in SBP and DBP, respectively, while an overall decline in all samples in DBP was 2mmHg and SBP was 4mmHg. Non-hypertensive patients at the onset demonstrated only a 3mmHg decrease in SBP but no change in DBP [18].

Another study by Garcia-Portilla et al. reported no significant change in HR, SBP, or DBP at the endpoint during a 52-week follow-up P3M study [19].

In a case report, a patient with autism was described as developing hypertension after starting treatment with aripiprazole, and the dechallenge test restored the high blood pressure to normal levels [20]. Authors suggested that aripiprazole might be causing hypertension through its potential vasoconstriction effect through 5-HT2A receptors or inhibition of microglial nitric oxide. [21]. Similarly, another case report demonstrated that hypertension in a patient was normalized after dechallenge and reappeared after the rechallenge. This case was reported to respond well to propranolol. Thus adrenergic hyperactivity was suggested as a possible mechanism by the authors [22].

Similarly, our study found no difference among any treatment groups regarding DBP and SBP. Due to controversial findings in the literature and the rise in blood pressure reported in several case studies, hypertensive patients were advised to be carefully monitored [23]. However our study results did not raise a concern regarding DBP, SBP and heart rate in our sample during the study period.

Another adverse reaction of antipsychotics may be observed as tachycardia. This occurs most possibly due to anticholinergic properties, sometimes related to orthostatic hypotension via α1-adrenoreceptor antagonism or a feature of secondary neuroleptic malign syndrome or myocarditis [24, 25].

There are increasing numbers of case reports for paliperidone and paliperidone palmitate leading to increased heart rate and related morbidity. One of the case reports described a patient who developed tachycardia with risperidone, and the adverse condition was aggravated by paliperidone palmitate injection [26].

This result might be related to the fact that risperidone and its metabolite paliperidone are documented to be associated with different tachycardia presentations, such as multifocal atrial tachycardia, sinus tachycardia, and QTc prolongation [27,28, 29]. Paliperidone was reported to be associated with tachycardia in 14% of the cases, which also may start the day after the administration of the agent [30,31].

On the other hand, aripiprazole was not accounted for cardiac risk factors in healthy subjects [32].

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Marmara Med J 2023;36(2): 157-161

Furthermore, QTc prolongation with aripiprazole is reported to be less significant than the conventional antipsychotics. [33]. However, a case report demonstrated that a 13-year-old girl with early-onset schizophrenia developed arrhythmia with aripiprazole [34]. In Another recent case report, tachycardia, headache, nausea, and high blood pressure was reported in a 53-year-old woman. Authors suggested a role for dopaminergic receptor interaction with the renin-angiotensin-aldosterone system [35].

In our study, HR was significantly higher in the P3M group compared with the AOM group. However, since the mean HR of the groups is within the physiological limits, this may not account for clinical significance.

The limitations of our study include our small sample size and statistical differences in the gender variable among P1M and P3M groups. Further studies are needed to address the SBP, DBP, and HR fluctuations to shed light on the controversial and limited data in the literature.

Conclusion

All three LAI preparations, AOM, P1M, and P3M, demonstrated a safe profile during four months with monthly assessments of blood pressure and heart rate in schizophrenia patients during the maintenance treatment. The heart rate was statistically significantly higher in P3M group compared to AOM however this difference was between physiological limits, thus a clinical significance was not noted. None of the paliperidone formulations were inferior to aripiprazole long acting formulation regarding vital signs. There was no significant difference among the 1 and 3 month sustained release formulations of paliperidone.

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Compliance with Ethical Standards

Ethical approval: Clinical Studies Ethics Committee approved the study protocol and amendments (09.2019.468, date: 03.05.2019) accordingly with the clinical approval of from the hospital and the local health authorities. The study was carried out in compliance with the Declaration of Helsinki and Good Clinical Practices. Informed consent was obtained from all subjects after detailed information was provided.

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Morphological and biochemical evaluation of effects of *Myrtus communis* L. extract on heart and aorta in high fat-diet-induced obese rats

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ABSTRACT

Objective: The purpose of this study was to examine the protective effects of *Myrtus communis L.* (MC) extract on high fat-diet (HFD) induced heart and aorta damage by evaluating oxidative stress and the endothelial nitric oxide system (eNOS).

Materials and Methods: Wistar albino male rats were divided into 3 groups (n=7) as control, HFD, and HFD+MC. Rats in HFD and HFD+MC groups were HFD fed for 16 weeks and in the last 4 weeks saline or MC (100 mg/kg) was administered orally (5 days/week). Triglyceride, cholesterol, and high-density lipoprotein (HDL) were estimated in blood serum. Tissue oxidative stress and inflammatory parameters were evaluated biochemically. Tissue morphologies, eNOS, inducible NOS (iNOS), and NADPH oxidase-2 (NOX-2)-immunopositive and apoptotic cells were evaluated histologically.

Results: Altered serum lipid profiles, degenerated heart, and aorta morphology, increased malondialdehyde, 8-hydroxy-2-deoxyguanosine, tumor necrosis factor-alpha, monocyte chemoattractant protein-1 and myeloperoxidase levels, and iNOS, NOX-2 immunopositive and apoptotic cells, decreased NO levels, eNOS-immunopositive cells in both tissues were observed in HFD group. All these parameters improved in the HFD+MC group.

Conclusion: This study revealed that HFD-induced obesity increased iNOS activation and oxidative stress in the cardiac and aortic tissues of the rats. MC improved oxidant/antioxidant balance and prevented heart and aorta damage via eNOS involvement.

Keywords: High-fat diet, Obesity, *Myrtus communis* L. extract, Heart, Aorta

1. INTRODUCTION

Based on the World Health Organization (WHO) definition, obesity is identified as excessive fat deposition that poses a risk to health. Approximately 1.9 billion people globally are overweight (Body mass index-MI≥25 kg/m2) or obese (BMI≥30 kg/m2). In many Western countries, one in every two people is considered to be either overweight or obese [1, 2]. Studies have shown that obesity can be caused by genetics, diet, environment, living conditions, and other factors [3]. It has been observed that cardiovascular diseases, atherosclerosis, Type 2 diabetes, cancer, sleep disorder, osteoarthritis, and some other diseases can develop as a result of obesity [4]. One of the main factors leading to obesity is consuming foods with high fat [5]. Oxidative stress is defined as reactive oxygen species (ROS) that cause lipid peroxidation and cellular damage in the organism due to the deterioration of the balance between oxidant and anti-oxidant systems in favor of oxidant systems [6]. It has been shown that oxidative stress induced by obesity is shown as the main mechanism responsible for obesity-related cardiovascular risk [7].

Numerous studies have shown that a high-fat diet leads to an increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 (NOX-2) and oxidative stress, which provides the production of free oxygen radicals [8]. Excessive amounts of ROS resulting from increased NOX-2 activation in obesity lead to endothelial dysfunction and ultimately to cardiovascular complications [9, 10]. It has been observed that increased ROS species as a result of a high-fat diet (HFD) lead to an increase in the synthesis of vascular constrictive factors while, reducing the release of relaxant factors such as nitric oxide (NO), which leads to endothelial dysfunction in the coronary arteries. However, antioxidant treatment applied in obese animals

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prevented changes in NO metabolism and improved coronary vascular response [10]. The development of hypertension and atherosclerosis is the most common cardiovascular complications that develop after endothelial dysfunction in obese and diabetic individuals [11]. Increased oxidative stress in the vessel wall stimulates the development of inflammation, enhancing the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and chemoattractant molecules such as monocyte chemoattractant protein-1 (MCP-1) [12, 13]. This results monocyte migration to the endothelial region and monocytes become active secretory macrophages. Endothelial dysfunction in obesity, a decrease in endothelial nitric oxide synthase (eNOS) activity and NO levels, also increase in inducible NOS (iNOS) activity, and consequently in peroxynitrite formation are observed [14]. In this case, vascular smooth muscle tissue deterioration occurs.

Herbal medicine including essential oils and flavonoids via antioxidative and anti-inflammatory activity may have many beneficial effects for many diseases associated with obesity, including cardiovascular failures [7, 15]. *Myrtus communis* L. subsp. communis (MC) is an aromatic plant commonly found in the Mediterranean region and the Middle East [16]. It is known to have anti-inflammatory, antihypertensive, antitussive, antiallergic, antiemetic, and diuretic effects. It has beneficial effects in experimental diabetes and hyperlipidemia [16, 17, 18]. Its ability to suppress inflammatory processes and scavenge oxygen-free radicals suggests that it is suitable for improving dysfunction in obesity [19]. Recent studies have shown that MC extract has antioxidative and antiinflammatory effects on experimental renovascular hypertension and obesity-induced renal and bladder damage [17, 20].

Based on the studies, we purposed to study the possible protective effects of MC on HFD-induced heart and aorta damage via biochemical and histological analysis. Cholesterol, triglyceride, and high-density lipoprotein (HDL) were measured in blood serum. Oxidative parameters as malondialdehyde (MDA), NO, 8-hydroxy-2-deoxyguanosine (8-OhdG), and inflammatory parameters as MCP-1, TNF-α, and myeloperoxidase (MPO) levels in heart and aorta tissues were measured using biochemical techniques. Cardiac and aorta morphology, apoptotic cells, eNOS, iNOS, and NOX-2-positive cells were evaluated using histological and immunohistochemical methods.

2. MATERIALS and METHODS

Experimental animals

Wistar albino male rats (2-3 month-old) were kept individually in a light – and temperature-controlled room on a 12-h/12-h light–dark cycle and fed a standard pellet lab chow. All experimental studies were allowed (23.2019.mar) by the Animal Care and Use Committee of the Marmara University School of Medicine.

Plant materials and preparation of MC extract

The plant samples used in this study were collected from the city of Manisa, (Turgutlu region). The samples were identified by a botanist in Marmara University, Faculty of Pharmacy. Voucher specimens were deposited in the Herbarium of Marmara University, Faculty of Pharmacy (MARE: 13006). Briefly, leaves of MC (100 g) were dried in the shade at room temperature. The dried powdered leaves were extracted with 96% ethanol using the Soxhlet device. After filtration, the extract was concentrated to dryness using a rotary evaporator. The powder MC extract obtained with a yield of 28.56% was kept in a dark glass bottle in a refrigerator (4°C) until use [21].

Experimental groups

Rats were divided into 3 experimental groups (n=7, in each group): 1) Control (C), 2) HFD, and 3) HFD+MC groups. A standard diet was applied to the C group. Rats in HFD and the HFD+MC groups were fed a high-fat diet for 16 weeks. MC extract (100 mg/kg/day, dissolved in saline) was given orally by gavage five days a week for the last month of the experiment to the rats in the HFD+MC group [21]. The weight of rats was measured weekly during the experiment. Rats were decapitated under ether anesthesia and blood was obtained for evaluation of lipid profiles. Heart and aorta samples were removed for histological, immunohistochemical, and biochemical evaluation.

Measurement of triglyceride, cholesterol, and HDL levels in blood serum

The serum concentration of triglyceride, cholesterol, and HDL levels was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Biont Rat ELISA kit, Shanghai, China). The results were given as mmol/L for total cholesterol and ng/ml for triglyceride and HDL

Measurement of MDA, NO, 8-OHdG, MCP-1, TNF-α and MPO levels in the heart and aortic tissues

At the end of the experiment, heart and aortic tissue samples were collected and the levels of MDA, 8-OHdG, TNF-α, MCP-1, and MPO were measured using the ELISA method. The tissues were removed after decapitation, kept at – 20 °C, and were brought to a temperature in the range of 2-8 °C before analyzing. A small amount of tissue samples was placed in a tube, phosphate-buffered saline (PBS) (pH 7.4) was added to 10% homogenate, and the samples were centrifuged at 3000 rpm for about 20 minutes with the help of a homogenizer. Then, the supernatants were collected and the commercial ELISA kit procedures (Bioassay Technology Laboratory, Shanghai, China) were applied. The absorbances were measured with the help of an ELISA Reader at 450 nm and the absorbance-concentration graph was drawn. NO levels were evaluated by measuring levels of accumulated total nitrate in the tissue samples using a commercial total nitric oxide assay kit (ENZO Life Sciences, Lausen, Switzerland). A standard curve was generated ranging from 0 to 100 µM using nitrate as standard and the total nitrate concentration was measured as µM for each sample via the calibration curve.

Light microscopic preparation and histopathological scoring

Tissue samples from the heart and aorta were removed and fixed in a 10% formalin solution. Thereafter, tissue samples were dehydrated with increasing alcohol series, cleared using xylene, and embedded in paraffin at room temperature. Paraffin sections (4 µm-thick) of the heart and aorta were stained with hematoxylin and eosin (H&E). Five similar areas in each section were evaluated in the experimental groups. H&E stained heart sections were scored semiquantitatively by a scale ranging from 0 to 3 (0, none; 1, mild; 2, moderate; and 3, severe) for each criterion including inflammatory cell infiltration and disruption of cardiomyocytes [22]. The aorta tissue damage was scored semiquantitatively from 0 to 4 according to the disorganization of the medial layer elastic network (0, none; 1, mild, only external elastic lamina disrupted; 2, moderate, external elastic lamina and outer medial layers degraded; 3, high, external elastic lamina and medial elastic layers breakage; 4, severe, all elastic layers breakage, and aortic rupture) [23]. The maximum score was 6 for heart sections and 4 for aortic sections.

eNOS, iNOS, and NOX-2 immunohistochemistry

Antigen retrieval was applied by microwaving the slides in citrate buffer (20 min) to the deparaffinized sections. Slides were cooled at room temperature (20 min). Endogenous peroxidase activity was blocked with 3% H_2O_2 (10 min), and then slides were washed in PBS. Blocking reagent (Histostain plus kit-Thermo Scientific Massachusetts, USA) was added to each slide and incubated (20 min) at room temperature in a humid chamber. Tissue sections were incubated overnight at 4 °C with primary rabbit polyclonal eNOS (1:300), iNOS (1:50), and NOX-2 (1:200) antibodies. The sections were washed in PBS three times (5 min) and then incubated in secondary biotinylated goat anti-rabbit antibodies (Novus, Abingdon, UK) at room temperature (30 min). After washing with PBS, streptavidin peroxidase-labeled reagent (Histostain-plus kit, Thermo Fisher Scientific, Massachusetts, USA) was applied (30 min) at room temperature in a humid chamber. Sections were incubated with 3, 3-diaminobenzidine tetrahydrochloride dihydrate (DAB) (ScyTek Laboratories Inc., Logan, UT, USA), and then slides were counterstained with Mayer's hematoxylin. After the dehydration procedure, they were covered with entellan. In each section, 5 similar areas were photographed at 40x objective of the light microscope. Staining intensity was evaluated by the Image J software (version 1.52a, Wayne Rasband, National Institutes of Health, USA). Immunohistochemical staining confirmation was performed using a negative control staining that was processed without primary antibodies.

Terminal transferase-mediated dUTP-biotin Nick end Labeling Method

The apoptotic cells were evaluated by terminal transferase-mediated dUTP-biotin Nick end Labeling (TUNEL) according to the manufacturer's manual (ApopTag Plus, In Situ Apoptosis Detection Kit, S7101, Millipore, Massachusetts, USA). The procedure was as follows: every fifth paraffine section (a total of five sections from each animal) was incubated with proteinase K (5 min), washed with distilled water, and incubated with 3% H_2O_2 in PBS (5 min). The sections were then washed with PBS, put in the equilibrium buffer (30 min), and incubated in recombinant terminal transferase TdT enzyme at 37 °C (1 h). The sections were agitated in washing buffer (15 s), washed in PBS, put into anti-digoxigenin conjugate (30 min), and then washed with PBS. DAB was applied (5 min) and washed with distilled water, stained with Mayer's hematoxylin. After the dehydration procedure, they were covered with entellan (Merck, Darmstadt, Germany). In each section, five similar areas were evaluated at the 40x objective of the light microscope.

All light microscopic sections were observed and photographed with the digital camera (Olympus C-5060, Tokyo, Japan) of a photomicroscope (Olympus BX51, Tokyo, Japan).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA). Differences between groups were determined with Tukey's multiple comparisons test, and the data were expressed as mean ± standard error of the mean (SEM). The significance of differences was taken at the level of $p < 0.05$. Calculations were performed using the Instant statistical analysis package (Prism 9.0 GraphPad Software, San Diego, CA, USA).

3. RESULTS

Body weight

The body weight of rats was not significantly different in the first week of the experiments among the experimental groups (268.40 ±3.71 g in the C group, 275.8 ± 4.58 g in the HFD group, and 260.6 ± 3.58 g in the HFD+MC group). At the end of 16 weeks, the rats in the HFD group showed the greatest increase in body weight. The body weight of rats in the HFD group (409.3 ± 4.8 g; $p<0.001$) was significantly increased compared to the C group (280.23 ± 4.20 g) and decreased in the HFD+MC group (337.3 ± 2.34 g: $p<0.001$) compared to the HFD group at the end of the experiment. The body weight of rats was not significantly different on the 16th week in the C and HFD+MC groups.

Triglyceride, cholesterol and HDL values

Serum triglyceride level was significantly increased in the HFD group ($p<0.001$) compared to the C and HFD+MC groups, while there was a significant decrease in the HFD+MC group ($p<0.01$) compared to the HFD group. Also, cholesterol level was significantly increased in the HFD group ($p<0.001$) compared to the C and HFD+MC groups, while there was a significant decrease in the HFD+MC group ($p<0.01$). However, HDL level was significantly decreased in the HFD group compared to the C and HFD+MC groups ($p< 0.001$) and this value significantly increased in the HFD+MC group ($p<0.05$) compared to the HFD group (Figure 1).

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Marmara Med J 2023;36(2): 162-170

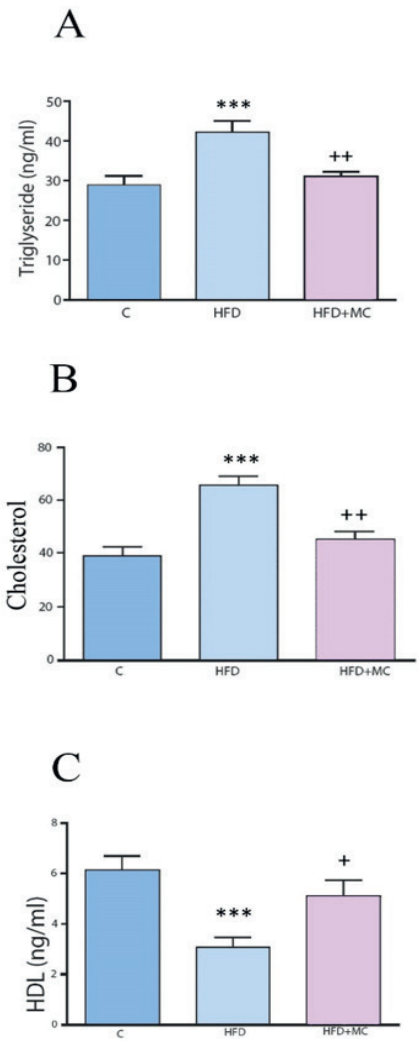

Figure 1. Triglyseride, cholesterol and HDL values in the experimental groups. *Serum triglyseride (A), cholesterol (B), HDL (C) levels are seen in the experimental groups. ***: p<0.001 compared to C group, +: p<0.05 ++: p<0.01 compared to HFD group.*

MDA, 8-OHdG, TNF-α, MCP-1, NO, and MPO levels in the heart and aorta tissues

Cardiac MDA (p<0.05), 8-OHdG (p <0.05), TNF-α (p<0.05), MCP-1 (p<0.05), and MPO (p<0.01) levels were higher and NO (p<0.05) level was lower in the HFD group than the C group. However, MDA (p<0.05), 8-OHdG (p<0.05) TNF-α (p<0.05), MCP-1 (p<0.05) and MPO (p <0.01) levels were lower and NO level (p<0.05) was higher in the HFD+MC group when compared to the HFD group in the heart homogenates.

Aortic MDA (p<0.01), 8-OHdG (p<0.01) TNF-α (p<0.01), MCP-1 (p<0.01) and MPO (p<0.01) levels elevated and NO (p<0.01) level reduced in the HFD group compared to the C group. However, MDA (p <0.01), 8-OHdG (p<0.01), TNF-α (p<0.01), MCP-1 (p<0.01), and MPO (p <0.01) levels elevated and NO level (p<0.05) reduced in the HFD+MC group compared to the HFD group in the aorta homogenates (Table I).

Histopathological findings

Normal heart morphology with regular cardiac muscle cells was seen in the C group. Myocardial fiber disorganization and degeneration of myofibrils in cardiomyocytes were seen in the HFD group. All these histopathological parameters were ameliorated in the HFD+MC group. The cardiac histopathological score was significantly increased in the HFD group (p<0.001) compared to the C group. However, the histopathological score decreased in the HFD+MC group (p<0.01) compared to the HFD group (Figure 2A-D). In the aorta sections regular intima, media, and adventitia layers were observed in the C group, thickened media layer with disorganized elastin lamella was observed in the HFD group. These structural changes were ameliorated in the HFD+MC group. The histopathological score in the aorta was significantly increased in the HFD group (p<0.01) compared to the C group. On the other hand, the histopathological score decreased in the HFD+MC group (p<0.01) compared to the HFD group (Figure 2A1-D1).

Table I. *MDA, NO, 8-OHdG, TNF-α, MCP-1 levels, and MPO activity results in the homogenization of the heart and aorta homogenates*

	MDA (ng/mg protein)	8-OHdG (ng/mg DNA)	NO (μM)	TNF-α (pg/mg protein)	MCP-1 (pg/ml)	MPO (U/g)
HEART						
C	98.48±2.72	12.72±0.65	42.45±6.89	331.6±20.66	378.3±36.10	0.458±0.04
HFD	122.05±1.26*	14.72±2.26*	38.64±10.82*	425.74±11.40*	480.8±18.48^{+}	0.556±0.03**
HFD+MC	110.42±3.32^{+}	10.27±0.65 $^{+}$	40.36±3.22^{+}	406.60±8.95^{+}	422.66±22.10^{+}	0.461±0.06^{++}
AORTA						
C	116.67±3.80	11.38±1.35	46.52±3.06	212.10±33.36	292.70±30.30	0.412±0.07
HFD	138.16±4.64**	12.08±1.01**	38.70±16.07*	353±11.55**	440.80±7.45**	0.458±0.04**
HFD+MC	122.82±2.38 $^{++}$	8.929±0.86^{+}	40.73±5.26^{++}	254.3±47.34^{+}	358.20±53.96^{++}	0.435±0.02^{++}

**: p<0.05, **: p<0.01 compared to C group, +: p<0.05 ++: p<0.01 compared to HFD group. MDA: Malondialdehyde, NO: Nitric oxid, 8-OHdG: 8-Hydroxy-2-deoxyguanosine, TNF-α: Tumor necrosis factor-alpha, MCP-1: Monocyte chemoattractant protein-1, MPO: Myeloperoxidase, C: control, HFD: High fat-diet, HFD+MC: High fat-diet+ Myrtus communis L.*

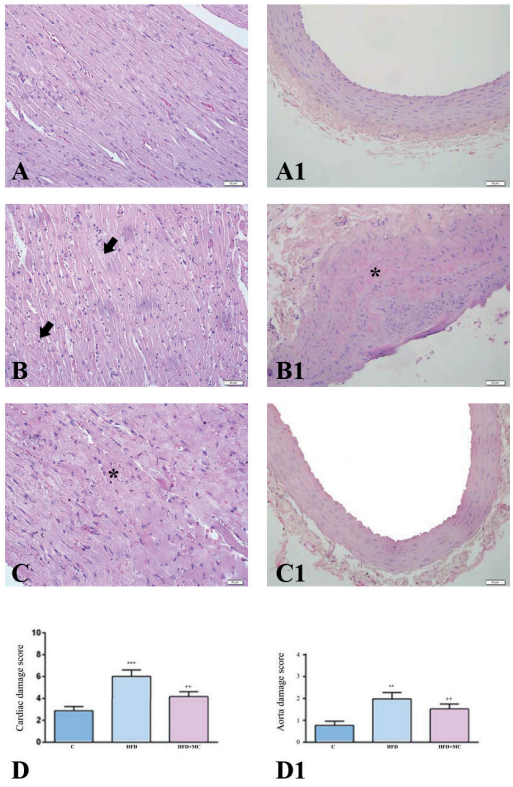

Figure 2. *Representative photomicrographs of H&E-stained cardiac (A-C) and aortic (A1-C1) tissues, cardiac (D) and aortic (D1) damage scores in the experimental groups. Normal cardiac morphology with cardiomyocytes are seen in the C (A) group. Degenerated cardiomyocytes with irregular arrangement of myofibrils (arrow) are seen in the HFD group (B). Regular cardiomyocytes (*) and decreased degenerated cardiomyocytes are seen in the HFD+MC group (C). Normal morphology of aortic wall with tunica intima, tunica media, and tunica adventitia are seen in the C (A1), HFD+MC (C1) groups. Irregular arrangement of elastin lamella and accumulation of eosinophilic material (*) in media region are seen in the HFD group (B1). **: p<0.01, ***: p<0.001 compared to the C group; ++: p<0.01 compared to the HFD group. Scale bar: 50 µm.*

eNOS, iNOS, and NOX-2 immunohistochemistry results

Brown-colored eNOS, iNOS, and NOX-2 immunopositive cells were observed in the heart and aortic tissues (Figures 3 and 4). eNOS – immunoreactivity (ir) was decreased in the heart and aorta tissues of the HFD group (p<0.05) compared to the C group. There was an increase in the eNOS-ir in the HFD+MC group (p<0.05) compared to the HFD group in both heart and aortic tissues (Figure 3A-3D, 4A-4D). However, iNOS-ir was increased in both tissues of the HFD group (p<0.01) compared to the C group. This value of heart and aortic tissues decreased in the HFD+MC group (p<0.05) when compared to the HFD group (Figure 3A1-D1, 4A1-D1). Additionally, NOX-2-ir of heart and aortic tissues increased in the HFD group (p<0.01) when compared to the C group. There was a decrease in the NOX-2-ir in the HFD+MC group (p<0.05) compared to the HFD group in both tissues (Figure 3A2-D2, 4A2-D2).

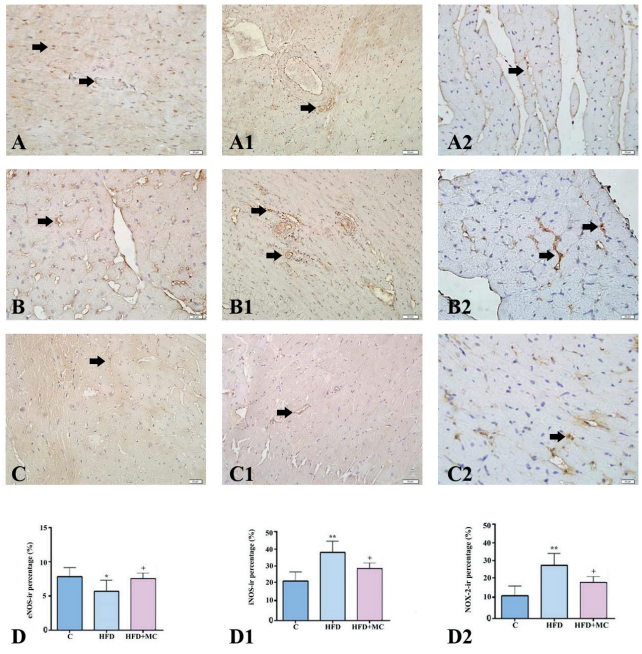

Figure 3. *Representative photomicrographs of eNOS (A-C), iNOS (A1-C1) and NOX-2 (A2-C2) immunostained cardiac tissue; percentage (%) of eNOS-ir (D), iNOS-ir (D1) and NOX-2-ir (D2) in the experimental groups. Arrow: eNOS, iNOS and NOX-2 immunopositive cells in control (A, A1, A2), HFD (B, B1, B2) and HFD+MC (C, C1 and C2) groups. *: p<0.05, **: p<0.01 compared to C group; +: p<0.05 compared to the HFD group. Scale bar: 50 µm.*

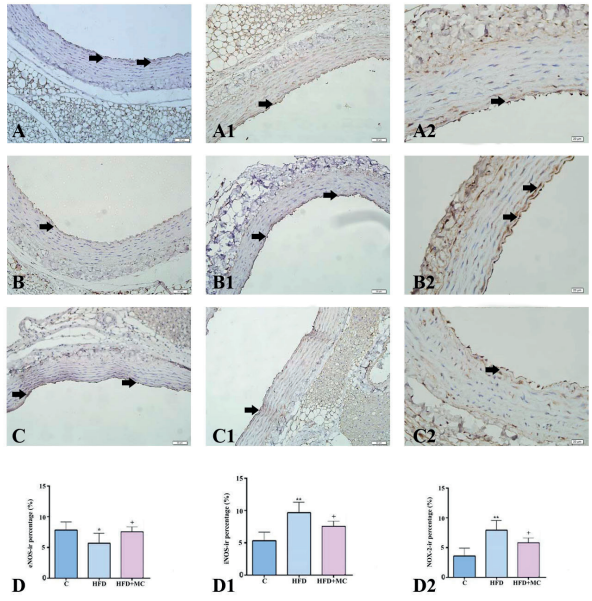

Figure 4. *Representative photomicrographs of eNOS (A-C), iNOS (A1-C1) and NOX-2 (A2-C2) immunostained aortic tissue; percentage (%) of eNOS-ir (D), iNOS-ir (D1) and NOX-2-ir (D2) in the experimental groups. Arrow: eNOS, iNOS and NOX-2 immunopositive cells in control (A, A1, A2), HFD (B, B1, B2) and HFD+MC (C, C1 and C2) groups. *: p<0.05, **: p<0.01 compared to the C group; +: p<0.05 compared to the HFD group. Scale bar: 50 µm.*

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Marmara Med J 2023;36(2): 162-170

TUNEL method results

TUNEL-positive cells were seen as dark brown in color in the cardiac and aortic tissues of the experimental groups (Figure 5). However, the number of TUNEL-positive cells and apoptotic index were higher in both the cardiac and aortic tissues of the HFD group ($p<0.01$) when compared to the C group. Additionally, there was a decrease in the number of TUNEL-positive cells and apoptotic index in both the heart and aorta tissues of the HFD+MC group ($p<0.05$) when compared to the HFD group (Figure 5).

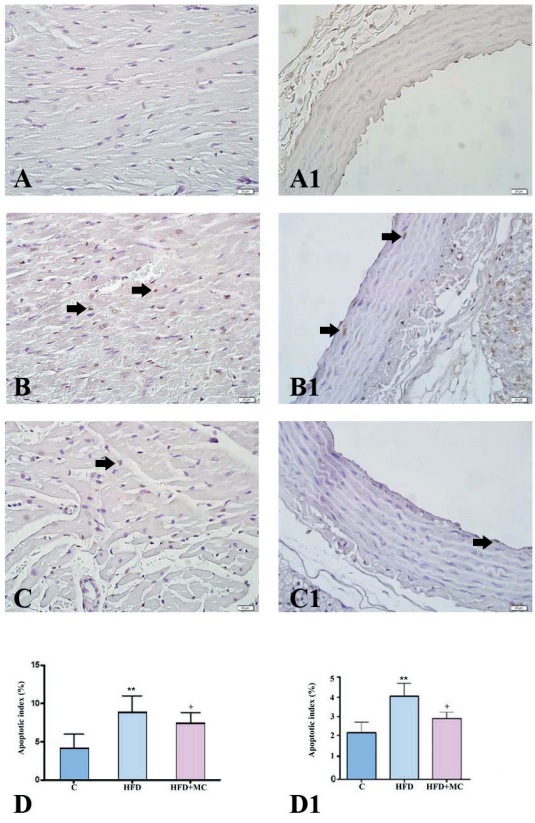

Figure 5. *Representative photomicrographs of TUNEL stained cardiac (A-C) and aortic (A1-C1) tissues and cardiac (D) and aortic (D1) apoptotic index in the experimental groups. Arrow: TUNEL positive cells in HFD (B, B1) and HFD+MC (C, C1) groups. **: $p<0.01$ compared to the C group, +: $p<0.05$ ++: $p<0.01$ compared to the HFD group. Scale bar: 20 µm.*

4. DISCUSSION

In the present study, the ameliorating effects of MC treatment on HFD-induced heart and aorta damage were evaluated by biochemical, histological, and immunohistochemical methods. This study demonstrated that HFD-induced obesity increased triglyceride, and cholesterol levels and decreased HDL levels in blood serum. Oxidative stress and inflammatory parameters including MDA and 8-OHdG levels and TNF-α, MCP-1, and MPO levels increased and NO levels decreased in the HFD group. Parallel with biochemical parameters, degenerated cardiac and aorta morphology, increased apoptotic, iNOS, and NOX-2 immunopositive cells, and decreased eNOS immunopositive cells were present in the HFD group. All these biochemical, histological, and immunohistochemical parameters improved in MC treated HFD group.

Previous animal and human studies have shown that HFD significantly increased body weight and oxidative stress has an important role in obesity and weight loss [7, 13]. For this reason, there are many studies on antioxidant treatments. It has been reported that MC extract has antiobesity effects [15, 24]. The present study showed that MC treatment reduces the body weight in HFD-induced obese rats. Also, previous studies have demonstrated significant alterations in lipid profiles such as an increase in serum triglyceride and cholesterol and a decrease in HDL levels in obese animals [7, 17]. MC treatment ameliorate biochemical alterations significantly and prevented dyslipidemia in HFD-induced obese rats [17]. Parallel to these findings, our study revealed an increase in triglyceride and cholesterol levels and a decrease in HDL levels in the blood serum, and MC treatment reversed these alterations.

Obesity is known to trigger cardiovascular diseases with the increased production of ROS [7, 8, 12]. In recent studies on obesity and the cardiovascular system, it revealed that cardiovascular dysfunction is often in regions where obesity is common [1]. Previous studies have reported that oxidative stress plays a key role in cardiovascular diseases [1, 7, 14]. One of the main factors of obesity formation is the consumption of foods with high-fat content. HFD increases oxidative stress formation due to the production of superoxide anions [25]. Obesity causes deterioration of the morphology in the heart via the inflammatory cell infiltration, diffuse fibrosis and collagen deposition, also leading to disruption of the structure of the endothelial cells in the vessels, resulting in endothelial dysfunction [26].

In recent years the use of plant derived antioxidants have been increased in research studies for the treatment and prevention of many diseases including cardiovascular diseases [27, 28]. Previous studies have shown that MC have, antiobesity, antioxidative, antiinflammatory, antiapoptotic effects against oxidative stress in various animal models. As a result of the researches, the effects of the aerial parts, leaves, fruits and essential oil of MC are revealed [15, 16]. Semi-myrtucommulone and myrtucommulone-A are the compounds of MC and their antiatherogenic effects are reported [29, 30]. However, there are few studies on the effect of MC treatment on obesity or body weight, heart and aorta studies are limited in the literature.

NO has an essential role in the continuation of cardiovascular homeostasis via protective effects against vascular injury, inflammation and thrombosis [13]. NO inhibits the adhesion of leukocytes to the endothelium, keeps vascular smooth muscle cells in a nonproliferative position, and limits the platelet aggregation [31, 32]. Common cardiovascular risk factors, such as hipertension and obesity impair the protective function of the endothelium [33]. Previous studies have shown that HFD induced obesity decreases eNOS activity and increases both NO levels and

iNOS activity by the excessive ROS production [13, 34]. In our study, we revealed a decrease in eNOS-ir cells and NO levels, and an increase in iNOS-ir cells in both heart and aorta in HFD induced obese rats. MC treatment ameliorated these NO levels, eNOS-ir and iNOS-ir in both heart and aorta tissues.

HFD increases the production of ROS and induces the pathological changes in tissues [35]. MDA is the key indicator of lipid peroxidation and it was reported that MDA levels increased in the HFD induced obesity [15, 17, 21]. Previous studies revealed that antioxidant agents decreased MDA levels in HFD induced obesity [18, 36, 37]. Similar to these studies, we observed increased MDA levels in HFD group and MC treatment ameliorated the alterations in both heart and aorta. Previous studies have shown that the main source of ROS is NOX-2, because activated NOX-2 transports electrons to oxygen causes the formation of superoxide anions [37]. Inhibition of this enzyme may play a role in protecting organs against oxidative damage [38]. Nutrition with a high-fat diet leads to the activation of NOX-2, which leads to the production of superoxide anions, and consequently to the increase of oxidative stress. NOX-2 is a major source of endothelial reactive oxygen species and expressed in the vessel in endothelial cells, adventitic fibroblasts and a smaller amount of smooth muscle cells. Excess oxygen radicals produced by NOX-2 cause endothelial dysfunction and cause vascular complications [39]. In our study we observed that HFD increased the number of NOX-2 immunopositive cells and MC treatment decreased the number of NOX-2 immunopositive cells.

Obesity increases ROS production and causes increased expression of proinflammatory cytokines such as TNF-α, MCP-1, and IL-6. Therefore, obesity is considered a chronic inflammatory disease [40]. In our study, HFD induced obesity increased inflammation-related markers via increasing ROS and detoriating anti-oxidant defense. TNF-α and MCP-1 levels increased in both heart and aorta tissues in HFD group, and it revealed that HFD induced damages were associated with inflammation. MC treatment ameliorated the levels of inflammatory markers including TNF-α and MCP-1, in both heart and aorta tissues.

Oxidative stress also affects the regulation of proliferation and apoptosis in the tissue. Thus, HFD induced oxidative stress causes an increase in apoptotic cell numbers in the heart and aorta. As it is well known, the alterations of apoptosis have been involved in pathology of the many diseases such as cardiovascular diseases [41]. In the present study, TUNEL positive cells counted and apoptotic index were used to evaluate apoptotic cells. The number of apoptotic cardiomyosites and endothelial and smooth muscle cells in aorta were the highest in the HFD. Increased apoptosis may be a consequence of HFD induced oxidative stress. MC treatment decreased apoptotic cells in both hearth and aorta via the strong antioxidative and antiinflammatory effects.

The increased ROS formation induced by a HFD may result in oxidation of both lipids and nucleic acids as well. Extensive oxidative damage interferes with replication and transcription, resulting in genetic instability and an increased mutations. Evaluation of the severity of oxidative damage to DNA commonly involves determination of the content of 8-OHdG which is an oxidized form of guanine [42]. 8-OHdG, is a marker of DNA damage which is formed by ROS in damaged tissue. Previous studies have shown that HFD induced obesity increases 8-OHdG in many tissues [43]. Moreover, it has been shown that HFD increased the number of apoptotic cells in the heart and aorta [44, 45]. In HFD induced obese rats, MC has been shown to increase anti-oxidant activity, reduce apoptosis and ameliorated the histopathological alterations [17, 18]. A previous study suggested that polyphenolic compounds have an important protective effect against cardiovascular diseases (46). LC-MS/MS analysis of MC ethanol extract in another previous study showed that the extract contained polyphenolic compounds such as myricetin hexoside, myricetin rhamnoside, ellagic acid, quercetin rhamnoside, myricetin, trihydroxy cinnamic acid derivative, cafeic acid derivative and sinapinic acid derivative [20]. In a previous study, it was found that MC extract had high total phenol content (368,68 mg/g extract as gallic acid equivalent) and total flavonoid (111,35 mg/g extract as catechine equivalent) with signifcant *in vitro* antioxidant activity [21]. Therefore, polyphenolic compunds in extract could be responsible for the curative effect of MC extract on HFD induced heart and aorta damage.

Parallel to these findings, an increase in 8-OHdG level and apoptotic activity in the heart and aorta tissues in the HFD group has been observed in our study. However, MC treatment reduced apoptotic activity and 8-OHdG level in both the heart and aorta tissues. MC extract amelioriated HFD-induced heart and aorta damage via inhibition of apoptotic activity. In this study it has been shown that MC extract might have antioxidant and antiapoptotic function in the heart and aorta tissue.

Conclusion

In conclusion, the present study revealed that HFD-induced obesity caused alteration in lipid profiles, histopathologic damage in the heart and aorta tissues by increased oxidative stress, NOX-2-ir and iNOS-ir, reduced NO activity. MC treatment has potent antioxidant, anti-inflammatory and antiapoptotic effects, it might ameliorate HFD induced cardiovascular damage via inhibiting the oxidative stress and regulating NO metabolism.

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Compliance with Ethical Standards

Ethical Approval: This study was approved by Marmara University, Animal Care and Use Committee (23.2019.mar).

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Conflict of Interest: The authors declare that there are no conflicts of interest.

Author Contributions: NOY, NBA, GS and FE: Contributed conception and design, NOY, NBA, GS, RK and FE: Performed experiments and did data collection, NOY, GS and FE: Analyzed data, NOY and FE: Contributed to the writing. All authors approved the final version of the article.

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The effect of serum activated ghrelin hormone on glycemic control in the diabetic patients with excessive body mass index

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ABSTRACT

Objective: In the literature, plasma ghrelin level was found to be lower in patients with obesity or diabetes in a few studies. However, there is no study comparing ghrelin level in non-diabetic and diabetic patients with overweight or obesity. We have two aims in this study; first to show whether plasma ghrelin levels in type 2 diabetes mellitus patients with excessive body mass index (BMI) decrease the level of a cumulative ghrelin which we expect in both diabetes-related and obesity-related conditions, secondly to study whether there is a correlation between ghrelin level and diabetes complications.

Patients and Methods: Ethics committee decision and written informed consent from patients were received before the study. 57 BMI≥25 type 2 diabetic patients treated and followed up in the diabetic outpatient clinic and 25 BMI≥25 subjects without diabetes mellitus (control group) were included in this case-control study. Pregnant women, patients with malignancy and under 18 years old were excluded. The results were evaluated by the SPSS statistical program.

Results: The ghrelin and BMI values of the diabetic patients with excessive BMI and the non-diabetic patients with excessive BMI were not statistically different. No statistical significant correlation between ghrelin and haemoglobin A1c (HbA1C), BMI, retinopathy, neuropathy, albuminuria, and macrovascular complications was found in the type 2 diabetic patients with overweight or obesity.

Conclusion: The presence of diabetes in addition to patients with excessive BMI does not cause ghrelin levels to decrease more than expected.

Keywords: Ghrelin, Diabetes mellitus, Obesity, Overweight

1. INTRODUCTION

Ghrelin was discovered in 1999 as a growth hormone releasing peptide [1]. It is released mainly by the gastric cells. Ghrelin hormone increases appetite, stimulates eating and gastric motility, but is also adipogenic [2-8]. Its blood level increases in fasting and hypoglycaemia conditions [9]. Intracerebroventricular ghrelin administration increases nitric oxide synthesis (NOS) levels in the hypothalamus. It is observed that ghrelin's effect on increasing food intake is inhibited by the administration of N-nitro-L-arginine methyl ester [10]. It has opposite effects than the effects of leptin and obestatin in the body [11].

Plasma ghrelin level is inversely proportional to body mass index (BMI) in non-diabetic patients with excessive BMI and type 2 diabetes mellitus patients [12,13].

Our objective in this study is to show whether plasma ghrelin levels in type 2 diabetes mellitus patients with excessive BMI decrease the level of a cumulative ghrelin that we expect in both diabetes-related and obesity-related conditions.

2. PATIENTS and METHOD

Fifty-seven BMI≥25 type2 diabetic patients treated and followed up in the diabetic outpatient clinic and 25 BMI≥25 subjects without diabetes mellitus (control group) were included in the study. Ethics committee approval (Ministry of Health Okmeydanı Education and Training Hospital's Ethics committee, 23-12-2008, number:156) and written informed consent from patients were received before the study. Before the

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start of the study, disease duration, age, sex, BMI, height, weight, waist, hip measurements, concomitant diseases were recorded in all patients.

Serum samples were obtained from patients for fasting blood glucose, HbA1C, urea, creatinine, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, activated ghrelin measurements.

Activated ghrelin was measured by using Linco Research's Human Ghrelin (activated) (Linco Research, Missouri, USA) Elisa Kit, sandwich ELISA method. The samples taken into the dry tube were centrifuged within half an hour, the plasma was separated and the samples were frozen and stored. After all patient and control serums were collected, samples were analysed in accordance with the stages of kit prospectus.

The BMI of the patients was calculated with the formula weight (kg) / height^2 (meters).

Haemoglobin A1c: was measured by high performance liquid chromatography (HPLC) method on the Bio DPC Adams A1c device (Arkray Inc., Kyoto, Japan). Normal reference intervals were 4.6-5.2%.

Glucose and urea: were studied by photometric method in the Olympus AU2700 autoanalyzer (Beckman Coulter Inc, CA, USA).

Total cholesterol, triglyceride, HDL, LDL, VLDL-cholesterol: were studied by photometric method in the Roche modular V2 autoanalyzer.

Statistical Analysis

Statistical analysis was performed in two stages. The first stage is for patient group data and the second stage is for the analysis of both the patient and the control group integrated data set. The patient group consisted of a total of 57 diabetic patients with excessive BMI, while the non-diabetic patients with excessive BMI group consisted of 25 subjects. Firstly, the Jarque-Bera test was applied to test the normal distribution of the data. Since, the test result was $p<0.05$, the H1 hypothesis suggesting abnormal distribution was accepted. Thus, it was considered that non-parametric methods should be used in the analysis. SPSS (24 version) statistical program was used to analyse data. Kendall's Tau-b, Mann-Whitney U and Wilcoxon W tests were done.

3. RESULTS

The diabetic patients with excessive BMI included in the study were 17 males, 40 females, and the control group consisted of 9 males and 16 females. The mean age of the patient group was 56, mean BMI was 31.1, mean HbA1c was 7.65 and mean ghrelin was 69.5. The mean age of the control group consisting of patients with excessive BMI without diabetes was 53, mean BMI was 32.2, mean HbA1c was 5.4 and mean ghrelin was 70.4. The values of the diabetic patients with excessive BMI index group and the control group consisting of patients with excessive BMI without diabetes are summarized in Table I.

Table I. *Comparison of the values the diabetic patients with excessive body mass index and the control group consisting of patients with excessive body mass index without diabetes*

Patients with excessive body mass index	Diabetic (n=57)	Non-Diabetic (n=25)	P value
	Mean (SD)	Mean (SD)	
Age (years)	56.14 (10.73)	53.04 (10.94)	0.781
BMI (kg/m2)	31.18 (3.81)	32.28 (3.37)	0.703
HbA1c(%)	7.65 (1.49)	5.46 (0.33)	0.001
Ghrelin (pg/mL)	69.59 (9.99)	70.45 (13.89)	0.080
Height (cm)	160.60 (7.93)	165.32 (7.45)	0.956
Weight (kg)	81.29 (12.92)	88.84 (9.88)	0.112
Waist (cm)	103.84 (10.43)	105.68 (8.22)	0.465
Total Chol (mg/dl)	192.11 (43.09)	200.6 (30.73)	0.184
TG (mg/dl)	170.84 (107.8)	162.96 (126.23)	0.839
HDL Chol (mg/dl)	47.61 (12.29)	48.6 (9.22)	0.107
LDL Chol (mg/dl)	111.2 (33.85)	119.84 (27.14)	0.275
VLDL Chol (mg/dl)	34.21(21.62)	32.68 (25.26)	0.813
FBG (mg/dl)	158 (55.84)	91.88 (8.72)	0.001
Urea (mg/dl)	32.61(10.26)	26.92 (8.62)	0.486
Creatinin (mg/dl)	1.04 (0.45)	0.8 (0.16)	0.140

BMI: Body mass index, HbA1c: Hemoglobin A1c, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: very low-density-lipoprotein, FBG: Fasting blood glucose,

The ghrelin and BMI values of the diabetic patients with excessive BMI group and the patients with excessive BMI group were not statistically different ($p>0.05$).

Ghrelin and HbA1c, BMI, retinopathy, neuropathy, microalbuminuria, and macrovascular complications were compared in diabetic patients with excessive BMI. No statistical significant relationship was found between ghrelin and HbA1C, BMI, retinopathy, neuropathy, albuminuria, and macrovascular complications in the group consisting of obese type 2 diabetic patients ($p>0.05$). The results are summarized in Table II.

Table II. *Corelation between Ghrelin level and metabolic parameters and complications in diabetic patients with excessive body mass index*

Ghrelin	n	P value
HbA1C	57	0.520
BMI	57	0.671
Retinopathy	24	0.929
Neuropathy	11	0.503
Microalbuminuria	28	0.084
Macrovascular complications	19	0.696

HbA1c: Hemoglobin A1c, BMI: Body mass index

4. DISCUSSION

Although, there is no study in diabetic patients with obesity, a few studies about blood ghrelin levels in patients with obesity or diabetes are available in the literature; Shiiya et al., showed in their study that plasma ghrelin levels decrease as BMI increases

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[14]. On the contrary, Verdesh et al., showed in their study that there is a positive correlation between weight and ghrelin [15]. Katsuki et al., showed in their other study that plasma ghrelin levels inversely decreased in type 2 diabetics with abdominal obesity, plasma insulin levels and insulin resistance [16].

The euglycemic clamp study in normal subjects and type 1 DM patients on intensive insulin treatment showed that hyperinsulinemia suppressed plasma ghrelin secretion [17,18]. It was observed that plasma ghrelin concentration increased before meals and decreased rapidly after meals and after intravenous glucose infusion. These studies showed that ghrelin secretion is suppressed in short-term hyperglycemia or hyperinsulinemia.

In a study performed by Ueno et al., the relationship of plasma ghrelin concentration with glycemic control in diabetic patients was studied. 56 male and 52 female patients, 11 of them with type1 diabetes, 97 with type 2 diabetes were included in this study. The selected patients were low-weighted, normal-weighted and obese. The study showed that plasma ghrelin level is inversely proportional to HbA1c. This study showed that poor glycemic control decreased ghrelin levels. However, it could not be observed whether plasma ghrelin level decreases due to poor glycemic control, or decreased ghrelin impairs blood sugar regulation. As known, hyperinsulinemia and obesity decrease plasma ghrelin levels. In this study, patients with obesity and patients without obesity were evaluated in the same group. Since, ghrelin level is affected by BMI, in this study it could not be observed whether plasma ghrelin level decreases due to poor glycemic control, or due to BMI level [19].

Unlike other previous studies, for the first time, blood ghrelin levels were compared in diabetic and non-diabetic patients with excessive BMI in our study. Plasma ghrelin levels of diabetics and non-diabetic patients with excessive BMI are similar, a cumulative ghrelin decrease induced by both diabetes and obesity could not be detected. This shows us that obesity is the major factor affecting ghrelin levels.

Besides, no statistically significant correlation was found between ghrelin and microalbuminuria, neuropathy, retinopathy and macrovascular complications in the diabetic patients with excessive BMI group in our study. Therefore, ghrelin cannot be associated with diabetic complications. On the other hand one study showed that the ghrelin given exogenously has a protective effect in a degree on renal complications in newborn diabetic rats [20].

There is a limitation in this study that could be addressed in future research, studies with a larger number of patients would achieve better results.

The results of this study showed that there are other factors that affect ghrelin in the obesity condition such as other adipokines including leptin and obestatin, and other unknown mechanisms which may play a major role, therefore, additional studies are needed to clarify this issue.

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Compliance with ethical standards

Ethical Approval: The study protocol was approved by the Ministry of Health Okmeydanı Education and Training Hospital's Ethics committee (23-12-2008, number:156). Written informed consent was received from the patients.

Conflict of Interest: We declare that we have no conflict of interest.

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Authors' Contributions: All listed authors contributed to this study accordingly.

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Increased D-dimer is associated with disease progression and increased mortality in Turkish COVID-19 patients

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ABSTRACT

Objective: Coagulopathy is thought to play an important role in the development of severe COVID-19. High D-dimer levels have been reported in Chinese cohort studies. However, ethnicity has significant implications for thrombotic risk. Our aim in this study is to determine the effect of D-dimer measurements on disease prognosis and mortality in Turkish patients with COVID-19.

Patients and Methods: The study was designed retrospectively. Patients over the age of 18 who were admitted to our hospital were included in the study.

Results: The study included 226 patients. According to the World Health Organization staging, 75(33.2%) patients, according to the staging of Siddiqi et al., 67 (29.7%) patients progressed. In the ROC analysis performed to predict mortality, AUC value for D-dimer was found to be 82.25% (95%CI 74.8%-89.71%). When the cut-off value for D-dimer was accepted as ≥3.25mg/L, specificity was 94.15%, correctly classified rate 88.5%, positive likelihood ratio as (LR):5.69, negative LR:0.71.

Conclusion: As a result, similar to the Chinese cohorts, elevated D-dimer measurements increase disease progression and mortality in Turkish patients with COVID-19. D-dimer levels of 3.25 mg/L and above, strongly determine the risk of increased mortality in the Turkish Caucasian ethnic group.

Keywords: COVID-19, D-dimer, Mortality, Ethnicity

1. INTRODUCTION

At the end of 2019, a new coronavirus was identified as the cause of pneumonia cases in China. World Health Organization (WHO) defined this disease as coronavirus disease 19 (COVID-19) [1]. During the course of COVID-19, coagulation abnormalities are frequently seen that affect the pathogenesis of the disease [2]. Coagulation anomalies do not only increase thrombotic events but also affect mortality. In autopsy studies of individuals who died from COVID-19, diffuse thrombotic microangiopathy limited to the lungs was observed. Similarly, while no embolism was detected in the pulmonary arteries, it was found that the right ventricles of these patients were enlarged [3, 4]. The amount of new vessel growth in the lungs of COVID-19 patients is higher than in the lungs of patients with influenza [5].

D-dimer is a product of cross-linked fibrin showing increased thrombin formation and plasmin and fibrin dissolution. Multivariate regression analysis in COVID-19 cohorts showed that high D-dimer levels are an important risk factor for poor prognosis [6, 7]. Moreover, anticoagulant therapy with low molecular weight heparin (LMWH) appears to be associated with a better prognosis in patients with significantly increased D-dimer [7].

Race and ethnicity have a significant impact on thrombotic risk. Europeans have a significantly higher incidence of venous thromboembolism (VTE) compared to Asian populations [8].

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Most of the studies investigating the prognostic importance of D-dimer measurement in COVID-19 patients have been published in China, where the disease originated. In a study conducted with COVID-19 patients of the Caucasian race, a coagulopathy proportional to the severity of the disease was shown. However, this study showed that LMWH did not significantly affect the increase in D-dimer levels observed in patients with severe COVID-19 [9].

To our knowledge, there is no data on D-dimer cut-off level that best predicts mortality in the Turkish population. Our primary goal in this study is; to determine whether high D-dimer levels have an effect on mortality and disease progression in Turkish patients infected with COVID-19. Our second goal; is the determination of the D-dimer cut-off value, which increases the risk of mortality.

2. PATIENTS and METHODS

The study was designed retrospectively. The data of 543 patients over the age of 18 who applied to our hospital between March and June 2020 were analyzed. Individuals with positive COVID-19 polymerase chain reaction (PCR) tests were included in the study. In addition, pregnant women and patients who did not have laboratory data and lung computed tomography (CT) at the time of application were not included in the study. As a result, data of 226 patients were used. The study was approved by the ethics committee of our hospital (12.06.2020 approval number: 09.2020.697).

Medical treatments related to COVID-19 of the patients were carried out by the recommendations updated by the Turkish Ministry of Health [10].

For hypoxemic patients; Oxygen titration was performed such that the initial target oxygen saturation (SpO2) was ≥94% and for maintenance oxygenation ≥90%. For most critically ill patients, the lowest possible fraction of inspired oxygen (FiO2) required to meet oxygenation targets was preferred, ideally targeting a SpO2 of 90 to 96 percent if possible. Considering this goal, treatment with a nasal cannula, mask, high flow nasal cannula (HFNC), or noninvasive mechanical ventilation (NIMV) was given, and intubation was performed in patients for whom clinical goals could not be achieved [11].

Patients who developed acute respiratory distress syndrome (ARDS) and septic shock were treated by the recommendations in international guidelines [12]. Anticoagulation treatments of the patients were arranged according to the guidelines updated regularly by the Turkish Ministry of Health and the local hospital guides [10]. Since, the recommendations in the guidelines are dynamic recommendations updated with new information, there have been changes in the treatment of patients from time to time.

The patients' symptoms and laboratory values including clinical, radiological, and coagulation tests at the hospital admission, and the worst laboratory values were recorded during the hospitalization. The clinical severity scores of the patients were calculated with two separate scoring systems which are the WHO and Siddiqi et al. and at least 1 step worsening in this scoring system during follow-up was accepted as progression [13, 14].

Siddiqi et al., classified the early infection period as the 1st stage, the lung involvement period as the 2nd stage, and the hyperinflammation period as the 3rd stage [13].

D-dimer measurements were made with the American Beckman Coulter device purchased from TURMED, Istanbul, Turkey. Immuno-turbidometric method was used [15].

In addition, supportive treatments and anticoagulation treatments, initial symptoms, and comorbidities were recorded.

Statistical Analysis

STATA 15.1 software was used for statistical analysis. Since, continuous variables did not show normal distribution, the median and interquartile range (IQR) were reported with minimum and maximum values. Categorical variables were reported with numbers and percentages.

Mann-Whitney U and Kruskal-Wallis tests were used to determine the differences of continuous variables between independent groups, and Chi-square and Fisher's exact tests were used for comparisons between categorical variables.

Odds ratios were given with 95% confidence interval (CI) when necessary. Sensitivity, specificity, correct classification, positive and negative predictive values, positive and negative likelihood values were reported for the cut-off value determined by the non-parametric receiver operating characteristic (ROC) test. A p-value less than 0.05 was considered statistically significant.

3. RESULTS

A total of 226 patients with a mean age of 54 (min-max 20-95) were included in the study (Table-I). All patients were of Turkish Caucasian ethnicity. When the values recorded as the highest values from the laboratory values checked during the first application and follow-up of the patients were examined, the median of the admission D-dimer values was 0.6 Interquartile Range (IQR) 0.67, min-max 0.14-20), and the median of the highest D-dimers in their follow-up was 0.895 (IQR 1.93, min.-max 0.14-20) (Table-II).

In the ROC analysis performed to predict mortality, the area under the curve (AUC) value for D-dimer was found to be 82.25% (95% CI 74.8% – 89.71%). When the cut-off value for D-dimer was accepted as 3.25 mg/L and above, the specificity was calculated as 94.15%, sensitivity 33.33%, correctly classified rate 88.5%, positive likelihood ratio (LR) 5.69, negative LR 0.71. The negative predictive value was 93.24% and the positive predictive value was 36.84% (Figure).

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Marmara Med J 2023;36(2): 175-181

Table I. *Demographics and clinical characteristics of patients*

Demographics and clinical characteristics		Statistics	Total
Count		n (%)	226 (100.0%)
Age		Mean (SD±) Min-Max	54 (15) 20-95
Gender			
Female		n (%)	99 (43.81%)
Male		n (%)	127 (56.19%)
Treatment			
LMWH*	0**	n (%)	69 (30.53%)
	1**	n (%)	97 (42.92%)
	2**	n (%)	60 (26.55%)
Symptoms			
Cough		n (%)	121 (53.54%)
Fever		n (%)	81 (35.84%)
Dyspnea		n (%)	94 (41.59%)
Weakness – Fatigue		n (%)	23 (10.18%)
Diarrhea		n (%)	19 (8.41%)
Nausea – Vomiting		n (%)	24 (10.62%)
Chills		n (%)	35 (15.49%)
Headache		n (%)	23 (10.18%)
Myalgia		n (%)	23 (10.18%)
Comorbidities			
Hypertension		n (%)	83 (36.73%)
Diabetes mellitus		n (%)	64 (28.32%)
Chronic obstructive pulmonary disease		n (%)	16 (7.08%)
Coronary artery disease		n (%)	20 (8.85%)
Chronic renal failure		n (%)	8 (3.54%)
Asthma		n (%)	17 (7.52%)

LMWH: Low molecular weight heparin, 0: LMWH never given, 1**: LMWH given a single dose, 2**: LMWH given a double dose, p<0.05*

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Marmara Med J 2023;36(2): 175-181

Table II. *The effects of laboratory data of patients on both disease staging and mortality*

Laboratory findings		WHO[14] Classification			Siddiqi et al. [13] Classification			Mortality		
	Median(IQR*) Min-Max	0	1	p	0	1	p	Alive	Death	p
D-dimer (at admission)	0.6(0.67) 0.14-20	0.51(0.54) 0.14 – 20	0.81(1.39) 0.19-20	<0.0001	0.52(0.56) 0.14-20	0.78(1.12) 0.19-20	0.0005	0.55(0.53) 0.14-20	1.46(2.59) 0.45-11.27	<0.0001
D-dimer (at peak)	0.895(1.93) 0.14-20	0.62(0.82) 0.14-20	2.54(5.61) 0.25-20	<0.0001	0.66(0.87) 0.14-20	2.42(5.97) 0.25-20	<0.0001	0.73(1.08) 0.14-20	8.61(16.53) 1.49-20	<0.0001

0: no disease progression, 1: there is disease progression, IQR: Interquartile range*

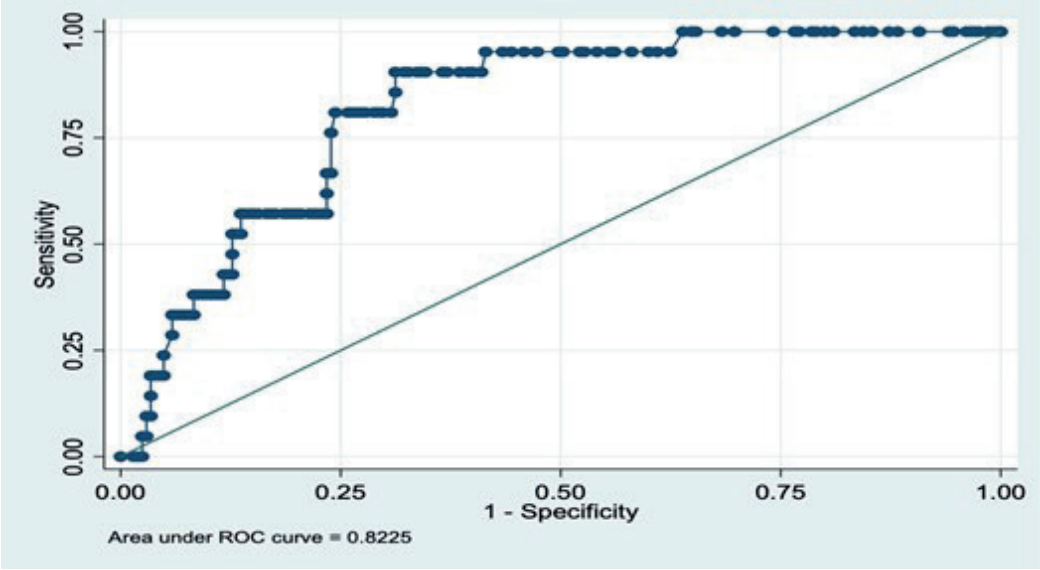

Figure. *Receiver operating characteristic (ROC) analysis for D-dimer in predicting mortality.*

4. DISCUSSION

Our study showed that increased D-dimer measurement was associated with disease progression and mortality in Turkish patients of Caucasian origin with COVID-19. A D-dimer level above 3.25 mg/L was most likely associated with mortality. In addition, the mortality-reducing effect of treatment with LMWH could not be demonstrated in patients with increased D-dimer levels.

The distinguishing feature of our study is that it is the first study to investigate the cut-off level of D-dimer, which increases mortality in the Turkish Caucasian race.

High D-dimer levels are known to increase in infections and sepsis. It has been reported that increased D-dimer level is associated with 28-day mortality in patients with infection or sepsis in the emergency department [16]. The most common anomalies in coagulation parameters in patients with COVID-19 infection is high D-dimer. In a study where five hundred and sixty cases were investigated, they found abnormally high D-dimer levels in 260 (46.4%) of the patients, and this rate was 60% in severe patients [14]. In another series, elevated D-dimers were likewise associated with a poor prognosis. In the study conducted by Zhou et al., it was observed that D-dimer higher than 1 μg/ml was associated with the fatal outcome of COVID-19 [6]. In the study conducted by Rodelo et al., it was shown that D-dimer levels above 2.0 mg/L can predict mortality with 92.3% sensitivity and 83.3% specificity [16].

Similarly, in our patient group, it was observed that the increase in D-dimer affected mortality. The same effect has been demonstrated in disease progression. It has been observed that having D-dimer >3.25 μg/mL increases mortality with high reliability.

Therefore, the use of LMWH has been recommended in this patient group since the beginning of the pandemic. It is known that LMWH has anticoagulant activity as well as anti-inflammatory and endothelial protective activity. Thus, in the studies performed by both Tang et al. [7] and Yin et al. [17], it was shown that LMWH treatment reduced mortality in the

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group with D-dimer >3.0 µg/mL. However, the use of LMWH has not been shown to reduce mortality in our patient group. In fact, these patients were more progressive and more mortal. This may be because high levels of D-dimer were seen in the severely ill patient group and the therapeutic dose of LMWH was given. LMWH at therapeutic dose has not yet been shown to reduce mortality in large studies, and its routine use is controversial [18, 19]. In our study, mortality in patients receiving therapeutic dose LMWH, was statistically significantly higher than those who did not receive therapeutic or those who received prophylactic doses. However, in staging according to both disease severity, the progression rate of those who received therapeutic dose LMWH was higher than those who did not receive therapeutic or those who received prophylactic doses. We think that the reason for this result is that the more severe patients in our study received therapeutic dose LMWH.

The elevation of plasma D-dimers was initially considered an indicator of coagulopathy [7] and was postulated as an indicator of diffuse intravascular coagulation (DIC). However, these patients do appear to have a clear DIC according to the International Society for Thrombosis and Hemostasis (ISTH) criteria [20] and fibrinogen levels are very high [7]. Alternatively, the origin of D-dimer is thought to be a direct result of acute lung injury seen in COVID-19 pneumonia by some authors [21]. It is known that the hallmark of acute lung injury is the accumulation of intraalveolar fibrin and that fibrin levels are controlled by alveolar epithelial cells that produce urokinase and regulate extravascular proteolysis. Urokinase then converts plasminogen to plasmin, which breaks down local fibrin. In addition; It has been described that increased macrophages in lung tissue, another marker of COVID-19 pneumonia, also produce fibrinolysis by an alternative route [22].

It is estimated that the lower incidence of venous thromboembolism in Asians is due to the lower prevalence of genetically induced anomalies that predispose to venous thromboembolism, such as Factor V Leiden [23]. Factor V Leiden is the most common genetic mutation that predisposes to venous thromboembolism [24, 25]. When thrombophilia screening was performed in Turks who had their first VTE attack, Factor V Leiden was detected in 15-20% of cases and the prothrombin gene mutation was detected in 5-7% of cases [26]. This frequency is reported to be 10% in the general Turkish population, which is higher than in the European population [27]. Differences in coagulation factors between Asians and Caucasians can be attributed to environmental factors, particularly diet and smoking, as well as genetic differences [25, 28]. Asians have been shown to have more effective inactivation of coagulation through activated protein C or greater fibrinolytic activity [23]. Most of the data on COVID-19 coagulopathy have been reported from China, where the incidence of venous thromboembolism is approximately 3 to 4 times lower in Chinese patients [29]. However, since the disease affects Caucasian individuals and Chinese individuals several times more than other ethnic groups, it is important to know the thrombogenic risk related to ethnic origin. Caucasians have a higher thrombotic risk than Chinese and other Asian populations, and the risk is even higher in African American and Hispanic patients in the United States [8, 30, 31]. In this case, the lower threshold value of D-dimer in Caucasians may increase mortality and require closer monitoring of these patients. Therefore, our study aims to determine whether the predictive value and cut-off point of D-dimer for mortality risk in the Turkish population are different from those in other races. In a study by Tang et al., examining laboratory parameters associated with poor prognosis, especially in patients with COVID-19 pneumonia who died in the late phase, it was found that fibrin-associated markers (D-dimer, fibrin degradation products) were moderately or significantly elevated [32]. This increase had significant effect on mortality, which is more than 2 times the normal value. The slightly higher predictive value we found may be related to the genetic, ethnic, and lifestyle differences between the Turkish and Asian populations.

Since, retrospective data were analyzed, other laboratory tests that could predict risk were not included in our study. The lack of effective antivirals and the lack of routine use of dexamethasone, which was known to reduce mortality at that time, may have contributed to poor clinical outcomes in some patients. The interpretation of our findings may be limited by the sample size. However, by including all adult patients identified for COVID-19, we believe our study population represents cases diagnosed and treated in the Turkish population.

The prognosis of COVID-19 is variable and poor in some patient groups. Unfortunately, an effective, globally accepted treatment algorithm for COVID-19 treatment has yet to be established. Additionally, the importance of anticoagulant therapy is increasing as thrombotic events play an important role in mortality. Our study shows that high D-dimer levels are strongly associated with disease severity and increased mortality. In the Turkish Caucasian ethnic group, D-dimer levels of 3.25 mg/L and above, strongly determine the risk of increased mortality. Future studies are needed to investigate whether anticoagulation treatment strategies reduce morbidity and mortality in COVID-19.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Marmara University, School of Medicine Clinical Research Ethics Committee (12.06.2020 approval number: 09.2020.697).

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

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The factors affecting the quality of life among women during the postpartum period

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ABSTRACT

Objective: As healthcare has become increasingly patient-centered, outcomes such as disease-specific quality of life (QoL) have become increasingly important. This study aimed to determine the factors affecting the QoL of postpartum women and which factors make a difference and affect the QoL.

Patients and Methods: A total of 175 postpartum mothers participated in this study. The Euro QoL 5 Dimension 5 Level (EQ 5D-5L) scale was used to measure the health-related QoL of postpartum women.

Results: The QoL of women differed in age, delivery type, venous thromboembolism risk factors, parity, gravida, number of live births, and use of anticoagulant medication. According to multiple regression analyses, the "age" variable had a significant effect on the QoL. However, the variables of education, social security, employment status, and monthly income of the family were not significant determinants of QoL. Also, the "number of live births" variable did not significantly affect the QoL, other obstetric and clinical variables had a significant effect on the QoL. The gravida increased the QoL but the number of miscarriages and the venous thromboembolism risk score decreased the QoL.

Conclusions: This study shows that, the QoL of women varies according to obstetric, socio-demographic, and clinical factors, and "age, gravida, the number of abortions and the venous thromboembolism risk score" variables have a significant effect on the QoL.

Keywords: Quality of Life (QoL), Pregnancy, Postpartum period, Pregnant women, EQ-5D-5L

1. INTRODUCTION

According to the United Nations, an average of 255 women give birth every minute worldwide [1], and approximately 385,000 babies (140 million per year) are born every day [2]. The puerperium period (postpartum period) (initial postpartum, early puerperium, and delayed postpartum period) covers the first 6-week period after birth, which starts with the expulsion of the placenta and ends at the end of the 6th week following birth [1, 3]. Six to 8 weeks after this period is called the "late postpartum phase," [4] and the physiological changes that occur during pregnancy and birth return to pre-pregnancy conditions [5].

The postpartum period involves various physical and mental problems [6]. Some women can be extremely sensitive to visible changes such as postpartum body shape and size changes, pregnancy scars, weight gain, and skin and hair loss, and may be affected by these changes. In addition, invisible internal changes such as postpartum depression can occur in many women [7]. In some cases, changing health conditions after childbirth can last up to 2 years after birth [6]. The most common problems in the postpartum period are infection, anemia, wounds, headache, back pain, constipation, hemorrhoids, urinary/fecal incontinence, and sexual problems [8]. Since, women have to cope with all these changes in the postpartum period, their quality of life (QoL) can be affected [9]. These postpartum health problems may lead women to take sick leave or quit their jobs due to long-term illness [10]. Changes in women's QoL in the postpartum period can affect various aspects of maternal and infant health [9, 11]. Therefore, health systems should adopt effective healthcare interventions that prevent and/or treat comorbidities and complications associated with pregnancy and postpartum disease [12].

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Women's subjective perceptions of their health-related QoL are a fundamental measure of the quality and effectiveness of maternal and child health interventions. Although, traditionally used methods for measuring the outcomes of the pregnancy and postpartum period, such as pregnancy-related morbidity and mortality rates, continue to be used as a basis, they are no longer adequate. Popular health depends on saving lives and improving the QoL [13]. Evaluation of QoL in clinical trials to investigate the effectiveness of preventive and treatment programs in pregnant and postpartum women has become increasingly important in pregnancy and postpartum periods [14].

Reliable, valid, and sensitive QoL measures are required to appropriately examine the effectiveness of interventions in pregnancy and postpartum periods. This study was conducted to compare the postpartum hospitalization process of women and the QoL post-discharge from the hospital. The study aimed to determine the differences in the QoL of women (women groups who gave birth by vaginal delivery and cesarean section) according to delivery type, the differences in obstetric, clinical, and sociodemographic factors, and those affecting the QoL of patients.

2. PATIENTS and METHODS

Study design

This descriptive and cross-sectional study used convenience sampling which is non-probabilistic. This sampling method was thought to be more appropriate since women in the postpartum period had to deal with issues such as feeding their newborns and suffering pain or stress just after birth. The women who agreed to participate in this study during the hospital pre-discharge and post-discharge in the postpartum (42 to 49 days) data collection period and were over the age of 18 were included in the sample. There were no exclusion criteria within the scope of the research. Women gave birth in a third-level hospital in July 2019 after uneventful pregnancies. Information about the women's QoL was collected by the researchers through face-to-face interviews with patients during the hospitalization period and telephone interviews between 42-49 days after the patients were dismissed from hospital. The QoL of postpartum patients was then compared based on the period when postpartum women were hospitalized for an average of 3 days and 6 weeks after delivery.

Measures

To measure the QoL of postpartum patients, the Turkish version of the EuroQoL (EQ 5D-5L) scale, was used (euroQoL .org). The Turkish version of the scale was translated into 171 languages by the EuroQoL group [15]. Although, the scale does not have a Cronbach alpha value calculated by EuroQoL, the Cronbach alpha value reported in international studies is observed as above 0.80 [16]. This scale is used in many studies in the national and international literature [17-22]. The EQ-5D-5L QoL scale is a tool to measure the EQ-5D index, which is "an index scale that evaluates the health status of patients in terms of five dimensions of health" with "five perceived health status levels per dimension" (5L). The EQ-5D index from the five scale parameters (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) with 1 to 5 for each parameter consists of the health status of the mother. The number 1 given to the questions indicates the best state of health, and the number 5 indicates the worst state of health. As a result of this method, a 1-digit number is obtained that expresses the level selected for this parameter. The digits of the five parameters are combined into a 5-digit number (for example, 11111), which describes the patient's health status. These five health status values are then converted into a weighted score used to calculate quality-adjusted life years (QALYs) ranging from zero (0) to one (1). A score of "0" represents death, and a score of "1" represents perfect health. However, during this transformation, it is important for each country to determine the transformation weight values in accordance with its sociological and cultural structure. Since, the coefficients describing the health status of each patient were not made for Turkey, they were evaluated using German values, which is one of the other countries whose coefficients were produced accordingly. There are many studies in the literature using the weights of other countries, and there are also studies using Germany's weights [23, 24]. Based on this evidence, in this study, the QALY weight values of Germany were used.

All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and ethical standards. This study was approved by the Hacettepe University Non-Invasive Clinical Research Ethics Committee (date: 19.03.2019, approval number: 2019/09-30). A written informed consent form was obtained from all participants.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (version 20.0) statistical program. Descriptive statistics are shown with numbers and percentages. The Kolmogorov-Smirnov test was used to test if the collected data showed a normal distribution pattern in order to decide on appropriate statistical tests in comparing groups. It was observed that the coefficient of variation was below 30%, the skewness and kurtosis coefficients were in the range of – 1.5 and +1.5 distribution, and the data were in accordance with the normal distribution. The results of normal distribution tests showed there was no bias against the use of parametric tests since the data was normally distributed. In addition, the differences in the QoL values according to the sociodemographic, obstetric, and clinical characteristics of the patients, the significance test of the difference between two independent groups (Student's *t*-test), the significance test between two dependent groups (paired samples t-test), and one-way variance analysis (one-way ANOVA) were used. The post-hoc LSD test was used to determine the group that made a difference between the groups in the parameters of the one-way ANOVA test. In the study of sociodemographic parameters, the patient's age, educational status, social security, and working status were used, while the average monthly income of the family, obstetrics, and clinical

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Marmara Med J 2023;36(2): 182-191

parameters of patients' gravida, parity, number of alive children, gestational week, indication for cesarean section, the risk of venous thromboembolism, and anticoagulant drug use were used to determine the post-discharge status score. To determine the main determinants of the QoL in the study, multivariate regression analyses were performed, and the backward regression method was applied during these analyses. In the established model of multiple regression analysis, the quality-of-life score was determined as a dependent variable, and other obstetric and clinical variables were determined as independent variables. Statistical significance was set at a p value <0.05.

As a result of the analyses, the QoL of postpartum women was examined according to various sociodemographic, obstetric, and clinical characteristics of patients, according to their type of delivery, the health status parameters that determine the QoL, and various other factors (age, education level, social security, employment status, monthly income of the family, the number of gravida, the number of live births, the number of miscarriages, and VTE risk scores) affecting the QoL.

3. RESULTS

Of the 175 postpartum women whose data were analyzed, 42.9% (n=75) were aged <25 years. This rate was followed by women over the age of 30 (n=54; 30.9%) and those in the 25–30 age group (n=46; 26.3%). According to the level of education, 54.9% (n=96) of the patients consisted of those who had received an education of maximum 12 years, while 45.14% (n=79) consisted of patients who received more than 12 years of education. Of the patients, 90.9% (n=159) did not work, and 96% of the women (n=168) were registered with the Social Security Institution. In addition, 1.1% (n=2) of the women did not have any insurance and paid a fee, 2.3% (n=4) benefitted from maternity insurance, and a mother under the age of 18 was also eligible for the benefit. The average monthly income level of 56% of the women (n=98) was over 2500 ₺ (Turkish liras). When the obstetric and clinical data of the cases were examined, it was determined that 37.1% (n=65) of the mothers had their first pregnancy, and 40.6% (n=71) had their first birth. A total of 58.29% of the patients (n=102) already had at least one live birth. In 56.6% (n=99) of the patients, the delivery occurred after the 37th gestational week was completed, that is, a mature newborn. A total of 33.1% (n=58) of the births was performed by cesarean section, 47.4% (n=27) of the cesarean sections were performed in emergency, and 52.6% (n=30) of the births were performed in elective (planned) conditions. Of the patients, 12.6% (n=22) had had at least one miscarriage, and the percentage of those who underwent elective (voluntary) abortion was only 2.9% (n=5). Low molecular weight heparin (LWMH) was administered as an anticoagulant (anticoagulant drug) in 40.6% (n=71) of the cases during hospitalization in the postoperative period and in 39.4% (n=69) within 6 weeks after delivery. According to the venous thromboembolism (VTE) risk scoring, the risk score of 41.7% (n=73) of patients was 0, while the risk scores of 21.1% (n=37) were 1, 27.4% (n=48) were 2, and 9.7% (n=17) were 3 and higher. A total of 39.4% of postpartum mothers continued to use LMWH post-discharge because the VTE risk score was 2 or higher.

Comparison of the QoL scores of postpartum women

When the pre-discharge QoL scores were examined according to the sociodemographic characteristics of the cases, the difference in scores was statistically significant only according to age group (p=0.002). The QoL score was significantly higher in postpartum women under the age of 25 years (Table I). In other sociodemographic characteristics (education, social security, working status, and average monthly income of the family), the QoL scores were similar between the groups (p>0.05).

When the mean pre-discharge QoL scores according to the obstetric and clinical characteristics of the patients were examined according to the method of delivery, it was found that the QoL scores were significantly higher in cases where vaginal delivery occurred (0.835 vs. 0.794, p=0.036). QoL scores were significantly higher in the groups with a VTE risk score of 0 or 1 (p=0.039; Table I). Also, there was a difference according to the VTE scores, the mean of QoL scores those with a VTE score of 1 point is higher than the others. There was no difference in QoL scores between the groups in terms of other obstetric and clinical characteristics (p>0.05).

According to sociodemographic parameters, postpartum women's QoL scores differed significantly according to age group post-discharge (p=0.008) (Table I). As in the case of pre-discharge, it was significantly higher in the under-25 group than in the 25-30 and above 30 years. In other sociodemographic variables, the QoL scores between the groups were similar.

According to the obstetric and clinical factors shown in Table I, the QoL score averages of the patient's post-discharge according to their gravida, parity, number of miscarriages, number of livebirths, and LWMH use status differed significantly between the groups (p<0.05). The QoL score was highest in those with a first pregnancy (i.e., gravida =1), while it was significantly lower in the group with gravida ≥3 (p=0.041). The QoL score was found to be significantly higher in women with a first birth (i.e., parity=0) than in other groups of women who had previously given birth and whose child had lived (i.e., parity=1 and ≥2) (p=0.014 and 0.015, respectively). The QoL score was found to be significantly higher in women who had 0 and 1 miscarriages. The QoL score was also significantly higher in those who used LMWH, an anti-clotting drug, post-discharge (0.966 vs. 0.960; p=0.035). The mean QoL score of the patients according to the week of delivery, type of delivery, indication for cesarean section, and VTE risk scores was similar between the groups (p>0.05).

The QoL indicators obtained from the five dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) that indicate the health status of postpartum mothers were compared pre-discharge and post-discharge. A statistically significant difference was found in mobility, self-care, usual activities, and pain/discomfort dimensions (p<0.005; Table II).

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Marmara Med J 2023;36(2): 182-191

Table I. *Comparison of QoL scores pre-discharge and post-discharge according to socio-demographic, obstetric, and clinical characteristics*

	Characteristics	Groups (n=175)	QoL Score Pre-Discharge			QoL Score Post-Discharge		
			Mean ±SD	t/F	p	Mean ±SD	t/F	p
Socio-demographic	Age	<25 (n=75)	0.858 ±0.129	2.072	**0.002**	0.980 ±0.048	4.979	**0.008**
		25-30 (n=46)	0.773 ±0.205			0.941 ±0.127		
		>30 (n=54)	0.794 ±0.165			0.930 ±0.106		
	Education level	≤12 years (n=96)	0.832 ±0.164	0.555	0.580	0.948 ±0.093	0.902	0.368
		>12 years (n=79)	0.811 ±0.152			0.962 ±0.098		
	Social security	Social Security Institution (n=168)	0.824 ±0.156	0.162	0.688	0.954 ±0.097	1.145	0.286
		Other groups* (n=7)	0.799 ±0.226			0.967 ±0.054		
	Employment status	Unactive (n=159)	0.825 ±0.161	0.180	0.673	0.955 ±0.097	0.149	0.700
		Active (n=16)	0.798 ±0.136			0.945 ±0.075		
	Family's monthly income	≤2500 ₺ (n= 77)	0.811 ±0.182	0.538	0.466	0.952 ±0.096	0.331	0.566
		>2500 ₺ (n=98)	0.831 ±0.141			0.956 ±0.095		
	Someone who has attendant (responsible)	Yes (n=164)	0.829 ±0.154	1.196	0.233	0.953 ±0.097	1.055	0.468
		No (n=11)	0.766 ±0.197			0.973 ±0.062		
Obstetric	The number of gravida	1 (n=65)	0.823 ±0.128	0.008	0.992	0.964 ±0.104	7.013	**0.041**
		2 (n=54)	0.826 ±0.178			0.956 ±0.075		
		≥3 (n=56)	0.820 ±0.185			0.941 ±0.103		
	The number of parity (previous births)	0 (n=71)	0.828 ±0.128	2.185	0.120	0.965 ±0.101	4.342	**0.014**
		1 (n=62)	0.772 ±0.222			0.947 ±0.092		
		≥2 (n=42)	0.875 ±0.968			0.948 ±0.091		
	The number of miscarriages	0 (n= 153)	0.832 ±0.140	2.173	0.142	0.956 ±0.091	7.780	**0.006**
		1 (n=18)	0.823 ±0.158			0.954 ±0.095		
		≥2 (n=4)	0.762 ±0.247			0.942 ±0.122		
	The number of live births	0 (n=73)	0.820 ±0.148	1.471	0.237	0.966 ±0.100	4.314	**0.015**
		1 (n=62)	0.787 ±0.203			0.944 ±0.093		
		≥2 (n=40)	0.877 ±0.101			0.949 ±0.091		
Clinical	Gestational week	≤37th gestational week (n=76)	0.827 ±0,135	0.167	0.861	0.960 ±0.077	0.735	0.444
		>37th gestational week (n=99)	0.820 ±0,174			0.950 ±0.107		
	Delivery type	Vaginal delivery (n= 117)	0.835 ±0,150	2.119	**0.036**	0.955 ±0.106	0.852	0.396
		Caesarean delivery (n= 58)	0.794 ±0,176			0.953 ±0.071		
	Cesarean section indication	Emergency (n= 27)	0.767 ±0,183	-0.702	0.491	0.952 ±0.078	-0.047	0.963
		Elective (n= 30)	0.819 ±0,173			0.953 ±0.066		
	Use LMWH	Yes (n=71)	0.786 ±0,193	-1.440	0.157	0.966 ±0.088	-2.129	**0.035**
		No (n= 104)	0.845 ±0,131			0.960 ±0.100		
	VTE risk score	0 (n=73)	0. 835 ±0,138	2.576	**0.039**	0.962 ±0.099	1.732	0.145
		1 (n=37)	0.863 ±0,126			0.946 ±0.103		
		2 (n=48)	0.776 ±0,214			0.949 ±0.095		
		≥3 (n=17)	0.780 ±0,080			0.949 ±0.069		

p=p-value; SD=standard deviation; t=t value; F=F value, LMWH :low molecular weight heparin, VTE: venous thromboembolism
**The women did not have any insurance, paid a fee, benefited from maternity insurance, and a mother under the age of 18 who was eligible for the benefit*

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Marmara Med J 2023;36(2): 182-191

Table III presents the comparison in the QoL scores according to the delivery types pre-discharge in cases who delivered. The results showed a statistically significant difference based on all sub-dimensions ($p<0.05$), while the mobility sub-dimension was not statistically significant ($p>0.05$). Accordingly, when compared with the cases who gave birth by cesarean section, the self-care, usual activities, pain/discomfort, and anxiety/depression scores were significantly higher both pre and post discharge ($p<0.05$). In general, the QoL scores of patients with vaginal delivery were higher. When the QoL scores of the patient's post-discharge were evaluated according to the delivery type, the mean QoL scores differed between those who delivered vaginally and those who delivered by cesarean section based on all sub-dimensions except for normal activities ($p<0.05$). Accordingly, there was a significant improvement in the QoL of patients after hospital discharge.

Determination of the effect of sociodemographics on the QoL score

According to the results of the four-stage regression analysis performed to determine the sociodemographic variables that affect the score of postpartum patients' post-discharge, the variables with a statistically significant effect on the QoL are given in Table IV.

Table II. *Comparison of women's' scores pre-discharge and post-discharge according to QoL parameters*

QoL subdimensions	Groups (n=175)	Mean ± SD	t	p
Mobility	Pre-discharge	1.88 ±0.839	8.202	**<0.001**
	Post-discharge	1.25 ±0.647		
Self-Care	Pre-discharge	1.35 ±0.780	4.391	**<0.001**
	Post-discharge	1.07 ±0.339		
Usual Activities	Pre-discharge	1.57 ±0.931	6.792	**<0.001**
	Post-discharge	1.09 ±0.384		
Pain/Discomfort	Pre-discharge	2.15 ±0.916	12.592	**<0.001**
	Post-discharge	1.24 ±0.587		
Anxiety/Depression	Pre-discharge	1.40 ±0.871	1.475	0.142
	Post-discharge	1.29 ±0.653		
Total QoL Scores	Pre-discharge	0.812 ±0.168	7.175	**<0.001**
	Post-discharge	0.916 ±0.115		

p=p-value; SD=standard deviation; t=t value. QoL: quality of life

Table III. *Comparison of pre-discharge and post-discharge QoL scores of women according to delivery types*

QoL subdimensions	Delivery types	QoL Score Pre-Discharge			QoL Score Post-Discharge		
		Mean ±SS	F	p	Mean ±SD	F	p
Mobility	Vaginal delivery	1.95 ±0.847	1.109	0.450	1.26 ±0.697	0.291	**0.015**
	Caesarean delivery	1.85 ±0.826			1.24 ±0.540		
Self-Care	Vaginal delivery	1.93 ±0.798	11.921	**<0.001**	1.08 ±0.375	0.124	**0.008**
	Caesarean delivery	1.39 ±1.074			1.07 ±0.256		
Usual Activities	Vaginal delivery	1.84 ±0.602	3.645	**<0.001**	1.09 ±0.415	0.725	0.686
	Caesarean delivery	1.30 ±0.365			1.07 ±0.317		
Pain/Discomfort	Vaginal delivery	2.36 ±0.894	1.127	**0.030**	1.24 ±0.611	0.889	**0.002**
	Caesarean delivery	2.04 ±0.931			1.24 ± 0.540		
Anxiety/Depression	Vaginal delivery	1.62 ±0.743	18.491	**0.018**	1.30 ±0.606	0.834	**0.023**
	Caesarean delivery	1.29 ±1.057			1.28 ± 0.744		
Total QoL Scores	Vaginal delivery	0.835 ± 0.151	4.310	**0.010**	0.916 ±0.121	0.144	**0.025**
	Caesarean delivery	0.766 ± 0.192			0.917 ±0.104		

p= p-value; SD=standard deviation; F=F value. QoL: quality of life

http://doi.org/10.5472/marumj.1302417
Marmara Med J 2023;36(2): 182-191

The established regression model was statistically significant (F=2.710; p=0.02). Accordingly, the "age" variable has a significant effect on the QoL. However, the variables of education, social security, employment status, and monthly income of the family are not significant determinants of QoL. According to age groups, the QoL scores of mothers between the ages of 25 and 30 were higher than those of mothers over the age of 30 and under the age of 25, and the total QoL score decreased as the age increased.

Determination of obstetric and clinical factors effect on the QoL score

Table V shows the variables that have a statistically significant effect on the QoL according to the results of the 5-stage regression analysis performed to determine the obstetric and clinical variables affecting the QoL score of the patients' post-discharge.

The established regression model was statistically significant (F=3.309; p=0.01). Accordingly, although the "number of live births" variable did not significantly affect the QoL, other obstetric and clinical variables had a significant effect on QoL. So, the gravida increased the QoL (p=0.02) but the number of miscarriages (p=0.026), and the venous thromboembolism risk score (p<0.001) decreased the QoL. These variables explain 7.20% of the QoL.

Table IV. *Sociodemographic parameters impacting the QoL score*

	B	Std. Error	β	t	p	
Constant	74.316	6.169		12.047	**<0.001**	
Age						
<25	**Ref.**					
25-30	-5.851	2.988	-0.159	-1.958	**0.050**	
>30	-1.221	2.870	-0.291	-3.562	**<0.001**	
Education level						
≤12 years	**Ref.**					R=0.272
>12 years	6.019	2.964	0.163	2.031	0.840	R^2=0.07
Social security						
Social Security Institution	4.924	2.244	0.059	0.789	0.431	$F_{(5, 175)}$ =2.710 (p=0.02)
Other groups*	**Ref.**					Durbin Watson=1.900
Employment status						
Active	**Ref.**					
Inactive	2.927	1.430	0.050	0.661	0.510	
Monthly income of the family						
≤2500 ₺	**Ref.**					
>2500 ₺	1.331	0.533	0.041	0.525	0.600	

Constant: constant value; B: unstandardized B coefficients; Std. Error=Standard error; β =Standard regression coefficients, t = t value; F: F value; p=p-value; Ref: Reference group; R2: R-squared value.
**The women who did not have any insurance, paid a fee, benefited from maternity insurance, and a mother under the age of 18 who was eligible for the benefit*

Table V. *Obstetric and clinical parameters with an effect on QoL score*

	B	Std. Error	β	t	p	
Constant	69.645	3.426		20.331	**<0.001**	
Number of gravida	6.461	2.754	0.471	2.246	**0.020**	R=0.269
Number of live children	-5.424	3.157	-0.298	-1.718	0.088	R^2=0.07
Number of miscarriages	-8.357	3.714	-0.230	-2.250	**0.026**	$F_{(4, 175)}$ =3.309 (p=0,01)
VTE risk scores	-3.359	1.201	-0.228	-2.796	**0.006**	Durbin Watson=1.914

VTE: venous thromboembolism, Constant: constant value; B: unstandardized B coefficients; Std. Error=Standard error; β =Standard regression coefficients, t = t value; F: F value; p=p-value; R2: R-squared value.

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4. DISCUSSION

Childbirth has a major impact on mothers' health-related QoL. The aim of this study was to determine the factors affecting the QoL of postpartum women (patient groups who gave birth by vaginal delivery and cesarean section) and which factors make a difference and effect the QoL.

According to the analyses performed to determine the sociodemographic factors affecting the QoL, the mean QoL score of the patients differed between the period of hospitalization pre-discharge and the period after 6 weeks post-discharge. Postpartum women's QoL improved post-discharge, and their QoL scores increased. Thus, while QoL differs according to age groups pre and post discharge, QoL does not differ according to other sociodemographic factors; in this study, it has been observed that the differences seen according to age are similar to other research findings [25-26]. This finding may partly explain the high average QoL of women under 25 years of age in this study. In the same study, the effect of physical activity level and sleep quality on the QoL in pregnant women was examined, and it was found that while there was no significant relationship between inactivity seen during pregnancy and quality of life, sleep changes were associated with the QoL [25-26]. In a study conducted by Mousavi et al, on 356 pregnant women, the QoL scores of postpartum patients was examined at the level of some variables; it was concluded that age affected the QoL at the environmental level, and age had no effect on the QoL scores at the physical, mental, social, and global levels. In addition, education and income variables did not have a significant effect on the QoL of patients in any dimension [27].

According to the analyses performed to determine the clinical factors affecting the QoL, the mean QoL scores of women before and post discharge differed according to the delivery type (vaginal or cesarean delivery). One of the most important goals of antenatal and postnatal care in developed countries is to improve the QoL of the mother [28], and a study on cesarean section reported that the risk of maternal morbidity increased cases of hysterectomy, bleeding, infection, thrombosis, and postpartum depression in patients who delivered by cesarean section (intrapartum). The odds ratio for cesarean section was 2.0 (95% CI 1.6–2.5) and 2.3 (95% CI 1.7–3.1), respectively [29]. In addition, the results of some studies show that fatigue, headache, insomnia, anemia, urinary tract infection, and other conditions requiring treatment in the first 8 weeks after delivery are higher in women who gave birth by cesarean section than in those who delivered by vaginal birth. Pain and fatigue can affect the QoL after birth [30]. According to Abedian et al., in a comparison of the QoL of the patients with vaginal delivery and with cesarean delivery in Iran, the QoL scores of the cesarean section group were found to be lower than the those of the vaginal delivery group, according to the mental and physical sub-dimensions [31]. These results show that the method of birth has an effect on the QoL, and there may be a difference in the QoL score of women according to the type of birth. Another finding in this study is that the QoL of patients differs according to the VTE risk score pre-discharge, and the QoL score was found to be higher in patients with a VTE risk score of 0 or 1 than in those with a high-risk score. This difference is believed to be associated with the absence of additional risk factors such as comorbidity, obesity, preeclampsia, postpartum hemorrhage, and the risk of immobility in the postpartum period in patients with a low risk score.

Post discharge, the mean QoL score differs according to parity, gravida, number of live births and the use of anticoagulant LMWH. However, QoL scores of the patients were similar regarding the week of delivery, type of delivery, indication for cesarean section, and VTE risk scores. The difference in QoL scores according to the number of parity was determined in the patients who had given birth at least once before, and the mean QoL score of those patients was higher than that of the other groups. There are differences between patients who gave birth at least once before and those who gave birth three or more times. Park and Choi stated that reproductive history can affect women's health and QoL [32]. Most studies have evaluated the relationship between birth and QoL [33, 34]. Unlike these results, in a study conducted by Dehcheshmeh et al., it was found that parity was not associated with birth in terms of pregnancy outcomes [35]. In this study, it was observed that the difference seen according to the number of gravida existed between the patients who had three or more pregnancies and those who were pregnant at least once or twice. In a similar study, Fatemeh et al., found that the number of gravida differed significantly according to the QoL [36]. In addition, another finding in this study is that there are differences between the patients who have at least one child and those who have three or more children according to the number of livebirths. Women who have more motherhood experience and more than one child adapt to motherhood more naturally after the birth of a second child [37]. In a study investigating the factors affecting the postpartum functional status of women, it was observed that as the number of children increased, the self-care activities of the mother decreased. As the postpartum period increases, the functional status of women who receive help in baby care and housework decreases in the 6th week and the 3rd and 6th months after birth [38]. These conditions can affect the QoL of women in various ways. In this study, there were differences between the patients who used and those who did not use anticoagulant drugs according to their status. The mean QoL scores were higher in patients using anticoagulant medication. This difference found after discharge is thought to be related to the information given to the patients by their physicians about the purpose of using LMWH before they are discharged from the hospital. Women using LMWH after discharge may have felt more confident when they considered the information of their physicians, which may explain the higher QoL scores in patients using LMWH compared to the group that did not use LMWH. In addition, LMWH is an important factor in the prevention of maternal mortality due to venous thrombosis [39-41].

The postpartum period can have significant physical, emotional, and social effects on a woman's QoL. Most postpartum research has focused on physical complications, and only a few studies have specifically investigated the QoL pre and post discharge. Therefore, this study is important [30, 31, 27, 42].

http://doi.org/10.5472/marumj.1302417
Marmara Med J 2023;36(2): 182-191

In this study, the QoL scores of the patients pre and post discharge differed based on all sub-dimensions (mobility, self-care, usual activities, and pain/discomfort) except for the anxiety/depression dimension. In a study conducted by Torkan et al., on the postpartum patient group based on 6 to 8 weeks, it was concluded that the QoL differed only in physical health indicators and the QoL did not change based on other dimensions [30]. In a study by Mousavi et al., in which they examined the QoL in the cesarean and vaginal delivery groups, differences were observed in all dimensions except environmental factors, and it was concluded that the QoL scores were higher in the vaginal delivery group than in the cesarean section group [27].

In this study, the effects of various sociodemographic, obstetric, and clinical variables on QoL in postpartum women were investigated, and it was concluded that age, number of gravida, number of exaggerations, and venous thromboembolism risk score were predictors of QoL, but the effect of the number of live births on QoL was not significant. In addition, while the number of gravida positively affects the QoL, the variables of age, number of livebirths, number of exaggerations, and venous thromboembolism risk score negatively affect QoL. In Akın et al.'s study examining the QoL of postpartum women according to the sociodemographic and fertility characteristics of women, similar to the results of this study, the age variable is an important determinant of QoL [25]. In a study conducted by Calou et al., the profession, the number of parities, support from their spouse, and marital status are predictors that positively affect their QoL [43]. In a study comparing the QoL after vaginal and cesarean section, Mousavi et al., concluded that parity is a predictor of QoL [27]. In their analysis, Da Costa et al. studied the determinants of the physical health status of women in the postpartum period; the parity number of the patients showed a significant effect on the QoL, and all the variables together explained 20% of the variance [44]. De Oliveira et al., examined the QoL of mothers after birth and found that, unlike the results of this study, number of live births and parity variables did not effect QoL [42]. In this study, the gravida has a positive effect on the QoL in relation to the fact that women with previous pregnancies are more naturally compatible with motherhood. In a study by Küçükkaya et al., in which they examined the concerns of women regarding the birth and postpartum period, the increase in the gravida in pregnant women in the 1st and 2nd trimesters decreased their anxiety scores regarding depression [45].

According to the findings of this study, an increase in VTE risk scores negatively affected patients' QoL scores. Most of the factors that make up the risk score for VTE include concomitant disease or additional risk factors. This may explain the negative impact of the VTE risk factor found in this study on QoL. In addition, Erickson et al., examined the relationship between various factors and QoL in patients with venous thromboembolism and found that most of them had a QoL score below the average QoL [46]. In the same study, patients with a history of VTE had higher depression and anxiety scores.

Conclusion

In this study, the QoL of women in the postpartum period was evaluated using the EQ 5D-5L "mobility, self-care, usual activities, pain/discomfort, and anxiety/depression." To evaluate the effect of women's sociodemographic and clinical characteristics on their QoL, their quality-of-life scores were compared immediately after delivery and 6 weeks after discharge. According to the results obtained, the QoL of women differs during the period of hospitalization and within six weeks of receiving home care. When the literature is examined, most studies focus on postpartum women's ability to perform their physical activities and daily normal activities. Studies examining the QoL pre and post discharge are rare. This study is important in terms of addressing this comparison and revealing the sociodemographic and clinical factors that are important in terms of QoL. However, since this was a cross-sectional study, the results obtained from this study are limited to the sample of the study, and these findings may differ in studies with larger sample sizes. Therefore, to understand the QoL of women in the postpartum period, practitioners should consider many factors together with their various aspects. Also measuring the QoL is widely perceived to have a substantial effect, but results are partially dependent upon study methods and outcome variables of interest.

The roles and responsibilities of mothers in the care of their newborn babies create a big difference in the QoL of women in this period. Postpartum women experience a range of physical symptoms associated with QoL, such as fatigue, back pain, perineal pain, dyspareunia, hemorrhoids, urinary incontinence, and psychological changes, such as an increased risk of depressive disorders. In this sense, healthcare research needs to comprehensively define the factors affecting the QoL of postpartum women. Cognitive-behavioral interventions (including communication, problem-solving, self-disclosure, and empathetic responsiveness) healthcare providers should consider postnatal cognitive-behavioral interventions. Therefore, it can be recommended that health care providers develop comprehensive interventions to improve the QoL of women in the postpartum period. In this context, longitudinal studies are required to precisely investigate which factors may affect postpartum women's health and their experiences within the Turkey maternity system.

Compliance with Ethical Standards

Ethical Approval: This study has been approved by the Hacettepe University Non-Invasive Clinical Research Ethics Committee (date: 19.03.2019, approval number: 2019/09-30). A written informed consent form was obtained from all participants.

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Is neurofibromatosis type 1 diagnosed in every patient who presents with café au lait macules? A single-center experience

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ABSTRACT

Objective: Neurofibromatosis type 1 (NF1) is the most common hereditary neurocutaneous syndrome. The most crucial morbidity of NF1 is tumors that may develop. Cases with café-au-lait macules (CALMs) which is the first clinical finding of NF1, due to the anxiety of its associated morbidity, are referred to the pediatric oncology clinic. In this study, we aimed to examine the characteristics of the patients who applied to our outpatient clinic with CALMs.

Patients and Methods: The data of 157 pediatric patients who applied to our institution with the diagnosis of CALMs between June 2010 and November 2020 were analyzed retrospectively.

Results: There were 157 pediatric cases referred to us for CALMs. According to the National Institutes of Health (NIH) diagnostic criteria, 109 (69.4%) cases were diagnosed with NF1. The diagnosis of 22 cases with NF1 were supported by genetic examination. Optic glioma was detected in 39 (24.8%) cases. In 15 (38.4%) of cases with optic glioma, visual functions were also affected. Second diagnostic criterion did not develop during the follow-up period, except for macules, in 48 cases (30.5%).

Conclusion: In cases with multiple CALMs, the probability of NF1 diagnosis is high, and close and regular follow-up is of great importance in catching the development of the second clinical criterion and minimizing its morbidity.

Keywords: Café-au-lait macule, Neurofibromatosis, Children, Tumor

1. INTRODUCTION

Café-au-lait macules (CALMs), are used to name the milky brown skin macules with different sizes on the skin, which are congenital or acquired. While its incidence is 2.7% in the neonatal period, more than three CALMs can be seen in 1% of children [1,2]. These macules can be the first sign of various genetic syndromes and neurocutaneous diseases. The most well-known syndrome is neurofibromatosis type 1 (NF1).

Neurofibromatosis type 1 is the most common hereditary neurocutaneous syndrome. The incidence is reported as 1 in 2500 births [3]. Clinical findings occur due to mutations in the NF1 gene, which is a tumor suppressor gene. Although, it usually occurs due to germline mutations showing autosomal dominant inheritance, it can also rarely be encountered with de novo mutations and can be the first case in the family. The NF1 gene is located on the 17q11.2 chromosome and encodes a protein called neurofibromin [4]. This protein acts as a negative regulator of the Ras proto-oncogene. Therefore, as a result of mutations in the NF1 gene, the frequency of mostly benign and malignant tumors of the central and peripheral nervous system, gastrointestinal stromal tumors, breast cancer, pheochromocytoma, leukemia, lymphoma, and rhabdomyosarcoma are also higher in these cases compared to the normal population [4]. The most crucial morbidity of NF1 is tumor that may develop and gliomas in the optic tract, which can cause vision impairment. Neurofibromas are the most frequently detected tumors, and more rarely, plexiform neurofibromas, which have the risk of malignant transformation, can be seen [5]. Although, not among the diagnostic criteria, hamartomatous lesions in the central

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nervous system (CNS) are typical radiologically and usually not symptomatic. However, these patients may also experience regression in neurocognitive functions. Apart from neurological findings, skeletal deformities, cardiovascular pathologies, and endocrine disorders are among the other findings. Diagnosis is made by meeting two or more criteria from the National Institutes of Health (NIH) criteria (Table I) [6].

Table I. *NIH consensus criteria for diagnosis of NF1 (Two or more criteria are required for diagnosis) [6]*

Six or more café-au-lait macules over >5 mm diameter in prepubertal individuals and >15 mm diameter in post pubertal individuals
Two or more neurofibromas or 1 plexiform neurofibroma
Freckling in the axillary or inguinal regions
Optic glioma
Two or more Lisch nodules
A distinctive osseous lesion
A first degree relative with NF-1

NIH:National Institutes of Health, NF-1: Neurofibromatosis type 1

Cases with CALMs, which is the first clinical finding of NF1 due to the anxiety of NF1 and its associated morbidity, are referred to the pediatric oncology outpatient clinic, and NF1 scans are performed. In this study, we aimed to examine the characteristics of the patients who applied to our outpatient clinic with CALMs, the degree of compliance with the NF1 diagnostic criteria, the frequency of developing benign or malignant tumors, and the follow-up results in treatment retrospectively.

2. PATIENTS and METHODS

The data of 157 pediatric patients who applied to our institution with the diagnosis of CALMs between June 2010 and November 2020 were analyzed retrospectively. Demographic characteristics of the patients, NF1 family history, NF1 diagnostic criteria, benign/malignant tumors if advanced, visual function evaluation results with central nervous system and orbital MRI, and endocrine, neurocognitive function, skeletal system, and genetic evaluation results were analyzed. This study is a retrospectively designed descriptive type of study. Mean, median, minimum and maximum values and numbers (n) and percentages (%) were used for data description. Patients whose parents refused to give consent were excluded from the study. Study was approved by the institute's ethic committee (09.2022.119).

3. RESULTS

One hundred and fifty-seven pediatric patients referred to our clinic for CALMs. The median age of the cases was 96 months (range, 1-210 months). Ninety-three (59.2%) cases were male, and 64 (40.7%) were female. Characteristic findings of the patients are listed in Table II. Median follow-up period of all cases was 33.5 months. While, there was a family history of NF1 in 39 (24.8%) cases, 6 (3.8%) patients had an undiagnosed family member with widespread CALMs. Presenting complaint of all cases was widespread CALMs on the body. At the time of admission, apart from the macules, the headache was present in 5 (3.1%), visual problems in 7 (4.4%), and swelling in the body in 6 (3.8%) cases. Regarding the distribution of the macules, data of 4 cases could not be reached. In one hundred and fifty-one (96.1%) cases, widespread macules were present, and in 3 (1.9%) of them, sizes of the spots were 0.5 cm-1 cm, in 18 (11.4%) cases 1-1.5 cm, in 136 (%86,6) cases larger than 1.5 cm. There was only one macule in two cases, and their sizes were 2x2 cm and 3x5 cm, respectively. In addition to spots, 30 (19.1%) cases had axillary/inguinal freckles. Lisch nodules were detected in 35 (22.2%) cases in eye examinations. When patients were evaluated according to the other NF1 diagnostic criteria; neurofibroma developed in 27 (17.1%) cases, one of which was spinal, and the others were cutaneous. The mean age of the patients who developed neurofibroma was 10.1 years. Ten (6.3%) cases had plexiform neurofibroma, and the mean age was 7.68 years. Malignant transformation was not observed in any patients with plexiform neurofibroma during their follow-up. When evaluated in terms of a skeletal anomaly; Skeletal system anomaly was present in 19 (12.1%) cases, and scoliosis was the most common skeletal system pathology with 9 (47.3%) cases. Neurological findings were present in 19 (12.1%) cases. Of these, 15 (78.9%) had neurocognitive dysfunction, 4 (21%) epilepsy, and 1 (5.2%) autism spectrum. When evaluated in terms of endocrine pathologies; precocious puberty was found in 5 (3.1%) cases, short stature in 3 (1.9%), hypothyroidism in 1 (0.6%), and diabetes insipidus in 1 (0.6%) cases. In the cardiac evaluation results; aortic coarctation in 2 (1.27%) cases, 4 (2.5%) mitral valve prolapse and 1 (0.6%) atrial septal defect were followed up in the cardiology outpatient clinic. According to the NIH diagnostic criteria, 109 (69.4%) cases were diagnosed with NF1. There were only 22 (14%) cases whose diagnosis was supported by genetic examination.

According to the NIH, a second criterion did not develop during the follow-up period, except for stains, in 48 cases (30.5%). There were hamartomas in 19 (39.5%) cases on brain imaging of these cases. When the stain characteristics were examined, only 2 cases had one stain, while 46 cases had widespread stains over 1 cm. Twenty-seven (56.25%) of these cases did not come to regular follow-up. The mean age of 21 patients who followed up regularly was 5.74 years.

When evaluated in terms of non-neurofibroma tumoral formations; Optic glioma was detected in 39 (24.8%) cases. In 15 (38.4%) of these cases, visual functions were also affected. Three cases had received chemotherapy due to progressive visual impairment and were being followed up for stable disease. Visual functions of 12 patients who did not receive treatment continued to be stable. One patient was followed up for pilocytic astrocytoma outside the optic tract, and another was followed up for a cardiac mass.

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Table II. *Clinical Characteristics of Patients*

	n (%)
Total number of patients	157(100%)
Age (months)	96 (1-210)
Sex	
Male/Female	93(59.2%) /64(40.7%)
Family History	39 (24.8%)
Other NF1 Diagnostic Criteria	
Neurofibroma	
Plexiform Neurofibroma	10 (6.3%)
Cutaneous Neurofibroma	26 (16.5%)
Spinal Neurofibroma	1.0 (0.6%)
Skin-Fold Frecking	30 (19.1%)
Lisch Nodules	35 (22.2%)
Optic Glioma	39 (24.8%)
Visual Function Affected	15 (9.5%)
Chemotherapy	3.0 (1.9%)
Bone Lesions	19 (12.1%)
Hamartoma	87(55.4%)
Cardiac Malformation	7.0(4.4%)
Mitral valve prolapse	4.0 (2.5%)
Aortic coarctation	2.0 (1.27%)
Atrial Septal Defect	1.0 (0.6%)
Endocrine Disorders	10(6.3%)
Precocious puberty	5.0(3.1%)
Short stature	3.0(1.9%)
Hypothyroidism	1.0(0.6%)
Diabetes Insipidus	1.0(0.6%)
Neurological Problem	20(12.7%)
Neurocognitive Dysfunction	15 (9.5%)
Epilepsy	4.0(2.5%)
Autism spectrum	1.0(0.6%)
Genetic Diagnosis	22 (14%)

NF-1: Neurofibromatosis type 1

4. DISCUSSION

Out of 157 cases who applied to our outpatient clinic with the complaint of CALMs, 109 of them were diagnosed with NF1 as a result of median follow-up of 33.5 months. CALMs are usually the first sign of NF1 syndrome, and other diagnostic criteria other than family history emerge over the years. While, CALMs usually occur in the first two years of life, axillary and inguinal freckles develop in 5-8 years, Lisch nodules in 5-10 years, neurofibromas in late childhood, and plexiform neurofibromas in the first ten years [7]. Optic gliomas, which can be seen in the first seven years, and skeletal dysplasia, which can be seen in the first seven years, can be diagnosed early by suspecting the disease with these clinical findings, thus, minimizing the risk factors for morbidities that may occur due to the disease. In conclusion, CALMs have an important place in the early diagnosis of NF1 disease. In a study in which 110 individuals with CALMs were evaluated, it was determined that 23% of these cases with six or more CALMs did not develop NF1 diagnostic criteria. It was emphasized that the number of CALMs alone was not sufficient for the diagnosis of NF1 [8]. In this study, while the median age at presentation was 96 months and the median follow-up period was 33.5 months, a second criterion did not develop in 30.5% of the cases (n=48) during the follow-up period. Since, 56.2% of these cases did not come to regular follow-up, this rate of no follow-up made the possibility of developing diagnostic criteria with a higher rate controversial. The median age of 21 cases that were followed up regularly was 5.74 years, and they were being followed up in terms of the possibility of developing the second criterion. Another remarkable feature in these 48 cases was the presence of hamartomatous lesions in 39.5%, which were not included in the NIH diagnostic criteria, but were pathognomonic for NF1 on brain MRI imaging. 55.4% of 157 patients had hamartomas on brain MRI imaging. In a study from Korea, hamartomatous cranial lesions were found in 20% of the 42 NF1 cases on brain MRI [9]. In the literature, it was stated that in 43% of the cases with NF1 diagnosis, hamartomas could be seen on brain MRI, growth may occur in less than 10%, and it was emphasized that after the age of 10 years old, a biopsy may be considered to rule out tumor formation in cases with enlarged hamartomas [10]. In this study, the cases with hamartoma were stable, and none of them needed a biopsy.

In another study in which 19 cases were evaluated, it was emphasized that 9 of these cases admitted with CALMs were diagnosed with the NIH diagnostic criteria, and four were diagnosed with genetic analysis. It is recommended that the diagnosis of NF1 should be persistently considered in cases presenting with multiple CALMs [11]. In our study, the cases diagnosed according to the NIH diagnostic criteria were 64.9% (109 cases) of all cases and in addition to macules, the second most common criterion for diagnosis was family history, with 24.8% of the cases. Although, there was no definite diagnosis in the family, genetic analysis was performed in only one of the 6 cases with multiple CALMs, and a mutation was detected. Of the remaining five patients, two were diagnosed with plexiform neurofibroma, one with cutaneous neurofibroma, and another with Lisch nodule, which was later diagnosed as NF1. According to this result, we would like to remind that assessment of a family member with multiple CALMs other than the diagnosis of NF1 should be included in anamnesis of cases. Although, NF1 is stated to be an autosomal dominant disease in literature, 50% of NF1 cases originates from de novo mutations without presenting a family history [7]. Diagnosis was supported by genetic analysis only in 22 (14%) cases in our study. Since, genetic analysis was

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Marmara Med J 2023;36(2): 192-196

not performed on every patient, a correct assessment of the genetic transmission rate could not be made for this study.

Optic glioma was the most common clinical diagnostic criterion after family history. Low-grade glial tumors occur with a frequency of 15-20% in cases with NF1, and 80% of them are seen in the optic tract [5]. These tumors can occur from birth, and only 5% of them can be symptomatic [7]. Early diagnosis is crucial in preserving visual functions in cases with optic glioma. Diagnosis is made by visual acuity, visual field, and orbital MRI examinations when the disease is suspected. MRI imaging in young children is not accessible due to the need for anesthesia, and visual function evaluations gain importance in this period. Visual functions should be evaluated regularly in cases with NF1 diagnosis. Children with NF1 had a better visual acuity outcome than sporadic optic glioma [12]. In our study, optic glioma was detected in 24.8% of the cases, consistent with the literature [5]. Visual functions were also affected in 38.4% of these cases. While patients with stable disease were followed, 5 patients with progression received chemotherapy treatment. After treatment, these cases continued to be followed up with stable disease. In our study, low-grade glial tumor formation outside the optic tract was detected in only one case and it was followed up as stable disease.

The third most common diagnostic criteria were neurofibromas (17.1%) and plexiform neurofibromas (6.3%), which are skin findings. Studies have shown that the incidence of cutaneous neurofibromas is 99% in cases with a diagnosis of NF1. While plexiform neurofibromas can occur from birth to 18 years of age, it generally occurs above 7 years of age [5]. In our study, mean age of the patients with neurofibroma was 10.1 years and it was found only in 17.1% of the patients. In literature, it is stated that 30% of plexiform neurofibromas are visible by naked eye and 50% of them are diagnosed by imaging studies [5]. In our study, the mean age of patients with plexiform neurofibroma was 7.69 years, while the incidence was 6.3%, and all of them were diagnosed with imaging studies. The lower rate can be explained by the fact that the mean age of the cases is still low. Since the incidence of scoliosis is 10-26% in patients with NF1 diagnosis, spinal examination gains importance in physical examination [13]. In a single center study, skeletal problems were seen in 14 of 52 patients with NF1 [14]. In our cases, skeletal system disorders were detected in 12.1% of cases, and 47.3% of them were scoliosis. We think that it should be kept in mind during the physical examination since it is a finding that can be missed if attention is not paid to the examination.

Although, there are few studies in the literature, the frequency of endocrine problems in cases diagnosed with NF1 is reported to be 1-3% [15-17]. In a multicentric study in which 116 cases were examined, endocrine problems were found in 27.6% of the cases, and 71.9% were central precocious puberty [18]. In our single-center study, 6.3% of 157 cases were found to have endocrine problems. Among them, the most common problem was precocious puberty followed by short stature. Due to the study's retrospective nature, the presence of cases whose hormone data could not be reached, limits the study in this respect. Previous literature reported frequency of cardiac pathologies to be 2.3% and 18.8% among NF1 cases [19, 20]. In the study of İncecik et al., it was reported that among 65 NF1 patients cardiac anomaly was observed in 15.3% of the cases, and mitral valve regurgitation was the most common anomaly which was observed in 5 cases [21]. In this study, the cardiac anomaly was detected in 7 patients, while mitral valve prolapse was found to be the most common anomaly in 4 (2.5%) cases. However, proper cardiac assessment record of some patients could not be accessed. Therefore, number of patients with cardiac anomaly was found to be limited in our study.

In conclusion, NF1 is a neurocutaneous syndrome that can cause severe morbidity by affecting many systems. Due to CALMs, which is its first finding, morbidity can be minimized with close follow-up, early diagnosis, and treatment. In cases with multiple CALMs, the probability of NF1 diagnosis is high, and close and regular follow-up is of great importance in catching the development of the second clinical criterion.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Marmara University, School of Medicine Ethics Committee (09.2022.119).

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Authors' Contributions: NE: Data acquisition, data analysis, data interpretation, manuscript preparation and revisions, NE, BTT, ES and AGT: Data interpretation, manuscripts preparation, BTT and ES**:** Data acquisition, data analysis, manuscript revisions. All authors approved the final manuscript.

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T1 relaxation time in the evaluation of liver fibrosis; with native MR relaxometry

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ABSTRACT

Objective: Non-invasive methods have been investigated as an alternative to biopsy in assessing liver fibrosis. This study aimed to evaluate the relationship between liver T1 relaxation time and liver fibrosis as a non-invasive alternative method.

Patients and Methods: This study analyzed 1.5T magnetic resonance (MR) images of 86 patients retrospectively. The participants were divided into two groups: patients with chronic hepatitis and the control group. Native variable flip angle (VFA) T1 mapping technique was used to estimate liver T1 relaxation time. T1 mapping sequence, T2* mapping sequence, and image analysis were performed. The liver size, the spleen size, the liver T1 relaxation time, and the liver T2* relaxation time were recorded.

Results: The T1 relaxation time was 758.4 ± 121.1 ms in the chronic hepatitis group and 600.2 ± 67 ms in the control group. The T1 relaxation time of the patient group was significantly higher than that of the control group ($p<0.001$). The spleen size of the patient group was statistically significantly larger than the control group ($p<0.001$). There was a significant positive correlation between liver T1 relaxation time and Ishak score ($r=0.683$, $p<0.001$). Also, a significant positive correlation was observed between T1 relaxation time and histological activity index score ($r=0.542$, $p<0.001$).

Conclusion: A native T1 map is a non-invasive method that works as an alternative to biopsy in the follow-up and diagnosis of chronic hepatitis. Moreover, this method can be used to measure liver T1 relaxation time in patients with liver fibrosis.

Keywords: Fibrosis, Liver, MRI, Relaxometry, T1

1. INTRODUCTION

Hepatitis is an inflammation of the liver that develops due to many reasons. Chronic hepatitis is characterized by inflammation lasting for at least six months. The processes that cause morbidity and mortality in chronic hepatitis include liver fibrosis, cirrhosis, hepatocellular carcinoma, and portal hypertension [1]. Magnetic resonance imaging (MRI) is one of the most commonly used methods for liver imaging in clinical practice in both focal and diffuse liver diseases. Chronic hepatitis can be diagnosed by MRI findings such as nodularity in the liver parenchyma, irregular contours, heterogeneous parenchyma intensity, decreased liver size, increased spleen size, and increased portal vein calibration [2].

Liver fibrosis is an essential indicator of disease progression in patients with chronic hepatitis. Liver biopsy is an invasive method that has been used to grade fibrosis. However, the biopsy may result in complications such as pain, bleeding, perforation, and other complications that may be developed during the surgical procedure [3]. The mortality rate of a liver biopsy ranges between 1/10000 and 17/10000 [4]. As a result, non-invasive methods that can be used as an alternative liver biopsy have been investigated. MRI can be used to make quantitative measurements of the liver parenchyma. Therefore, magnetic resonance (MR) relaxometry can be used to determine the T1 relaxation time of the liver parenchyma [5] and to assess fibrosis in patients with chronic hepatitis [6].

Magnetic resonance relaxometry sequences that allow quantitative evaluation of relaxation times have been developed for qualitative assessment with MRI [7]. MR relaxometry

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can review the quantitative information in diagnosing some heart, liver, brain, and skeletal system diseases and follow-up. In recent years, MRI sequences that display T1, T2, or T2* relaxation times as parametric color maps, which can be used for quantitative evaluation of tissues via the direct region of interest (ROI) analysis on images, have been developed [8]. In addition, quantitative information about tissues can be obtained by measuring T1 relaxation time in milliseconds (ms) [9]. Furthermore, prolongation at the T1 relaxation time of the tissue indicates conditions characterized by irregular collagen deposition, such as fibrosis [10].

T1 relaxation time can be derived from contrast differences in color maps using the variable rotation angle (VFA) T1 mapping technique using two or more gradient-echo datasets obtained from different rotation angles [11]. The VFA T1 mapping method is frequently used in liver T1 mapping. Two different turning angles were used in a T1-weighted volumetric interpolated breath-hold examination (VIBE) sequence. Liver T1 relaxation time can be measured from pre – and post-contrast images on the 3-dimensional gradient-echo sequences [12]. As a result, T1 relaxation time can be used in ms as quantitative data to assess pathologies like liver fibrosis and predict liver function. Fibrosis, inflammation, and hepatosteatosis prolong the T1 relaxation time. However, since chronic hepatitis is associated with inflammation and fibrosis, the liver T1 relaxation time is predicted to be prolonged [13].

This study aims to determine the predictive value of T1 relaxation time as a non-invasive method that can be used as a biomarker of liver fibrosis in chronic hepatitis patients. In addition, it is investigated whether T1 relaxation time could be an alternative to biopsy in diagnosing and following liver fibrosis in chronic hepatitis patients. For this purpose, we compared liver T1 relaxation time in patients with chronic hepatitis and the control group using native liver T1 mapping. In addition, we evaluated the relationship between histopathological results and T1 relaxation time.

2. PATIENTS and METHODS

Upper abdomen MRI cases were investigated retrospectively in the Department of Radiology, Ankara Health Application and Research Center MRI unit using a 1.5 Tesla MRI device (Magnetom Aera, Siemens Healthcare GmbH, Erlangen, Germany) between April 2017 and December 2017. We performed liver native T1 mapping sequence in routine examination protocol.

A total of 86 cases were included in the study population. Patients with fatty liver and iron overload in both control and chronic hepatitis groups were excluded from the study. Patients with a liver fat fraction greater than 5% were excluded from the study. Therefore, all cases included in the groups had free fatty liver. Liver fat fractions were calculated using dual-echo T1 images obtained with a single excitation. Patients with iron overload were also excluded from the study because the calculation of liver fat fraction could be misinterpreted in the case of iron overload. T2* relaxation times were obtained by routine liver T2* mapping in the protocol.

Our study consisted of two groups, the control, and the chronic hepatitis group. The data from each group were measured and compared as a mean. Patients in the control group have MR findings, clinical findings, and laboratory data inconsistent with chronic hepatitis and do not have a prior diagnosis of parenchymal liver disease. In addition, there were 44 patients in the control group. The second group was comprised of chronic hepatitis patients who had MRI findings, clinical findings, and pathological diagnoses consistent with chronic hepatitis. In addition, there were 42 patients in the chronic hepatitis group. In addition, 19 patients in the second group had liver biopsy results six months before and after the MRI. There were no biopsy results in 23 patients during this period. However, the clinical diagnosis, laboratory findings, and MRI findings all pointed to chronic hepatitis. All of the scans were done on a 1.5 Tesla MR scanner with a 32-channel superficial body coil and a phased array (Magnetom Aera, Siemens Healthcare GmbH, Erlangen, Germany).

T1 Mapping Sequence

Parameters of T1 mapping sequence (without contrast agent) obtained using two different rotation angles in one breath attitude by VFA technique; TR: 4.76 ms, TE: 2.08 ms, 3.5 mm cross-sectional thickness, 2^{o}-14.9^{o} turning angles, FOV: 310x310 mm, voxel size: 1,6x1,6x3,5 mm^3, bandwidth: 1955 Hz / pixel and matrix size: 96x77 mm.

T2 Mapping Sequence*

T2* mapping parameters obtained using multi-gradient echo sequence in single breath attitude; TR: 200 ms, TE: 0.93 to 14.24 ms in 12 different echo times (0.93+ (Nx1,11msn), 10 mm section thickness, 20^{0} turning angle, FOV: 325x400 mm, voxel size: 3.1x3.1x10 mm^3, bandwidth: 1953 Hz / pixel and matrix size 128x104 mm.

Image Analysis

T1 and T2* relaxation time were measured and averaged using a volumetric ROI of 1 cm3 from all liver segments. Furthermore, in-phase liver and spleen intensity and out-phase liver and spleen intensity were measured with the help of ROI. The liver fat fraction was calculated using single excitation dual-echo T1 images. The liver and spleen sizes were measured, and values were recorded in the control and chronic hepatitis groups using conventional MR images.

Statistical Analysis

IBM® SPSS® 23 program was used to analyze the data obtained in the study. Data were expressed as mean ± standard deviation (SD). Normality was tested using the Kolmogorov-Smirnov test. Student t-test or One-way ANOVA followed by post-hoc Fisher's LSD test were used to compare the data between the groups. Pearson correlation test was used to evaluate the correlation between the data. Comparisons were evaluated by ROC curve

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analysis to determine cut-off values for T1 times. p<0.05 was accepted as statistically significant.

3. RESULTS

Our study had 86 participants, 42 of whom were in the control group and 44 in the chronic hepatitis group. The general population ranged in age from 16 to 78 years old, with a mean age of 50.14±15.6 years.

The control group consisted of 15 male and 29 female participants. The mean age of the control group was 53.93±14.9 years. The chronic hepatitis group comprised of 15 female and 27 male patients. The mean age of the patient group was 46.52 ± 15.7 years.

T1 mapping images were analyzed in control and chronic hepatitis groups in each case. Fig 1 and Fig 2 show the T1 map image of one case each from the control and patient groups, respectively.

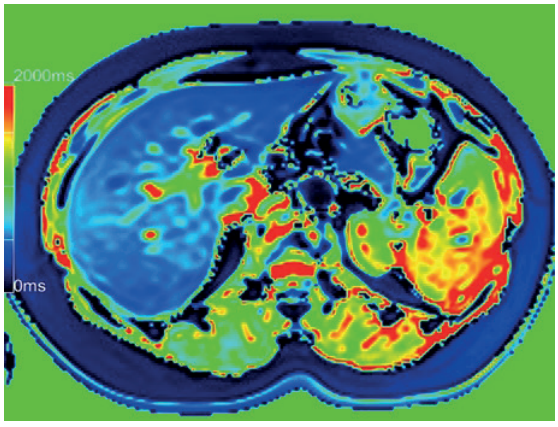

Figure 1. *A 38 year-old woman with normal liver. T1 relaxation time of the liver was 642 ms.*

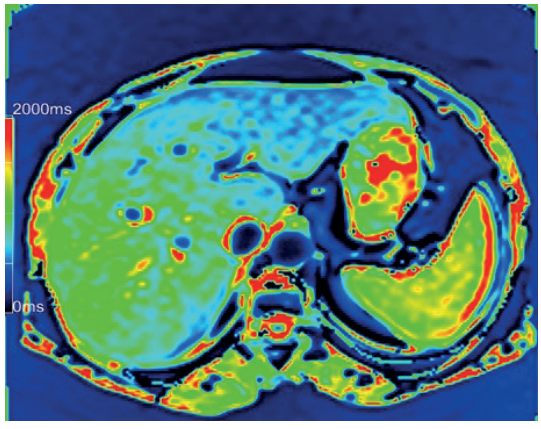

Figure 2. *A 54 year-old man with chronic hepatitis. T1 relaxation time of the liver was 988 ms.*

The mean liver size, mean spleen size, mean liver T1 time, and mean liver T2* time was recorded in both control and chronic hepatitis groups. The mean liver T1 time, liver T2* time, spleen size, and liver size were expressed in Table I.

Table I. *Demographic and clinical data of study groups*

	Control Group (n=44)	Chronic Hepatitis Group (n=42)	P value
Age	53.93 ± 14.9	46.52 ± 15.7*	<0.05
Liver size (mm)	113.9 ± 18.3	106 ± 21.3	>0.05
Spleen size (mm)	107.5 ± 20.6	133.2 ± 29.8**	<0.001
Liver T1 time (ms)	600.2 ± 67	758.4 ± 121.1**	<0.001
Liver T2* time (ms)	29 ± 3.4	29.5 ± 4.1	>0.05

Data are presented as mean ± SD. (*) Statistically significant compared to control group (p<0.05). (**) Statistically significant compared to control group (p<0.001).

The mean liver sizes of the control and chronic hepatitis groups were 113.9 ± 18.3 mm and 106 ± 21.3 mm, respectively. The mean liver size of the control group was larger than that of the patient group. However, the difference between the groups was not statistically significant (p>0.05).

The mean spleen sizes of the control and chronic hepatitis groups were 107.5 ± 20.6 mm and 133.2 ± 29.8 mm, respectively. The results are expressed in Fig 3. There was a statistically significant difference in spleen sizes between the control and chronic hepatitis groups. The mean spleen sizes of the patient group were statistically significantly greater than the control group (p<0.001).

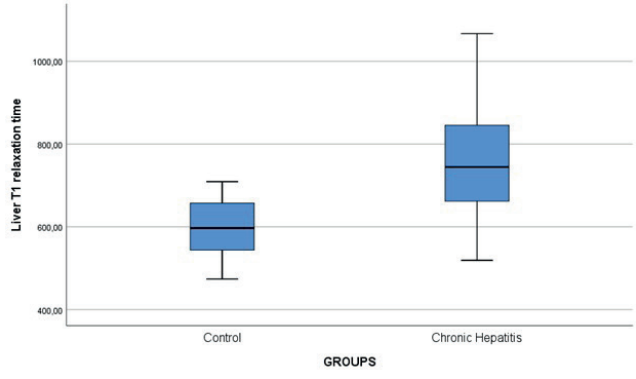

Figure 3. *Liver T1 relaxation time of control group and chronic hepatitis group*

The mean liver T1 relaxation time was 600.2 ± 67 ms and 758.4 ± 121 ms in the control and chronic hepatitis groups, respectively, as shown in Fig. 4. The T1 relaxation time of the patient group is statistically significantly higher than the control group (p<0.001).

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Marmara Med J 2023;36(2): 197-202

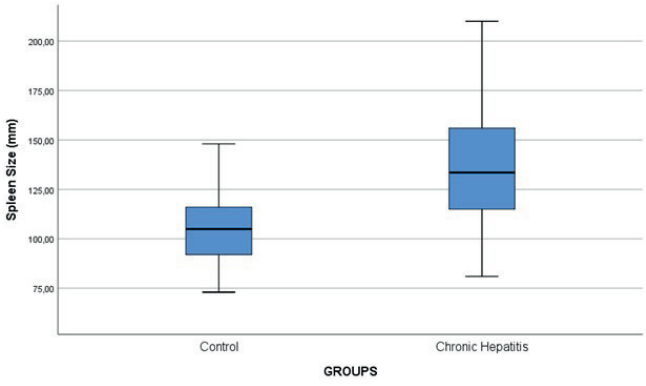

Figure 4. *Spleen size of control group and chronic hepatitis group*

The mean liver T2* time of the control and chronic hepatitis groups were 29 ± 3.4 and 29.5 ± 4.1 ms, respectively, as expressed in Fig 5. The mean liver T2* time was higher in the chronic hepatitis group than in the control group. However, there was no statistically significant difference between groups regarding liver T2* time.

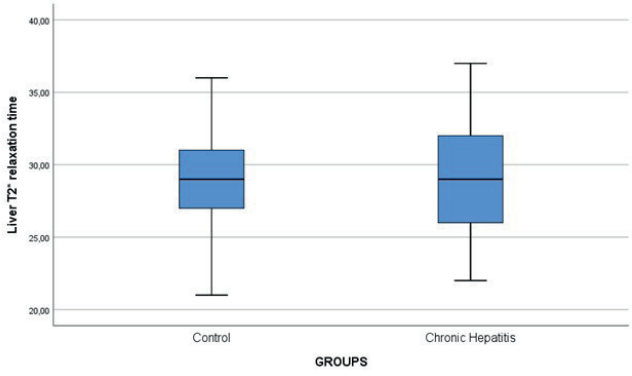

Figure 5. *Liver T2* relaxation time of control group and chronic hepatitis group*

The correlation analysis was performed between age, mean liver size, mean spleen size, mean liver T1 time, and mean liver T2* time. Correlation analysis revealed a statistically significant positive correlation between liver T1 time and spleen size (r=0.447, p<0.001). However, no statistically significant correlation was observed between age and any parameters, including mean liver size, mean spleen size, mean liver T1 time, and mean liver T2* time.

The correlation analysis was conducted on 19 chronic hepatitis cases with liver biopsy results. A positive and significant correlation was found between liver T1 relaxation time and ISHAK score (r=0.683, p<0.001). Moreover, a positive and significant correlation between liver T1 relaxation time and histological activity index score was also observed (r=0.542, p<0.001)

ROC analysis was performed to evaluate the predictive value of T1 relaxation time. The results of ROC analysis indicated that liver T1 relaxation time was statistically significant in differentiating the control group from the chronic hepatitis group (AUC= 0.877 ± 0.037; p <0.001) (Fig. 6). The sensitivity and specificity of liver T1 relaxation time for predicting chronic hepatitis were calculated at different cut-off values. The cut-off value of 661 ms for the liver T1 relaxation time resulted in 76.2% sensitivity and 79.5% specificity. The cut-off value of 678.5 ms for the liver T1 relaxation time resulted in 71.4% sensitivity and 88.6% specificity.

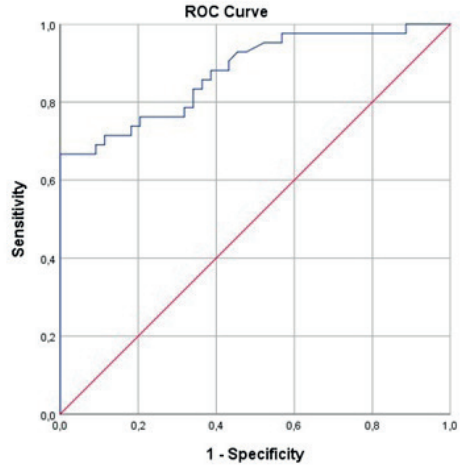

Figure 6. *ROC analysis of liver T1 relaxation time*

4. DISCUSSION

The most important recent advances in MRI tissue quantification include T1, T2, and T2* mapping. Tissue quantification can be performed using the native T1 mapping method without contrast material. T1 relaxation time can reveal important and crucial details about the characteristics of the tissue [14]. T1 relaxation time is known to be elongated by fibrosis, while T2* relaxation time is shortened by iron accumulation [15]. However, recent studies have also shown that fatty liver patients have longer T1 relaxation time [16].

The degree of fibrosis plays a vital role in the treatment plan in patients with the chronic liver parenchymal disease. Several studies reported that non-invasive methods could be used to evaluate liver fibrosis, including MR elastography, diffusion-weighted imaging, MR spectroscopy, MR perfusion, diffusion tensor imaging, and double-contrast MRI. Venkatesh et al., found a strong correlation between MR-elastography-measured liver stiffness and fibrosis stage (r=0.945, p<0.0001) [17]. Huwart et al., reported that the degree of fibrosis (low, moderate, and high) was correlated with liver stiffness, and there were statistically significant differences between the groups (p<0.05) [18].

T1 and T2* relaxation times vary depending on magnetic field strength and device. Cassinotta et al., reported that the liver

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Marmara Med J 2023;36(2): 197-202

T1 time of healthy cases was 500 ± 79 ms tested using 1.5 Tesla MRI [19]. De Bazelaire et al., measured 586 ± 39 ms [20], and Henninger et al., 592 ± 11 ms in the study with 1.5 Tesla MRI [9]. Our study measured the T1 and T2* times of the liver parenchyma in chronic hepatitis and control groups. The mean liver T1 time was 758.4 ± 121 ms in the chronic hepatitis group and 600.2 ± 67 ms in the control group. This finding was similar to those of previous studies reported.

In our study, the mean liver T1 time was 758.4 ± 121 ms in the chronic hepatitis group and 600.2 ± 67 ms in the control group. The difference was statistically significant ($p<0.05$). In the literature, similar findings were reported. Cassinotto et al., reported a statistically significant difference between the control and cirrhosis groups regarding T1 relaxation time, and the liver T1 relaxation time in cirrhosis patients was 690 ± 147 ms. In addition, they used native T1 mapping to investigate liver fibrosis and found that the Child-Pugh degree correlated with liver T1 relaxation time in cirrhosis patients [19]. Banerjee et al., in their native T1 mapping study, found a strong correlation between liver fibrosis and T1 relaxation time ($r=0.68$, $p<0.0001$) [21].

In studies conducted using hepatocyte-specific agents, compairing T1 map measurements obtained pre – and post-contrast can provide information about liver function and predict hepatocyte damage in chronic liver parenchymal disease. Haimerl et al., reported no statistically significant difference between the cirrhosis patients and the control group in terms of precontrast T1 time. However, there were statistically significant differences between the groups in terms of post-contrast T1 time and T1 time reduction rate ($p<0.05$) [22]. In our study, T1 mapping was not performed after applying the contrast material. The results were evaluated with native T1 mapping, and we obtained similar findings to the literature. However, in our study, we could not assess the liver functions of the participants due to the lack of information about the post-contrast T1 relaxation time and the reduction rate of T1 relaxation time.

In the study population, the lowest liver T2* time was 21 ms, and the highest liver T2* time was 37 ms. Chandarana et al., stated that a liver T2* time less than 14 ms indicates hepatic iron accumulation with a 100% sensitivity and 97.3% specificity [23]. In our study, the lowest liver T2* time was 21 ms, and the highest was 37 ms. Thus, our participants had no iron accumulation based on these results. In the literature, studies reporting normal liver T2* time in normal cases were similar to our research. Anderson et al., measured mean liver T2* time as 33 ± 7 ms [24], and Pepe et al., measured it as 25.6 ± 3.4 ms [25] in normal cases.

Despite all these data, our study has some limitations. The first limitation is that the liver biopsy results were unavailable for most cases. Therefore, without biopsy results, we could not make a clear histological definition of the liver parenchyma of the control and the chronic hepatitis group. In addition, the lack of biopsy results prevented us from evaluating the relationship between T1 relaxation time and fibrosis stage in patients with chronic hepatitis. The second limitation is the sample size of our study groups. Therefore, our results cannot be generalized to the general population. Finally, since the T1 relaxation time of the liver can increase due to inflammation, it was impossible to state whether the increase in T1 times in the study population was due to fibrosis. There is a need for studies with a larger sample size with patients with a diagnosis proven by liver biopsy. For all these reasons, new studies should be conducted with a more significant number of cases who underwent a liver biopsy to support the data obtained from our research.

In conclusion, our study found the mean liver T1 relaxation time was longer in chronic hepatitis. In addition, T1 relaxation time and fibrosis grade were found to be correlated. In the literature, only a few studies evaluated liver T1 time in patients with chronic hepatitis. The previous studies supported our findings. Therefore, our study results will contribute meaningful data to the literature. However, there is a need for comparative studies with larger samples and longer terms to suggest the native T1 mapping method as a non-invasive alternative to biopsy in examining liver tissue.

Compliance with Ethical Standards

Ethical Approval: This retrospective investigation was authorized by The Human Research Ethics Council of Ankara Training and Research Hospital, Ankara, Turkey (11.07.2018/525).

Informed consent was obtained from the patients before the MRI examination.

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Endogenous maternal serum preimplantation factor levels in early-onset preeclamptic pregnancies

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ABSTRACT

Objective: Preimplantation factor (PIF) is a new peptide with many potential functions. We aimed to compare the maternal serum PIF levels among early preeclamptic patients with the healthy controls at the same gestational age.

Patients and Methods: Thirty-nine early-onset preeclamptic (< 34 gestational weeks) patients and 45 healthy expecting women were included to our study. Patients with or suspicion of any chronical maternal disease, gestational diabetes, twin pregnancies, fetal or placental anomalies or any other obstetric complication have been excluded. Competitive ELISA has been used to analyze the PIF levels in the collected samples. Gestational age, maternal age, gravida, parity, fetal growth, BMI, maternal weight and height, plasma PIF levels have been collected/measured and analyzed in both groups.

Results: The primary outcome of our study was that PIF was significantly higher in study group than the healthy controls (100.36 ± 41.92 vs. 83.14 ± 51.27. p=0.016).

Conclusion: Preimplantation factor levels were statistically higher in the study group. PIF levels might have a role in the progression and pathogenesis of the preeclamptic patients. Further studies with larger groups have to be planned and performed to reveal the real relation between PIF and preeclampsia.

Keywords: Preimplantation factor, Preeclampsia, Maternal serum

1. INTRODUCTION

Preeclampsia (PE) is a unique disease which occurs during pregnancy. The systemic inflammatory response, the pathogenesis of which stems from the implantation period, causes various clinical symptoms in each patient at the later stages of pregnancy [1]. Although, it has been the subject of many studies, the pathophysiology of PE is still poorly understood. Placental blood flow and remodeling of spiral arteries, imbalance in angiogenic factors and anti-angiogenic state, immune factors and inflammation, low oxygen tension, oxidative stress in gene expression have been the main focus of ongoing studies for years [2].

The preimplantation factor (PIF) is a recently discovered 15 amino acid peptide (MVRIKPGSANKPSDD) released from healthy embryos, thought to play a role in implantation and decidualisation [3]. The role of PIF in implantation has been studied in different experimental and animal models in various studies, and its synthetic version has also been produced as the synthetic preimplantation factor (sPIF). After its introduction by Barnea, as a novel peptide secreted as early as the 2-cell stage of viable mammalian pregnancies [4], its potential therapeutic effects and the role of its endogenous secretion have been evaluated in pregnancy related/reproductive diseases such as recurrent pregnancy loss, PE and endometriosis [5-7].

The preimplantation factor has proven effects on regulating local and systemic immunity, embryo adhesion – decidualisation improvement and trophoblast invasion enhancement [8]. The immunomodularity effects of PIF and its synthetic analog also brought up the question that it can be utilized in the treatment of different autoimmune diseases other than pregnancy related diseases [9]. Muller et al., studied the analogous sPIF which may promote neuro-protection in rodent models of experimental

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autoimmune encephalomyelitis and prevent perinatal brain injury [10].

Based on the data that PIF targets Kv1.3β – cortisone and causes a similar effect to cortisone according to Dr. Eytan Barnea, its role in the receptivity of a semi-allegenous or-in cases of donor pregnancies – allegenous embryo is better understood. It has been also shown by the same study that sPIF admission potentialized embryo protection and development by preventing oxidative stress and protein misfolding in embryo cultures [11].

Due to its various effects on immune-receptivity, PIF is found in the maternal circulation of bovine models by using chemiluminescent PIF ELISA. It was detected shortly after artificial implantation, by day 20, and correlated 100% with live pregnancy outcomes. On the other hand, its absence correlated at 100% with a non-pregnant status. By using anti-PIF monoclonal antibodies, Barnea et al., found that endogenous PIF is expressed mostly in the trophoblastic layer of bovine placenta [12]. The study conducted by Moindjie et al., in 2014, strengthened the theory that PIF is also secreted in human first-trimester placentas, to a lesser extent till the third-trimester human placentas. Their further achievement was the observation that PIF is localized in the syncytiotrofoblasts and extravillous-trofoblasts as evidence of the effects of PIF on the human placenta endocrine function [13].

In a more recent study, Dos Santos et al., evaluated the effects of sPIF on the endometrial stromal cell function, and found that it significantly upregulates the mRNA expression of IGFBP-1 and connexin-43, and prolactin secretion, which is essential in the decidualisation of human receptivity and a favorable pregnancy outcome [14].

Consequently, as current data strongly demonstrates, endogenous PIF secretion plays an important role in human placentation. PIF affects various steps, such as its role against oxidative stress, its promotion of implantation and trophoblast invasion and modulation of immune response. As critical as these factors are for implantation, they shape and cause PE pathogenesis and evaluation [7].

Considering its functions, the maternal serum levels of endogenous PIF levels and its response to preeclampsia is still not defined. Besides the potential therapeutical effect of synthetic PIF, endogenous PIF levels might play a role in the diagnosis and evaluation of preeclamptic patients. That is why; we conducted a case-controlled observational study to compare the endogenous PIF levels of early preeclamptic patients and healthy controls at the same gestational age. Our aim is to measure the PIF levels and define –if there is any – clinical correlation with the PE manifestation.

2. PATIENTS and METHODS

This study started with the approval of the local Clinical Research Ethics Committee, decision number 54 dated 10.03.2017. The study was carried out between March 2017 and October 2017 in our tertiary perinatology clinic.

Patient Selection

Preeclampsia has two major clinical presentations, early-onset and late-onset PE [15]. Its early-onset presentation is thought to be more related with the placental implantation and immunologic maladaptation [16]. Considering PIF's effect on placental invasion and immune modulation, we included patients who were diagnosed with early-onset PE in this study for study population. Early onset PE is defined as the onset of maternal hypertension and proteinuria after 20 and before 34 weeks of gestation (systolic blood pressure ≥140 mm Hg; diastolic blood pressure ≥90 mm Hg; spot urine protein/creatinine ratio ≥0.3).

The control group was chosen from patients with healthy pregnancies. All control cases had negative diabetes screening, normal amniotic fluid index and estimated fetal weight appropriate for gestational age.

Pregnant women diagnosed with polyhydramnios and anhydramnios, pregnant women diagnosed with diabetes mellitus and systemic medical disease were excluded from the study. After collection of samples, control cases were followed up until delivery. Samples of patients with any other obstetrical complication (preterm birth, antepartum bleeding etc.) or lost in follow-up were excluded.

The study was conducted in a tertiary center. Most of the cases diagnosed with PE included in the study were referred from an external center. Betamethasone (Celestone™) treatment was started in an external center in some of these cases who applied to our hospital. Although, at the beginning of the study it was planned to collect blood samples at the time of the patient's admission to the hospital, as the study progressed, it was decided to collect blood samples after the second dose of antenatal steroid so that all blood was collected at a similar time. Only 2 patients' samples were taken after the first dose, because they had emergency caesarean section for placental abruption.

In the literature review, no scientific article was found mentioning maternal serum PIF levels. Also, data on maternal serum PIF values in normal healthy pregnant women could not be obtained from the kit supplier regarding the examination of PIF values in maternal serum. (Elabscience Biotechnology Co™). Although, this makes the study unique, it makes it difficult to evaluate the results. After receiving the results of PIF values in our control group in pregnant women who were not diagnosed with PE, we assumed these values as normal and calculated 30% difference (positive or negative) with 80% power and p values smaller than 0.05 significant, we found out a sample size of 33 patients for each group (Confidence interval CI: 95%, Power 80%).

Informed consent was obtained from all participants.

Sample Collection

In order to ensure standardization in the study group, blood was taken from the patients in two separate 2 mL citrate tubes, after the betamethasone (Celestone™) doses were applied. Only 3 patients were delivered by emergency caesarean section due to the seriousness of their clinical situation. Blood sampling

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had to be done after 1 dose of betamethasone. Blood samples of the control group patients were taken in the outpatient clinics of our hospital. The collected blood samples were centrifuged at 1000 RPM for 30 minutes in a cold centrifuge device (-9 °Celsius). Plasma samples were collected and placed in 2 separate Eppendorf tubes. The tubes were labeled with the patient's name and case number, and then stored at – 40 °Celsius. In the hourly monitoring chart of the freezer temperature, it was observed that the temperature did not rise above – 39 °Celsius during the entire storage period.

Competitive PIF ELISA

Competitive ELISA (Elabscience Biotechnology Co™ USA) method was used to evaluate the collected plasma samples. 1000pg/mL standard solution included in the kit was diluted 15 minutes before the procedure and prepared in 8 different concentrations. 50-µL microliter samples taken from plasma samples stored in Eppendorf tubes were placed in a microplate. Biotinylated antibody was diluted to 50 µL microliters and added to all plates. It was then put in an incubator at 37 °C for 45 minutes and watched for antigen-antibody conjugation. After that automatic washing was made three times, 100 µL microliters of horse radish peroxide (HRP) (Horse Radish Peroxidase, Elabscience Biotechnology Co™ USA) conjugate was diluted and pipetted. It was put in the incubator again at 37 °C for 30 minutes. Afterwards, automatic washing was performed 5 times. After adding 90 microliters of substrate, samples were placed in an incubator at 37 °C for about 15 minutes to facilitate coloration reaction. Following the addition of the stop solution (Elabscience Biotechnology Co™) to stop the reaction, a spectrophotometric reading was performed at 450 nm wavelength. Values calculated according to standard chart (Figure 1) were reported as ng/mL (Nano grams per milliliters).

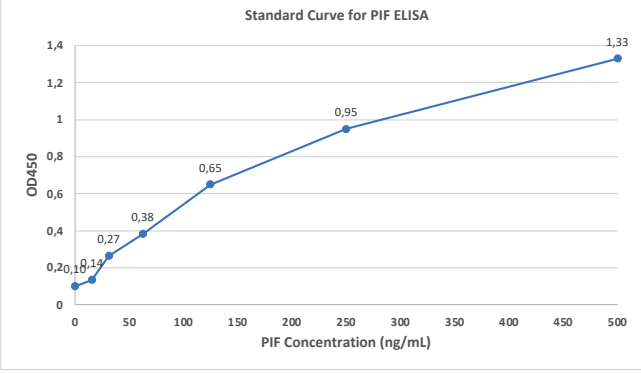

Figure 1. *Standard Curve for Competitive Preimplantation Factor (PIF) ELISA*

Statistical Analysis

Statistical analyses were performed using SPSS™ version 17.0 package software. The correlation between variables was analyzed using the Spearsman's rho correlation test. Descriptive analyses and categorical variables were given using the mean and standard deviation range. Variables not normally distributed were compared using the Mann-Whitney U test. For categorical variables, comparisons between groups were made using Chi-Square-Fisher tests. P-values less than 0.05 ($p<0.05$) were interpreted as statistically significant.

3. RESULTS

Considering the inclusion criteria in our study, 39 cases were included in the study group and 45 cases were included in the control group out of a total of 110 samples collected for evaluation. Age, body mass index (BMI), gravidity, parity, gestational age (GA), estimated fetal weight (EFW) and maternal serum PIF values were evaluated in all groups. Gestational age and EFW are given in correlated days for better explanation and statistical analyses. The results of these parameters are shown in Table I.

Table I. *Evaluation of Descriptive Variables between the groups*

Variable	Study (N1=39)	Control (N2=45)	p
Age	31.03 ± 6.46	29.29 ± 6.18	0.202
Gravida	2.44 ± 1.93	1.96 ± 1.22	0.41
Parity	1.05 ± 1.34	0.53 ± 0.79	0.088
BMI	*30.2 ± 3.88*	*27.2 ± 4.14*	*0.001[a]*
Gestational Age	214.64 ± 20.38	209.44 ± 24.1	0.348
EFW	205.46 ± 21.71	207.18 ± 22.94	0.713

a: Statistically significant, EFW: Estimated Fetal Weight in correlated days, BMI: Body Mass Index
All values are given as Mean ± Standard Deviation p-value less than 0.05 is considered to be statistically significant

Age, gravidity, parity, GA and EFW were similar between the preeclamptic patients and healthy controls (p=0.202, p=0.41, p= 0.088, p=0.348, p=0.713, respectively, all p>0.05).

The mean ± std BMI was 30.2 ± 3.88 kg/m2 in the study group (pregnant women diagnosed with PE) and 27.2 ± 4.14 kg/m2 in the control (healthy pregnant women) group and a statistically significant difference was found between the two groups in terms of BMI (p= 0.001 p< 0.05).

When the groups were evaluated in terms of maternal serum PIF values, mean maternal serum PIF value was statistically significantly higher in the study group (100.36 ± 41.92 vs. 83.14 ± 51.27 p=0.016) (Table II).

Table II. *Comparison of PIF Value Between Groups*

	Study (N1=39)	Control (N2=45)	
PIF	100.36 ± 41.92	83.14 ± 51.27	0.016[a]

a: Statistically significant, PIF: Preimplantation Factor

When the common complications of PE in the study group were evaluated; fetal growth restriction (FGR) was found in 15 cases (38.46%), abruptio placenta in 2 (5.1%), progression to HELLP syndrome in 3 (7.6%) cases. Doppler ultrasonography

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Marmara Med J 2023;36(2): 203-209

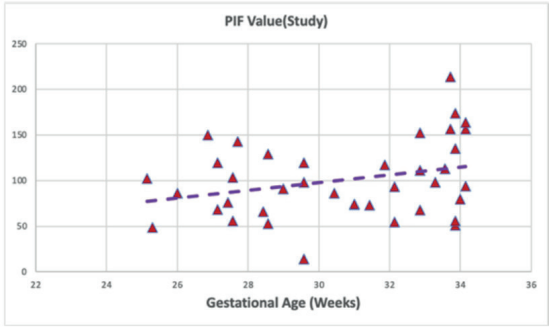
evaluation revealed bilateral uterine artery notch in 15 (7.6%) of 39 patients of cases (Table III).

Table III. *Distribution of Common Complications in Preeclampsia in the Study Group*

Variable	N	Percentage
FGR	15/39	38.5%
Ablatio Placenta	2/39	5.1%
HELLP	3/39	7.7%
Bilateral Uterine Artery Notch	15/39	38.5%

FGR: Fetal Growth Restriction, HELLP: Hemolysis Elevated Liver Enzymes Low Platelets Syndrome

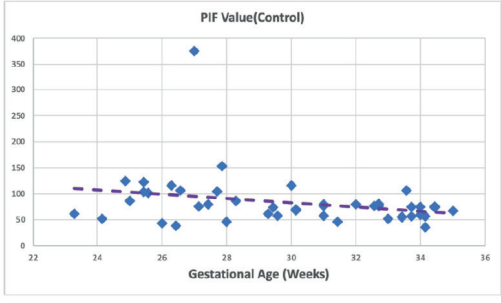
The distribution of maternal serum PIF values through GA in weeks for both groups is given in Figure 2 and 3. Cases diagnosed with PE are marked in the figure as triangles (Figure 2), and healthy controls are shown as diamonds (Figure 3). The interrupted line shows the trend through GA in weeks.



Interrupted line shows the trend in gestational age in weeks.

PIF: Preimplantation Factor

Figure II. *PIF Values of Preeclamptic Patients and Distribution through Gestation*



Interrupted line shows the trend in gestational age in weeks

PIF: Preimplantation Factor

Figure III. *PIF Values of Healthy Controls and Distribution through Gestation*

Correlation between maternal serum PIF and GA, EFW and maternal weight are shown in Table VI. In the analysis performed, there was no significant correlation between maternal serum PIF values and GA (p=0.097 p > 0.05) in the study group, whereas a significant and inverse (negative) correlation was observed between maternal serum PIF and GA in the control group. When all cases were evaluated, no statistically significant correlation was found between GA and maternal serum PIF (p=0.642 p > 0.05).

Table IV. *Correlation between PIF-Gestational Age, Fetal Weight and Maternal Weight*

		Study (N1=39)	Control (N2=45)	All groups (N=84)
Gestational Age	rho	0.27	-0.337	-0.051
	p	0.097	0.023[a]	0.642
EFW	rho	0.347	-0.35	0.047
	p	0.03[b]	0.018[a]	0.67
Maternal Weight	rho	-0.038	0.045	0.153
	p	0.817	0.767	0.166

a: Significant negative correlation, b: Significant positive correlation, EFW: Estimated Fetal Weight, p: p-value less than 0.05 is considered to be statistically significant rho: Spearman correlation between two variables

The correlation of maternal serum PIF value with EFW is also in Table IV. In the analysis performed, a significant and positive correlation was observed between maternal serum PIF values and EFW in the study group (p=0.03 p < 0.05), while a significant and inverse (negative) correlation was observed between maternal serum PIF and EFW in the control group as it was with GA. When all groups were evaluated, no statistically significant correlation was found (p=0.67 p > 0.05).

There was a predictable difference between the BMI values of the 2 groups. In the analysis made for a more valid explanation; there was no statistically significant correlation between maternal serum PIF values and BMI in the study group, control group and all cases (respectively p=0.817, p=0.767, p= 0.166, p>0.05 for all) (Table V).

When the maternal serum PIF values of the patients diagnosed with PE with and without bilateral notch in the uterine artery and/or FGR were compared, no statistical significant difference was found (p=1.00,p=0.2.respectively) (Table V).

Table V. *Correlation of Notch in Bilateral Uterine Artery Doppler and Fetal Growth Restriction with PIF Measurements*

	BUAN	N	Mean ±SD	P
PIF	+	15	102 ±43.67	1
	-	24	100 ±39.22	
	FGR	N	Mean ±SD	P
PIF	+	15	89.82 ±45.97	0.2
	-	24	106.94±38.71	

BUAN: Notch in Bilateral Uterine Artery Doppler, FGR: Fetal Growth Restriction

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Marmara Med J 2023;36(2): 203-209

4. DISCUSSION

The study was carried out in 2 groups consisting of healthy pregnant women and pregnant women diagnosed with early onset PE at similar gestational weeks. Considering demographic characteristics the study group was in the range of "obese" description and the mean value of the control group was in the "overweight" group [17]. Obesity alone is a risk factor for PE and obese women are at a higher risk for developing PE [18]. A local study among 986 pregnancies calculated the mean ± std for maternal weight in the third trimester 28.2 ± 4 and stated a rise of 4.6 in the BMI [19]. Pregnant women with PE tend to gain more weight cause of due to the oedema resulting from systemic vascular inflammation. The Norwegian fit for delivery trial shows a significant weight gain and a rise in the total body water in the third trimester in pregnancies diagnosed with PE when compared to the healthy ones [20]. These explain well why we have overweight patients even in the control group and correlates with our results in the controls.

Maternal serum levels of some pregnancy associated proteins and their associations with obesity have been studied before. Maternal serum Alpha-fetoprotein levels are lower in pregnant women with higher maternal weight than in those with normal maternal weight [21]. Obese pregnant women have lower maternal serum levels of human chorionic gonadotropin, pregnancy associated plasma protein-A, unconjugated estriol, most probably due to higher plasma volume and its dilutional effects [22]. For a better analysis we performed a correlation analysis between maternal weight and maternal serum PIF levels in all 84 cases. No significant correlation was found between maternal serum PIF value and maternal weight in both the study, control group and also in evaluation of all cases (respectively p=0.817, p=0.767, p= 0.166, p>0.05 for all) (Table IV). We found a significantly higher level of maternal serum PIF in the study group.

We found that maternal serum PIF levels become lower in healthy controls with advancing gestational age (r= – 0.337, p=0.023, p<0.05) (Table IV). This negative correlation is parallel to the findings of the study performed by Barnea et.al. [7]. In this study, correlation statistical analyses showed that the downward trend changed to an upward trend in the pregnant group diagnosed with PE. (r= 0.270, p=0.097, p>0.05) (Figure 2). This may support the altered maternal serum PIF level in PE group. The same shift in the downwards trend was calculated significantly between the correlation of EFW and PIF levels. (r= – 0.350, p=0.018, p<0.05 vs. r= 0.347, p=0.03, p<0.05) (Table 4).

Since, PIF plays a regulating role in the immunomodulation, its levels might be rising in PE due to the marked inflammation. Many pro-inflammation factors such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and Interleukin-6 are found to be elevated in PE [23]. This may be an explanation for the significantly high maternal serum PIF levels in the PE group. Simone et al., conducted a study on female Swiss mice, they modelled a lipopolysaccharide (LPS) induced placental inflammation and measured endogenous PIF levels and 22 cytokines/chemokines. They found that LPS induced inflammation causes a rise in placental PIF expression and results in increased levels of TNF-α (prime pro-inflammatory cytokine), growth related oncogene (GRO: neutrophil-attractive chemokine) and Interleukin – 18 (an inflammasome-related cytokine). This correlates with our findings in the PE group. They also add synthetic-PIF (s-PIF) and it was shown that s-PIF reversed the inflammatory response [24].

The one and only study conducted on human placenta samples related to PIF was carried out by Moindjie et al.[25]. By using PIF immunostaining, they compared placenta samples obtained from 8 normal pregnancies with samples of 8 pregnancies with diagnosis of FGR and 4 pregnancies with diagnosis of PE. They found lower PIF protein expression in normal third-trimester than in first-trimester placental villis. However, relative quantification of PIF by immunostaining indicated that PIF protein staining was lower in FGR and PE samples than in third-trimester control samples. This conflicts with our finding of higher maternal serum PIF levels in the PE group. The theoretical reason for that might be an altered/damaged trophoblastic layer due to inflammation which may raise the maternal serum levels, but lower the placental PIF protein content. This may even support our result, since we evaluated two very different samples. If we refer to the AFP example mentioned earlier, while its levels in fetal plasma and amniotic fluid starts to decline after the first trimester, the maternal serum levels of AFP keep increasing till the end of 35th week of gestation [26].

In our study, no statistically significant difference was found between FGR, BNUA and PIF levels. Considering the sample size of our study and the number of cases complicated with FGR, HELLP and abruptio placentae, it may have been insufficient to obtain a meaningful result.

The effect of corticosteroid injections on PIF levels remains unclear. There are no data concerning this issue. We collected the blood samples just after the antenatal steroid administration, but the maximum effects of betamethasone occur after 24-48 h of administration [27]. Secondly, considering other placental proteins like human placental lactogen and other placental steroids, antenatal steroid administration does not seem to effect placental lactogen levels even after 48 h of administration [28], and even lowers the levels of other placental steroids [29]. Considering its vital benefits [27], the antenatal steroid was not delayed in our patient group. An animal model might be useful for the further investigation of the possible effects of betamethasone on serum PIF levels.

Placental abruption occurred in 2 pregnant women included in the study group. The serum PIF values of these two pregnant women were found to be remarkably low. Their samples were collected just before emergency caesarean section, one patient had 14.1 ng/ml and the other one 48.7 ng/mL. These results are quite low considering the average serum PIF level of study group 100.4 ± 41.9. Since, there is no data considering the clearance and distribution of PIF in maternal serum, it is hard to interpret but given the fact that PIF is released by only living cells, this alteration can be regarded as an expected result [25].

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Limitations

Our study has some limitations;

1. There was no research funding and the study was only supported by the research team, limiting the size and power of this study,
2. For Competitive ELISA (Elabscience Biotechnology Co™ USA) the commercial manufacturer did not provide large scale validation results, lowering the reliability of our results,
3. Finally, as mentioned in the methods, we collected our blood samples after the corticosteroid injections. Although, not considered important, since PIF targets Kv1.3β – cortisone target, this may have altered our results.

Conclusion

We found higher maternal serum PIF levels in the preeclampsia group. Despite its limitations, our study is the first work regarding PIF levels in maternal serum. Larger scaled and multicenter studies may reveal the true connection between PIF and pregnancy complications. This can also shed light on the studies on the use of sPIF in the prevention and treatment of obstetric complications.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Zeynep Kamil Training and Research Hospital, Clinical Research Ethics Committee with the decision number 54 dated 10.03.2017. T

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Authors' Contributions: MAO and HAT: Concept and design of the study, MAO, HAT and EK: Acquisition and analysis of data, MAO, HAT and EK: Drafting the manuscript, tables and figures. All authors read and approved the final version of the article.

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Incidental findings detected on magnetic resonance imaging scans of the cervical, thoracic and lumbar spine of patients prediagnosed with discopathy

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ABSTRACT

Objective: To determine the frequency and types of incidental findings on magnetic resonance imaging (MRI) scans of the cervical, thoracic, and lumbar spine in patients with intervertebral discopathy.

Patients and Methods: This retrospective study included 1000 patients (513 females and 487 males, with a mean age of 50.5 years) with clinically suspected intervertebral discopathy who underwent MRI. Any abnormal findings and congenital anomalies/anatomical variations unrelated to the primary complaint were referred to as incidental findings. Frequency distributions of the assessed imaging characteristics were calculated.

Results: Of the 1000 patients, 192 (19.2%) patients were presented with incidental findings. The positive findings in the thoracic spine (26%) were higher than those in the lumbar (19.8%) and cervical spine (13.7%). The study found vertebral haemangioma to be the most common finding, followed by Schmorl's nodes in the thoracic and lumbar spine. Thyroid nodules constituted the most common finding in the cervical spine, followed by vertebral haemangioma. Renal cysts in the thoracic and lumbar spine and thyroid nodules in the cervical spine were the most frequent extraspinal findings.

Conclusion: Incidental findings are commonly detected during MRI examination of intervertebral discs, and most are benign findings. However, incidental findings including clinically essential findings can alter the patient's treatment or affect the patient's life. Therefore, it is crucial to systematically evaluate MRIs without focusing solely on the spine and report incidental findings detected on MRI.

Keywords: Incidental findings, Magnetic resonance imaging, Discopathy

1. INTRODUCTION

The term "incidental findings" (IF) refers to lesions detected incidentally during radiological assessments that are unrelated to the patient's primary complaint [1]. To detect spinal disorders, magnetic resonance imaging (MRI) is usually used. This instrument may reveal a clinically insignificant incidental abnormality or a significant non-spinal lesion explaining the patient's symptoms. The images for reporting purposes are commonly magnified around the vertebral column cropping out much of the structures within, the neck, back, and waist. While this procedure increases the probability of detecting spinal pathologies, it ignores possible extraspinal pathologies. Given how straightforward it is to create reconstructions with a wide field of view that includes these structures, the authors looked at the frequency and kind of extraspinal incidental findings (ESIF) that have been recorded [2].

An increased number of findings have been observed in spinal MRIs after the image archiving and communication system established for image evaluation in most hospitals became operational [3, 4]. In the daily practice of radiologists, it is reported that lesions detected incidentally in spinal MRI examinations are very high [1, 5]. Although, there are studies related to IFs in lumbar spinal MRIs in the literature, very few

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studies are associated with IFs detected in cervical and thoracic spinal MRIs [4, 6, 7].

In this study, we sought to emphasize the type, prevalence, and clinical importance of incidental findings in the lumbar, cervical, and thoracic MRI scans for intervertebral disc disease.

2. PATIENTS and METHODS

In our study we evaluated radiological images of 1000 patients with clinically suspected intervertebral discopathy who underwent MRI between 01/10/2020 and 01/10/2021 (cervical, thoracic and lumbar). A radiologist re-evaluated the spinal MRIs (cervical, thoracic, and lumbar spinal). Patients under the age of 18, those with recent acute trauma, known malignancies, a prior history of spinal surgery, and those with incomplete or subpar MR images were not included in the study. The local Institutional Review Board approved this retrospective study protocol. The study had no requirement for informed patient consent.

In this study, we defined any abnormal finding not related to the primary complaint as IF. IFs were defined separately according to the cervical, thoracic and lumbar regions: cervical (pituitary mass, perineural cyst, cerebellar hernia, schmorl's nodule, lymphadenopathy, syringomyelia, thyroid nodule and vertebral haemangioma), thoracic (lung mass, renal mass, hepatic haemangioma, stomach tumour, gallbladder stone, oesophageal disorder, nodule, syringomyelia, vertebral haemangioma and Tarlov cyst), lumbal (abdominal artery aneurysm, horseshoe kidney, renal stone, bladder stone, gallbladder stone, cysts and masses in the urogenital organs, retroperitoneal mass, schmorl nodule, syringomyelia, vertebral haemangioma and Tarlov cyst). Clinically significant findings (E3 and E4 according to a modified CT Colonography Reporting and Data System (C-RADS) classification), anatomic variations (C-RADS E1) and benign conditions (C-RADS E2) were noted during the review of the reports [8].

Statistical Analysis

All measurable data were summed up in a comparison table. Descriptive analysis was applied using the Statistical Package for the Social Sciences version 20 for Windows (IBM Corporation, Armonk, NY, USA).

3. RESULTS

Magnetic resonance images of a total of 1094 patients with clinically suspected intervertebral discopathy, were retrospectively evaluated. Nine of these images were excluded from the analysis because the patients were under the age of 18. 13 were excluded due to low-quality or incomplete imaging; while another 13 were excluded due to acute trauma. Additionally, 59 patients were excluded because they had either previously undergone spine surgery or had a known malignancy.

Out of a total number of 1000 patients, 487 were men (48.7%), and 513 were women (51.3%). The mean age of patients in our study was 50.5 years, ranging between 18 and 88 years. Overall, 192 patients (19.2%) had incidental findings. The mean age of the patients with IFs was 48.4 ± 14.7 years. The percentages of IFs on the cervical, thoracic, and lumbar spinal MRI were 13.7%, 26% and 19.8%, respectively. The study found high positive findings in the thoracic spine than those in the lumbar and cervical spine. Vertebral haemangioma and Schmorl's nodes in the thoracic and lumbar spines were the two most frequent findings. Thyroid nodules were the most typical discovery in the cervical spine, followed by vertebral haemangiomas (Table I, Table II, Table III). The most common extraspinal finding was renal cysts in the thoracic and lumbar spine and thyroid nodules in the cervical spine (Table I, Table II, Table III). The percentages of clinically significant findings on cervical, thoracic, and lumbar spinal MRI were 2%, 1.5% and 1%, respectively. In 77 cases, discopathy was not detected by MRI. 74 of these 77 patients had no findings, while 3 showed IF.

Concerning the lesions C RADS E4, only two aortic aneurysms, one adnexal mass, one endometrial thickening and 1 bladder wall thickening were found.

Table I. *Incidental Findings of the Lumbar Spine on MRI scans*

	Incidental Findings	Frequency (n)	Percentage (%)
Spinal Findings	Vertebral Hemangioma	40	8
	Schmorl's Nodule	15	3
	Perineural Cyst	4	0.8
	Syringomyelia	1	0.2
Urinary System Findings	Horseshoe Kidney	4	0.8
	RenaL Cyst	11	2.2
	Renal Stone	2	0.4
	Bladder Calculus	1	0.2
	Bladder Diverticulum	1	0.2
	Bladder Wall Hypertrophy	1	0.2
	Chronic Cystitis	1	0.2
Genital Organ Findings	Adnexal Mass	1	0.2
	Adnexal Cyst	2	0.4
	Uterine Myoma	7	1.4
	Ovarian Cyst	1	0.2
	Nabothi Cyst	2	0.4
	Endometrial Hyperplasia	1	0.2
Other	Retroperitoneal Mass	1	0.2
	Abdominal Aortic Aneurysm	2	0.4
	Cholelithiasis	1	0.2
	No Lesion Detected	401	80.2

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Table II. *Incidental Findings of the Cervical Spine on MRI scans*

Incidental Findings	Frequency (n)	Percentage (%)
Vertebral Hemangioma	9	3
Schmorl's Nodule	3	1
Perineural Cyst	2	0.6
Syringomyelia	6	2
Thyroid Nodule	14	4.6
Cervical Lymphadenopathy	1	0.3
Cerebellar Hernia	4	1.3
Pituitary Mass	2	0.6
No Lesion Detected	259	86.3

Table III. *Incidental Findings of the Thoracic Spine on MRI scans*

Incidental Findings	Frequency (n)	Percentage (%)
Vertebral Hemangioma	24	12
Schmorl's Nodule	8	4
Perineural Cyst	2	1
Syringomyelia	6	3
Lung Mass	2	1
Esophageal Dilatation	1	0.5
Gastric Cancer	1	0.5
Liver Hemangioma	3	1.5
Cholelithiasis	1	0.5
Renal Cyst	4	2
No Lesion Detected	148	74

4. DISCUSSION

MRI is frequently used to evaluate patients with neck, back and low back pain. Benign lesions are the IFs commonly seen on MRI scans [4]. However, it is unclear how these IFs affect human health [1, 3, 4]. Sometimes these IFs may be a more serious disease finding than the preliminary diagnoses that lead to an MRI request, and further investigations may be required [3, 9].

There are several studies in the literature about the IFs detected in lumbar MRI examinations. For instance, Park et al., detected 107 (8.4%) IFs in 1268 patients who were thought to have lumbar disc herniation [4]. Eroglu et al., found IFs in 82 (13.3%) of 613 patients who underwent lumbar MRI, considering that they had lumbar discopathy [10]. Ibrahim et al., reported 90 (22.5%) cases had incidental non-spinal findings in 400 patients [11]. In our study, IFs were detected in 19.8 % of cases submitted to lumbar MRI for low back pain (12 % spinal, 7.8% extraspinal). The rates of IFs differ for a variety of reasons. In the study of Park et al., only spinal IFs were investigated. Eroglu et al., investigated spinal and extraspinal lesions, while only extraspinal lesions were evaluated in the other two studies. In addition, while some findings such as hip lesions, prostatic enlargement, fluid in the Douglas cavity were included as IFs in some studies, they were not included as IFs in other studies referans .

In our study, the most common IFs in the lumbar region were spinal IFs. This is inconsistent with the findings of Park et al., who found fibrolipoma as the most common lumbar IF [4]. We did not find any fibrolipoma in the lumbar region in our study. Consistent with the study of Eroğlu et al., and Sobhan et al., the most common IF in the lumbar region was vertebral haemangioma [10, 12]. The frequency of vertebral haemangiomas was determined in the study of Barzin and Maleki to be 9.5% in autopsy reports, which is compatible with our findings (8%) [13]. Since, vertebral haemangiomas are age-related, the difference in the mean age of patients in the studies may explain these inconsistent rates. Tarlov cysts detected in our study were not associated with the patients' symptoms. Tarlov cysts predominated in the younger group, and the incidence of the lesion in our study was found to be 0.8%. In previous studies, researchers reported a 1–3.5% incidence [10, 14]. In our study, we found Schmorl's nodule in 3% of the patients and asymptomatic syringomyelia in only one.

Several studies have reviewed the frequency of incidental extra spinal findings on lumbar spine MRI scans. Variable prevalence of ESIF in the range of 8.1-68.8% has also been reported among different age groups [1, 3, 8, 15, 16]. In our study, this rate was 7.8%, which was lower than the percentages noted in previous studies. This is because some findings such as pelvic fluid, uterine septation defects, uterine cavity dilation, lymphadenopathies less than 1 cm and fibrinoids are not included. The majority of our ESIFs were renal in origin followed by genital organ pathologies. This is consistent with the studies of Tuncel et al., and Zidan et al. [6, 11]. Contrary to our study, Ibrahim et al., reported that most of the ESIFs were of the uterus and ovarian origin [15]. Simple renal cysts are the most common lesions in the kidney, which usually do not show clinical findings. They are seen incidentally because of radiological examinations. However, they rarely require treatment. According to Erolu et al., and Sobhan et al., incidence rates of renal cysts were 2.2% and 2.9%, respectively [5, 10, 12]. Our findings on the prevalence of renal cysts (2.2%) are in line with their findings. In contrast to a study conducted by Ciezanowski et al., the prevalence of renal cysts was found to be 25.1% [5]. Our study found uterine myoma (1.4 %) as the second most common ESIF. Most uterine myomas are benign and do not cause any problems. In different studies, the prevalence of uterine fibroids ranging from 2.6 to 4.5% has been reported [6, 11, 15, 17]. The difference in prevalence rates could be due to different sample sizes and female/male ratios in these studies. Four cases (0.8%) of horseshoe kidneys were registered as an incidental extraspinal congenital anomaly. Although, it is usually asymptomatic, it may be associated with some syndromes such as Turner syndrome [18].

Some ESIFs have significant clinical importance and require further examination and treatment. 5 (1%) ESIFs (C RADS E4), which are clinically significant were found in the current study, including 2 aortic aneurysms, 1 adnexal mass, 1 thickening of the endometrial, and 1 thickening of the bladder wall. An abdominal aortic aneurysm can cause life-threatening complications. Adnexal mass, endometrial thickening, and bladder wall thickening can signify cancer.

While many studies on the lumbar region are related to IFs detected on MRI, incidental findings in the cervical and thoracic spine have not been as extensively studied in the literature. Since, the liver, ovaries, uterus, kidneys, and vascular structures are

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Marmara Med J 2023;36(2): 210-214

located in the lumbar region, incidental extraspinal pathologies are more common in MRI of the lumbar region [19]. In our study, we detected IFs (spinal and extraspinal) most frequently in the thoracic spine, while we detected ESIFs more frequently in the lumbar spine. In the current study, 41 (13.6%) out of 300 patients were found to have IFs on MRI scans of the cervical spine (20 spinal, 21 extraspinal). The most common incidental pathologies in cervical spinal MRI are thyroid nodules [20]. In this study, the prevalence of thyroid nodules was recorded as 4.6% (n=14). We also noticed 6 (2%) clinically significant ESIFs: four cerebellar hernias and two pituitary masses. In addition, we saw clinically significant ESIFs more frequently in the cervical region than the thoracic or lumbar, at 2%, 1.5%, and 1%, respectively. These abnormalities may result in persistent head and neck pain, even though the patients' spine MRIs may be perfectly normal. Chronic neck pain commonly requires the use of an MRI for diagnosis. To prevent missing uncommon causes of persistent head and neck pain, cervical MRIs should be thoroughly evaluated.

According to our results, 52 (26%) out of 200 patients were found to have IFs on MRI scans of the thoracic spine. In three studies, thoracic IF rates were reported as 13.3%, 10.5% and 4.7%, respectively [7, 17, 21]. The rates of thoracic spine IFs were higher in our study as compared to findings in the existing literature. This might be because our study focused on all age groups. Nevertheless, considering Ramadorai et al., who focused on paediatrics groups, and the number of cases examined by Zidan et al., were still relatively low compared to our study [7, 21]. In addition, spinal findings such as vertebral haemangioma, Tarlov cysts and syringomyelia were not included in the study by Zidan et al., and Dilli et al., [7, 17]. This rate also was higher than IFs detected in the cervical and lumbar regions. However, only 12 of these findings were extraspinal, and three of them (one stomach cancer, two lung masses) were of clinical significance.

The major limitation of this study is its retrospective nature. Another limitation is the small number of cases in the thoracic region compared to the lumbar and cervical regions. Nevertheless, despite these limitations, the study's strengths are its relatively large patient population and comprehensive investigation of the cervical, thoracic, and lumbar regions.

In conclusion, reporting IFs detected in MRI may enable early diagnosis and treatment of a serious disease of the patient and prevent unnecessary further investigations. It is essential to systematically evaluate MRIs without focusing solely on the spine and report incidental findings detected in MRI, whether they are associated with the patient's pre-diagnosis for MRI. Also, it is necessary to learn about the frequency of incidental lesions, manage them, and determine their impact on patients' lives.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Clinical Research Ethics Committee of Kocaeli Derince Training and Research Hospital, University of Health Sciences (date: 25.11.2021, number: 2021-122).

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Authors' Contributions: KSS, GS: Surgical and medical practices, KSS, GS and HH: Concept, KSS, HH and SMA:Design, GS:Data collection or processing, HH, SMA and OM: Analysis or interpretation, HH, SMA and OM: Literature Search, KSS, OM: Writing. All authors approved the final version of the manuscript.

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Healing effects of L-carnitine on experimental colon anastomosis wound

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ABSTRACT

Objective: The purpose of this study is to examine the effects of L-carnitine on healing of experimental colon anastomosis injury in early and late period.

Materials and Methods: Forty female Wistar-Albino rats were used in this study. The rats were divided into 4 groups (CONT-3, CONT-7, CARN-3, and CARN-7). Injury healing was evaluated for CONT-3 group on the 3rd day and for CONT-7 group on the 7th day following the anastomosis. Following the operation, CARN-3 and CARN-7 groups were intraperitoneally administered with 100 mg/kg/day L-carnitine and injury healing was evaluated on the 3rd and 7th days. Injury strength, histological evaluation and antioxidant enzyme activities and oxidant damage were determined in tissue samples of anastomosis area.

Results: Bursting pressure levels and histological scoring values of CARN-3 group were found to be higher than the CONT-3 group ($p<0.05$). Antioxidant enzyme activities were found to be high in groups which were administered with L-carnitine, and oxidant damage was found to be significantly low in CARN-7 group ($p<0.05$).

Conclusion: It was seen that L-carnitine speeds up the injury healing process and increases the injury strength and antioxidant capacity in early period. Increase in antioxidant enzyme activities was observed to be continued in late period as well.

Keywords: L-carnitine, Colon anastomosis, Antioxidant, Bursting pressure

1. INTRODUCTION

Colorectal surgery is being widely used in colorectal cancers as well as ischemic colitis, ulcerative colitis, Crohn disease, mechanical intestinal obstruction, trauma, and recurrent diverticulitis [1]. Many serious and fatal complications can be seen in colon surgeries as well as in other major surgeries. Incidence of complications is 10-30% [2]. There are still cases which end up with death after taking out the tumor and performing end-to-end anastomosis for the remaining parts when the anastomosis area cannot heal completely and there is a leakage. Therefore, full and rapid healing of the anastomosis is very important.

One of the molecules effective in healing of injuries is L-carnitine [3,4]. L-carnitine is an aminoacid that has the structure of 3-hydroxy-4-N-trimethylaminobutyric acid. It facilitates the entry of long chain fatty acids into the mitochondrion and provides β-oxidation and consequently revealing of energy [5]. It was shown that L-carnitine restores membrane lipid bilayer by an indirect antioxidant effect [6,7]. Furthermore, it was suggested that L – carnitine is also effective in directly inactiviating superoxide and hydrogen peroxite [3,8,9]. It was determined that L-carnitine speeds up healing of skin injuries even under suppression of the immune system [10].

There are several studies suggesting that antioxidant activity is important in injury healing [11-17]. It was shown that antioxidant activity may also be important in surgical injury healing after colon anastomosis [12]. It may be suggested that L-carnitine contributes to injury healing after colon anastomosis by taking into consideration the positive effects of antioxidant

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activity on injury healing together with the fact that L-carnitine has an antioxidant effect.

Therefore, we examined how L-carnitine affects injury healing after colon anastomosis. For this purpose, we administered L-carnitine intraperitoneally to the rats, which were applied colon anastomosis, beginning from the date of anastomosis. We examined the effects of the treatment on healing of surgical injury in early period (3rd day after anastomosis) and late period (7th day after anastomosis).

2. MATERIALS and METHODS

Experimental Animals and Environment

The project was found in compliance with ethical board directives and approved by Canakkale Onsekiz Mart University Ethical Board of Animal Testing (Approval No. Of the Ethical Board: 2013/09-10).

In this study, 40 female Wistar-Albino rats of 6-8 weeks old were used. During the experiment period, the rats were observed in the laboratory with 21±3 °C constant room temperature, 60% humidity, and 12 hours day and night cycles. For their nutrition standard animal feed and tap water were used.

Experimental Groups

The rats were randomly divided into 4 groups, each group having 10 rats. The number of subjects in the groups used in the study was determined according to the "resource equation" method [18,19]. The groups were formed as follows:

Control 3 days (CONT-3): The rats were applied colon anatomosis and were injected with physiological saline solution intraperitoneally for 3 days. Injury healing was observed after 3 days (Control 3 days).

Control 7 days (CONT-7): The rats were applied colon anatomosis and were injected with physiological saline solution intraperitoneally for 7 days. Injury healing was observed after 7 days (Control 7 days).

L-carnitine-3 (CARN-3): The rats were applied colon anatomosis and were injected with 100 mg/kg L-carnitine [10] intraperitoneally for 3 days. Injury healing was observed after 3 days (L-carnitine 100 mg/kg 3 days).

L-carnitine-7 (CARN-7): The rats were applied colon anatomosis and were injected with 100 mg/kg L-carnitine intraperitoneally for 7 days. Injury healing was observed after 7 days (L-carnitine 100 mg/kg 7 days).

Performing Colon Anastomosis

Animals used in this study were anesthetized by subcutaneous injection of a mixture of Xylazine (5 mg/kg Rompun ®) and Ketamine (50 mg/kg-Ketalar®) following a 12-hour fasting [20,21].

Anesthetized animals were lied down on their back on the table with their abdomens facing up. For hygienic reasons, their abdominal region was shaved and cleaned with 10% povidone iodine. Anastomosis in the left colon region was performed in accordance with our previous study [22].

Actions Carried Out After the Anastomosis

The rats in CONT-3 group and CONT-7 group were injected with physiological saline solution intraperitoneally for 3 days and 7 days, respectively. The rats in CARN-3 group and CARN-7 group were injected with 100 mg/kg L-carnitine intraperitoneally for 3 days and 7 days, respectively.

Subjects were anesthetized by using either on the 3rd (CONT-3 and CARN-3) and the 7th (CONT-7 and CARN-7) day. The animals were sacrificed after blood samples were taken by cardiac puncture. Abdomens of the subjects were opened and 2 cm distal and 2 cm proximal of the anastomosis and intestine including the anastomosis line were resected and anastomosis bursting pressure at the anastomosis line was measured. After this measurement, colon including 0.5 cm distal and 0.5 cm proximal of the anastomosis was resected. After that, the samples taken were put into a – 80 °C freezer for measurement of malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, catalase activity, and hydroxyproline level.

Anastomosis Bursting Pressure Measurement

After the fecal content in the intestinal segment which was resected as described above, was washed with physiological saline solution and removed, one end of the colon was attached to the infusion pump and the other end was attached to the pressure transducer (Biopac MP 35 Data Acquisition System, USA) by 2/0 silk suturing. After the distal catheter was attached to the pressure transducer in the data collecting system, physiological saline solution infusion was made from the proximal catheter at a 4 ml/min speed via infusion pump. Meanwhile, pressure level reached during the bursting of anastomosis location was determined by recording changes in pressure in the intestinal segment continuously.

Measurement of Hydroxyproline (HPR) Level

Hydroxyproline in tissue samples was measured by hydroxyproline kit (Sigma-Aldrich® Hydroxyproline Assay Kit MAK008, St. Louis, MO, USA) spectrophotometrically at 560 nm as defined in the kit prospectus. Briefly, hydroxyproline concentration is determined by the reaction of oxidized hydroxyproline with 4-(dimethylamino)benzaldehyde (DMAB), which results in a colorimetric (560 nm) product, proportional to the hydroxyproline present. We homogenized aproximately 10 mg tissue in 100 µL of distiled water and added 100 µL of 12M hydrochloric acid to hydrolyze at 120 °C for 3 hours. After centrifugation at 10,000 g for 3 minutes, we transferred 50 µL of supernatant to a 96-well plate. We added 100 µL of the Chloramine T/Oxidation Buffer Mixture to each sample and standard well. Then, the sample plate was incubated at room temperature for 5 minutes. After adding 100 µL of the diluted DMAB Reagent to each sample and standard well and incubate for 90 minutes at 60 °C. Measurement was carried out the absorbance at 560 nm (A560).

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Marmara Med J 2023;36(2): 215-222

Measurement of Malondialdehyde (MDA) Level

Tissue MDA levels were determined according to the spectrophotometric method defined by Yagi [23]. The samples were kept frozen at – 80 °C until the working day. After the tissues dissolved at 4°C were weighed on a precision scale, homogenization was performed. 10% TCA (Trichloroacetic acid) and 0.675% TBA (Thio barbituric acid) were used in the determination. MDA was determined by spectrophotometric measurement at 532 nm of the pink colored complex formed by MDA, which is the end product of lipid peroxidation, which occurs as a result of incubation of tissue homogenate in a boiling water bath at pH: 3.5 in an aerobic environment for one hour.

Measurement of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity in tissue samples obtained was measured spectrophotometrically at 440-460 nm as defined in the kit prospectus by using SOD kit (Cayman Chemical Company Superoxide Dismutase Assay Kit 706002, USA). The tissues firstly were ringed by phosphate buffer saline (PBS – pH=7.4). After adding 10mM, pH=7.2 of cold HEPES buffer containing 1mM EDTA, 210mM mannitol and 70mM sucrose per gram tissue, we homogenized the tissue by Retsch MM400 (Haan, Germany). Then all samples were centrifugated at 1500 g, 5 minutes at 4°C. We obtained the supernatant containing extracellular and cytosolic SOD for assay. The pellet contains mitochondrial SOD. We diluted 20 µL of SOD standard with 1.98 mL of sample buffer. From the stock solution we prepared, we pipetted 10 µL of standard at various concentrations into the empty wells of the plate. Likewise, 10 µl of each sample was pipetted. Then 200 µl of radical detector was added to them. 20µl of diluted xanthine oxidase was added to all wells. The plate was incubated for 30 minutes at room temperature with gentle shaking. Absorbances were measured at 460 nm with a plate reader. SOD activities of all samples were obtained from the standard graph.

Measurement of Catalase (CAT) Activity

Catalase activity in tissue samples obtained was measured spectrophotometrically at 540 nm as defined in the kit prospectus by using catalase kit (Cayman Chemical Company Catalase Assay Kit 707002). Briefly, we washed the tissues in phosphate buffer with pH=7.4. Then, 50mM, pH=7.0, potassium phosphate buffer containing 1mM EDTA per gram tissue was added. Supernatants were taken by centrifugation at 10000 g for 15 minutes at 4°C, and the study was carried out by preparing the reagents in the kit as specified in the procedure. Different concentrations of formaldehyde were prepared as standard and 20 ml of both standard and samples were added to the empty wells of the plate. 20µl of hydrogen peroxide was added to all wells to initiate the reaction. The plate was covered and incubated for 20 minutes at room temperature. Then 30 µl of potassium hydroxide and then 30 µl of catalase purpald as chromogen were added. After incubation for 10 more minutes, 10 µl of catalase potassium periodate was added and incubation was performed for 5 minutes at room temperature. Absorbances were obtained at 540 nm using a plate reader.

Histopathological Examination

Histopathological evaluation was performed by a histologist who did not have the knowledge of which tissue belonged to which experimental group.

Tissues taken for histopathological examination were fixed in 10 % of formaldehyde. Tissues obtained were washed under tap water for one night long and then were embedded into paraffin after being subject to routine histological procedures. Sections of 5 µm taken from the paraffin blocks were taken onto slides. Tissue samples prepared were dyed with hematoxylin-eosin and evaluated under light microscope according to the criteria based on the method of Chiu et al. as shown in Table I [24].

Table I. *Histopathological evaluation criteria* [24]

(0)	No injury healing/adherence microscopically.
(+)	Non-adherent areas at the microscopic level at the anastomosis line or low level of healing
(++)	There is scar tissue at the anastomosis line. Anastomosis ends are active (Edema in tissue, congestion, hypercellular scar reaction, mononuclear cellular infiltration)
(+++)	Granulation tissue has formed at the microscopic level in the anastomosis area and there is a healthier appearance.
(++++)	Full injury healing, healthy look with epithelialisation

Statistical Analysis

Data obtained were indicated as average ± standard error (SE). Statistical significance levels of data were determined by using statistical package software "SPSS for Windows version 16" (Chicago, IL, USA). Multiple group comparisons were done by Kruskal-Wallis test. Mann Whitney U-test was used for comparison of two groups. For the interpretation of the result found, $p<0.05$ value was accepted as statistically significant.

3. RESULTS

No animals died or were excluded throughout the experiment.

Anastomosis Bursting Pressure Levels

Anastomosis bursting pressure levels (mmHg) were recorded as 16.43±4.86, 282.41±39.76, 48.42±7.60, and 261.66±20.69 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 1). There was a significant difference between bursting pressure levels of CONT-3 and CARN-3 groups ($p<0.05$). On the other hand, the difference between bursting pressure levels of CONT-7 and CARN-7 groups was not significant ($p>0.05$).

Biochemical Evaluation Results

Results of Hydroxyproline (HPR) Level Measurement

Hydroxyproline levels (µg/mg wet tissue) of scar tissue in groups were recorded as 2.32±0.27, 1.13±0.17, 1.96±0.47, 4.48±0.76 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively

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Marmara Med J 2023;36(2): 215-222

(Figure 2). It was seen that application of L-carnitine did not cause a significant change in hydroxyproline levels compared to the control groups on the 3rd day of the operation. However, hydroxyproline level of CARN-7 group was significantly higher than CONT-7 group (p<0.05).

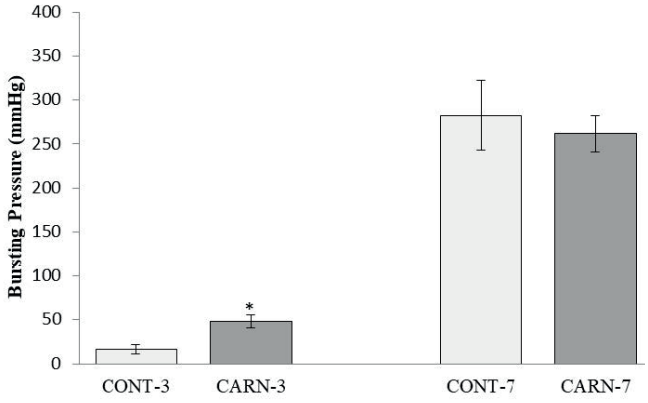

Figure 1. *Bursting pressure levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group.*

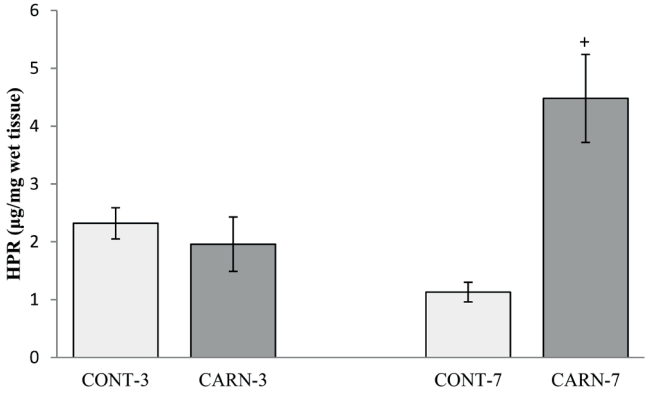

Figure 2. *Hydroxyproline (HPR) levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
+: p<0.05 versus CONT-7 group.

Results of Malondialdehyde (MDA) Level Measurement

MDA levels (nmol/mg wet tissue) of tissue samples were recorded as 6.49±1.09, 10.52±1.53, 4.87±0.90, 3.23±0.51 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 3). Three-day application of L-carnitine did not cause a significant change in MDA level compared to CONT-3 group. However, MDA level of CARN-7 group was significantly lower than CONT-7 group (p<0.05).

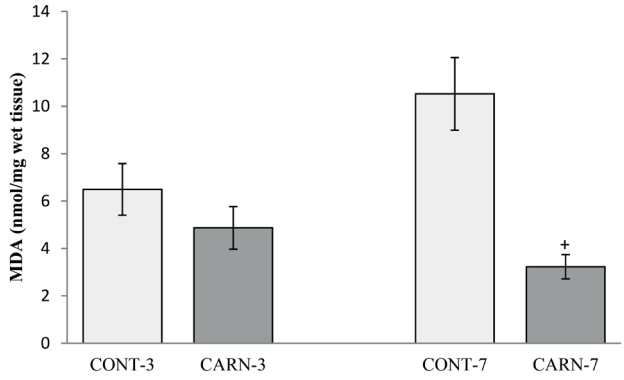

Figure 3. *Malondialdehyde (MDA) levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
+: p<0.05 versus CONT-7 group.

Results of Superoxide Dismutase (SOD) Activity Measurement

SOD activity levels (U/mg wet tissue) of scar tissue in groups were recorded as 4.63±0.51, 3.70± 0.33, 6.82±0.34, 8.59±0.50 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 4). A significant difference was observed between CONT-3 and CARN-3 groups, as well as between CONT-7 and CARN-7 groups (p<0.05).

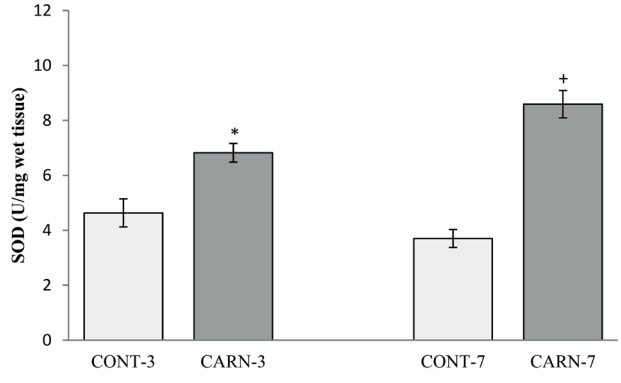

Figure 4. *Superoxide dismutase (SOD) activity levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group, +: p<0.05 versus CONT-7 group.*

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Marmara Med J 2023;36(2): 215-222

Results of Catalase (CAT) Activity Measurement

Average values and standard errors for catalase activity levels (U/mg wet tissue) of tissue samples were recorded as 10.61±0.99, 6.52±0.97, 17.12±1.66, 22.07±1.34 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 5). When we compared CONT-3 group and CARN-3 group, we found a significant diffference in favor of CARN-3 group with respect to injury healing ($p<0.05$). The same result was seen in comparison of CONT-7 group and CARN-7 group as well ($p<0.05$).

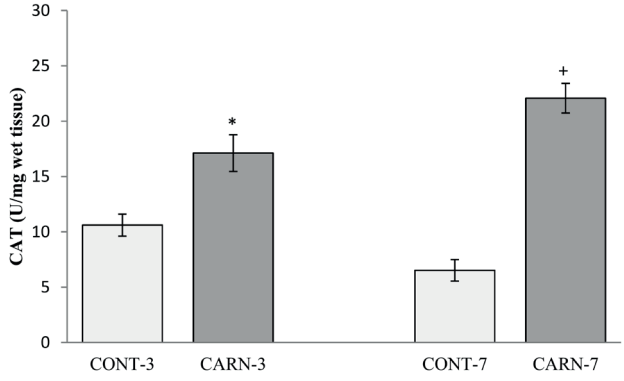

Figure 5. *Catalase (CAT) activity levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group, +: p<0.05 versus CONT-7 group.*

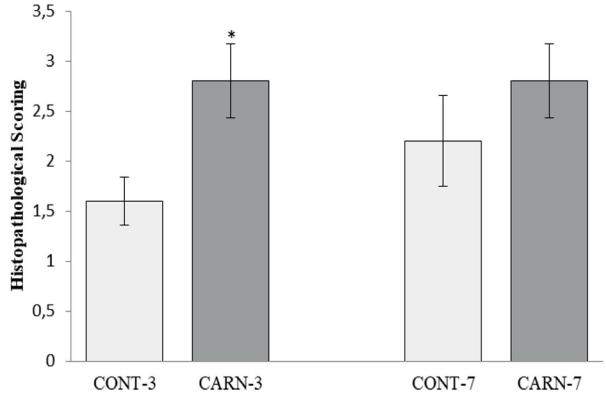

Figure 6. *Histopathological scoring of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group.*

Results of Histopathological Scoring

Average values and standard errors for histopathological scoring of tissue samples were recorded as 1.60±0.24, 2.20±0.45, 2.80±0.37, 2.80±0.37 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 6). A significant difference was observed between CONT-3 and CARN-3 groups ($p<0.05$). Histopathological sections stained with hematoxylin eosin belonging to different groups are shown in Figure 7.

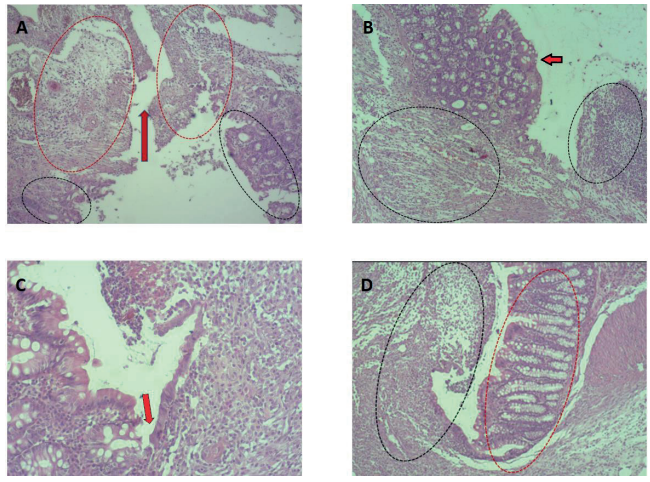

Figure 7. *Histopathological sections stained with hematoxylin eosin belonging to different groups.*
A: Histological section of the anastomosis site on the 3rd post-operative day in a rat belonging to the CONT3 group. The red arrow indicates the anastomosis line. Wound healing begins in the areas surrounded by red dots on both sides of the anastomosis line, but it is observed that there is no fusion between the ends. The two cut ends of the colon are also seen in the black dotted areas. Wound healing has begun in these areas, but fusion between the anastomotic ends has not yet occurred (Mag. 4x).
B: Histological section of the anastomosis site on the 3rd postoperative day of a rat belonging to the CARN3 group. The black dotted areas show the hypercellular healing tissue in the surgical incision area. There is intact epithelial tissue in the area marked with the red arrow (Mag. 4x).
C and D: Histological sections of the anastomosis site on the 7th postoperative day of a rat belonging to the CARN7 group. In C, the junction of the intact epithelium and the newly formed tissue is seen in the area marked with the arrow (Mag. 10x). In D, the colonic epithelium and glandular structure can be observed in the red dotted areas. The black dotted area shows the newly formed tissue at the anastomosis line. Significant mononuclear cell infiltration is seen in this multicellular area (Mag. 4x).

4. DISCUSSION

The main result of our study is that L-carnitine speeds up the anastomosis healing in the early period. High bursting pressures were recorded in CARN-3 group, which show the injury strength. This finding is also supported by the high level of histopathological scores of CARN-3 group. Another important finding of our study is that application of L-carnitine increases antioxidant capacity and significantly decreases the oxcidant damage, especially in the long term (CARN-7 group).

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Marmara Med J 2023;36(2): 215-222

Surgical intervention has an important role in treatment of colorectal cancers. Colorectal surgeries are applied widely, and the most common complication of these surgeries is anastomotic leaks [25]. Morbidity and mortality rates of colon anastomotic leaks are higher than the other anastomotic leaks [26]. Despite the developments in surgical techniques, these rates increase when there is a leakage from the surgical area after taking out the tumor and performing end-to-end anastomosis of the remaining parts and the anastomosis area cannot heal completely. Therefore, full and rapid healing of the anastomosis is very important. Although we did not evaluate whether there is a leakage from the anastomosis area, the healing of the injury site and injury strength are important signs of a healthy process. One of the most frequently used measurement methods of injury strength in experimental anastomosis studies is bursting pressure [27]. We also used bursting pressure for measuring injury strength in our study. We determined that bursting pressure value increased 3 days after the anastomosis in the group to which L-carnitine was applied but there was no significant change on the 7th day (Figure 1). By looking at these results, it is possible to say that application of L-carnitine in the early period increases injury strength in the model we used. Furthermore, as a result of the evaluation of injury healing in the anastomosis area by histopathological scoring, we determined a distinct healing in the CARN-3 group which represents the early period (Figure 6). According to the protocol we used in our study, a high histopathological score indicates that anastomosis healing is better. The higher histopathological scoring value compared to the CONT3 group explains the increase in bursting pressure in the CARN3 group.

One of the factors that affect injury healing is oxidative stress. Reactive oxygen species (ROS), which cause oxidative stress, are produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during normal metabolic incidents. The superoxide anion produced by the effect of this enzyme can cause tissue damage besides leading other ROS formations. Hydrogen peroxide (H_2O_2) is one of these and it is not a radical. However, it can cause serious damages in cells [28]. The existence of iron and copper ions in the environment causes H_2O_2 to form hydroxyl radicals. Another radical that comes out in this process is hypochloride, which occurs by the effect of myeloperoxidase. Majority of the formation of ROS is realized by phagocytal cells. ROS is an important defense mechanism for the injury area against pathogen microorganisms. In addition, superoxide anion occured in the injury area and H_2O_2 stimulate vascularization by regulating the microcirculation. By this means, healing is accelerated by satisfying the nutrition and oxygen needs of the injury area. It was also stated that ROS in low levels has a role in signal transmission in cells [29]. However, ROS can also cause tissue damage if its amount exceeds the level where the favourable effects are seen [30]. As a result of this, ROS and oxidants occurred in the injury area can deteriorate healing. They break down the proline and hydroxyproline which constitute the collagen and can change the adhesion, reproduction, and vitality of fibroblasts. Besides, they cause serious damages in fibroblasts by inhibiting the migration of H_2O_2 keratinocytes and signal transmission of epidermal growth factor (EGF) [31]. Increased inflammatory infiltration in the injury area indicates the existence of oxidative stress. For this reason, reducing the effects of ROS is very critical for injury healing. Thus, the antioxidant substances stand out. Today antioxidants are used for injury healing [32]. There are numerous studies that examine the contribution of antioxidants to healing of colon anastomosis injuries [12,13,15-17]. In these studies molecules such as acetylcysteine, resveratrol, and simvastatin were used as antioxidants. It was shown that acetylcysteine, which is one of the antoxidant molecules, increased the fibroblast proliferation [33]. Fibroblasts synthesize collagen and glycosaminoglycan, which is very important for injury healing. Existence of adequate fibroblast proliferation and collagen synthesis in injury tissue speeds up healing [34].

In our study, we examined the effects of L-carnitine, which is an antioxidant molecule, on injury healing in early and late period. It was already shown that L-carnitine application has positive effects on colon anastomosis healing [35]. Caecal ligation, formation of peritonitis by drilling, and colon anastomosis were made simultaneously in the mentioned study. The effects of L-carnitine on injury healing were evaluated by determining the bursting pressure values of the anastomosis area on the 5th day following the operation. Injury healing was positively affected by L-carnitine for both rats with and without peritonitis. We, on the other hand, compared the effects of L-carnitine in early and late period in our study. Evaluation of injury healing in the early period following the anastomosis is important as anastomosis leakages occur especially in the early period [36].

L-carnitine can decrease oxidant damage by increasing antioxidant capacity [3,37]. In the comprehensive compilation of Fathizadeh H, it is stated that although L-carnitine does not affect total antioxidant capacity, it causes an increase in SOD activity and a significant decrease in MDA levels, which is an indicator of oxidant damage [37]. Considering the same systematic review, the existence of studies in which the total antioxidant capacity increases in regard to sample size and dose of L-carnitine draws attention. Similar with these results, we also found that the antioxidant capacity in intestinal tissue was increased by L-carnitine. The findings show that SOD and catalase activities were significantly higher in CARN-3 group than CONT-3 group, and also higher in CARN-7 group than CONT-7 group. It can be said that the increase in SOD and catalase activities improves the defense capability of the tissue against oxidant damage.

Another finding of our study is that the increase in antioxidant enzyme activities causes a decrease in MDA levels in the tissue obtained from the anastomosis area. Although the difference between MDA levels of CARN-3 and CONT-3 groups was not significant, a tendency to decrease was determined. On the other hand, we determined a significant decrease in the MDA levels of CARN-7 group following a long-term L-carnitine application. This shows us that L-carnitine decreases oxidant damage in the long run. By looking at these results, it is possible to suggest that the balance between oxidative stress and antioxidant capacity does not fully change in favour of antioxidant capacity in the

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Marmara Med J 2023;36(2): 215-222

early period of injury healing and that antioxidant capacity can reach a level where it can overcome oxidative stress only in the late period of injury healing.

In our study, finding high bursting pressure levels in CARN-3 group, which was formed to determine the effects of L-carnitine in injury healing in the early period, is an important indicator of injury strength. However, hydroxyproline levels of CARN-3 group were not different from CONT-3 group. In a previous study, Cronin et al. stated that the measurements of anastomosis bursting pressure showed that the applied force increased gradually following the 3rd day of anastomosis and reached its maximum on the 7th and 10th days; hydroxyproline concentration in the injury area decreased by 40% during the first 3 days, approached to normal after approximately the 5th day, and reached a level above normal on the 10th and 14th days [38]. This shows that some factors other than hydroxyproline play an important role in improving injury strentgh. The results of our study are coherent with the study of Cronin et al [38]. Moreover, we determined that hydroxyproline levels of CARN-7 group, which represents the late period of injury healing, increasead significantly. Considering that injury healing is a long process which may take weeks, it is possible to say that L-carnitine application in the late period increases the hydroxyproline levels in the long run. Besides, one of the important findings of our study is that L-carnitine increases injury strength in the early period without an increase in hydroxyproline levels. However, more studies are needed to determine the molecular mechanisms of the increase in injury strength in the early period caused by L-carnitine.

Consequently, it is possible to say that L-carnitine application is useful in preventing anastomosis leakage, which is one of the most important complications that increase mortality rates after intestinal surgery. It is seen that L-carnitine speeds up injury healing, especially in the early period. Moreover, increase of injury strength in the early period comes out without a significant increase in hydroxyproline levels. With the application of L-carnitine, hydroxyproline levels increase and also oxidant damage decreases significantly in the late period of injury healing. It can be said that, for this effect to come out, the increase in SOD and catalase activities, which are antioxidant enzymes, and decrease in oxidant damage caused by L-carnitine play an important role. However, additional studies are needed to determine how L-carnitine affects the molecular mechanisms of injury healing process.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Animal Experimentation Ethical Committee of Canakkale Onsekiz Mart University (approval number: 2013/09-10)

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Conflict of Interest Statement: There is no conflict of interest.

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Formation and branching patterns of deep palmar arch

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ABSTRACT

Objective: The present study is to document and provide information about both normal and variable morphology of the deep palmar arch (DPA) in adult human cadavers by the dissection method.

Materials and Methods: We examined 12 upper extremities (6 cadavers). After the classification of the vascular patterns of DPA and its branches, measurements of the vessel diameters were carried out using a digital compass.

Results: Deep palmar arch was found as a completed arch (100%) in all cases. The anastomosis between the distal deep palmar branch of the ulnar artery (DPBUA) and the terminal branch of the radial artery (RA) was the most common type in our study. The incidence of the DPA was reported as a complete arch ranging from 54.9% to 100%. Palmar metacarpal arteries (MPAs) originating from the DPA were divided into four branches (25%) in three cases and three branches (75%) in nine cases. The mean diameter of the MPAs at the point of origin at the DPA was between 0.3 mm and 0.6 mm.

Conclusion: A comprehensive understanding of the DPA branching diameters in the hand will facilitate surgical and radiological approaches and contribute to a constantly expanding knowledge base in literature.

Keywords: Anatomy, Deep palmar arch, Hand, Radial artery, Ulnar artery

1. INTRODUCTION

Arterial blood supply to the hand is vital, comprising the vascular networks that provide anastomosis between the superficial palmar arch (SPA) and the deep palmar arch (DPA) [1-5]. These arches and their branches form an abundant network of blood vessels that provide oxygenated blood to all parts of the hand and fingers. The SPA is generally formed by the ulnar artery (UA) and the superficial palmar branch of the radial artery (SPBRA) [5,6]. The radial artery (RA) then continues its course, curving around to enter the dorsal aspect of the hand, passing the scaphoid and trapezium, and passing through the floor of the anatomical snuff box [7]. After reaching the snuff box, the RA passes between the two heads of the first dorsal interosseous muscle and proceeds to the palm, and shows continuity as the DPA (Figure 1). The DPA is dominated by the RA in the palm and is formed by the anastomosis of the distal deep palmar branch of the ulnar artery (DPBUA) and RA [6]. The DPA is localized deep in the flexor tendons of the hand. The palmar metacarpal arteries (MPAs), which anastomose with the common palmar digital arteries, emerge from the convexity of the DPA [8]. It is known that proximal and distal perforating branches, as well as MPAs, originate from the DPA (Figure 1). The DPA is located more proximally than the SPA [9]. The DPA supplies blood to the thumb and the lateral side of the index finger [7]. Injury to this area can cause considerable bleeding, but healing is rapid due to anastomoses. These two arches balance the circulation with some compensatory variations [10].

A thorough examination of hand function is the basic requirement of all hand surgeons during interventions. The vascular anatomy of the hand is a complex and challenging field, and has been the subject of many classification studies. One of the first classifications of the palmar arches was made by Coleman and Anson [11]. The human hand is highly developed in terms of its complexity and variation, which stems from embryologic development [12]. It is

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important to note that in a careful long-term analysis of arterial variations based on homogeneous criteria, these variations occurred with very similar incidences each year [13]. In previous studies, the completeness of the SPA ranged from 31.8% [14] to 100% [4], whereas the completeness of the DPA was reported to range from 54.9% [1] to 100% [7,15,16].

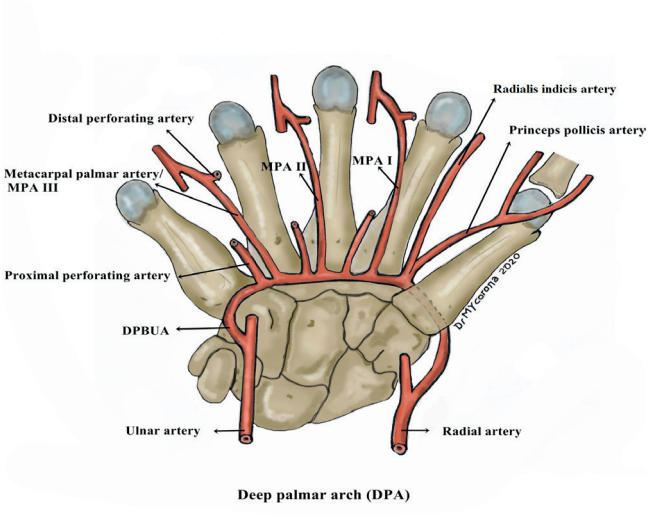

Figure 1. *Schematic representation of the deep palmar arch. (DPBUA: Deep palmar branch of the ulnar artery; MPA: Palmar metacarpal arteries)*

Aside from the well-known SPA, recent studies have shown that DPA and its branches are of sufficient size for some microvascular reconstruction procedures [7]. van Leeuwen et al. [17] recently concluded that although the incompleteness of the SPA is common, the digital blood supply is always preserved by a complete DPA. The ischemia potential and the variability of vascular formation are the most significant difficulties faced in these surgical procedures [18,19].

It is beyond doubt that an awareness and identification of the DPA variations in the hand are particularly important for the reconstruction of congenital anomalies, post-traumatic lesions and the use of the RA as an arterial by-pass graft [1-3,8,10]. Physical examinations, diagnostic studies and an evaluation of surgical interventions for the restoration of blood flow are necessary to prevent irreversible damage. Angiography, ultrasonography, and Doppler studies have been carried out as well as gross dissections and casting techniques with the aim of gaining an understanding to the complex and thin vascular structures in the palmar region [18,20,21].

The present study aimed to establish the morphometric parameters and the anatomical branching pattern of the DPA in human cadaver. To the best of our knowledge, there have been few studies to date focusing on the morphometry of the DPA branches in terms of surgical and anatomical relevance. Such information defining the DPA morphology could be substantially beneficial to the clinical and anatomical fields.

2. MATERIALS and METHODS

We examined 12 upper extremities (6 cadavers). The cadavers were both female and male (female 1/male 5), aged 30–70 years and had no orthopedic defects. All cadavers were fixed using phenol and formol, and routinely dissected by first-year medical students.

Measurements were repeated three times to minimize measurement error, and the average of the data obtained from different cases was used as the study data. The measurements were made using a digital caliper with a 0.01 mm accuracy.

The study was conducted to document and inform both the normal and variable morphology of the DPA. A skin incision was made to the dissected areas extending to the distal phalanxes, and the skin and fascia were then cut off. The RA and UA were dissected in the wrist region, and their branches were identified in the distal third part of the forearm (Figure 2). After the branch of the SPBRA that participates in the SPA and the proximal (distal, if any) DPBUA that joins the DPA, the superficial palmar region was dissected (Figure 2). The palmar aponeurosis was revealed, separated from the flexor retinaculum and divided proximally, and the septae were removed. The neurovascular structures were protected from the beginning of the flexor retinaculum with curved-tip dissecting scissors. Then the flexor retinaculum was dissected on both sides with a central incision down the middle of the retinaculum. The branches of the median nerve passing through the carpal tunnel, and the tendons of the flexor digitorum superficialis and the flexor digitorum profundus were carefully dissected.

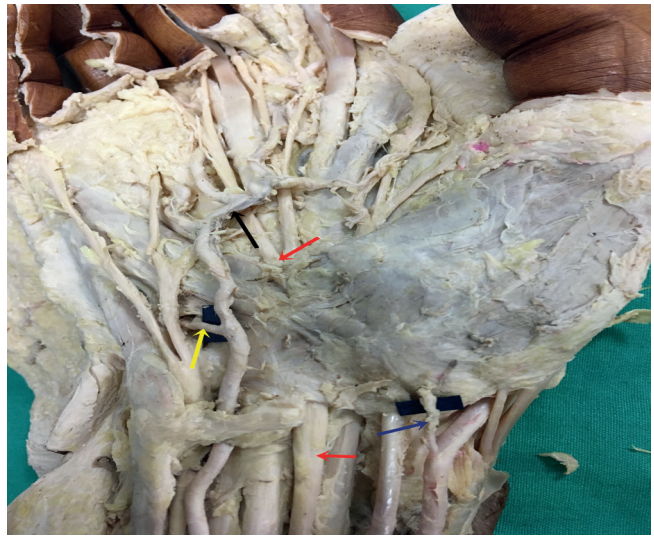

Figure 2. *Appearance of the vessels, nerves, and their branches in the palmar region of the hand (case-4). (Yellow arrow: Distal deep palmar branch of the ulnar artery; Blue arrow: superficial palmar branch of the radial artery; Black arrow: superficial palmar arch; Red arrow: median nerve)*

The dorsal course of the RA was examined before reaching the palmar region. The RA was dissected to reveal the course and branching of the first interosseous space in the anatomical snuffbox (Figures 3,4).

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Marmara Med J 2023;36(2): 223-229

Figure 3. *Branches are given by the radial artery after passing through the snuff box (A: case-2; B: case-5). (Yellow arrow: Deep palmar arch; Blue arrow: Princeps pollicis artery; Red arrow: radialis indicis artery; Green arrow: dorsal metacarpal artery I)*

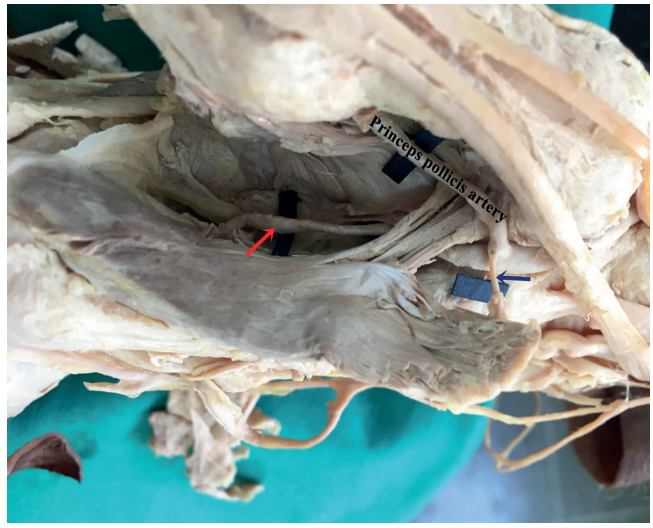

Figure 4. *The appearance of a perforating branch feeding the oblique head of the interosseous muscle and princeps pollicis and radialis indicis arteries originating from the radial artery (case-1). (Blue arrow: perforating branch; Red arrow: radialis indicis artery)*

Table I. *The mean diameters of vessels (mm) and number of MPAs originating from the DPA.*

Specimen	Case	Sex	R/L	RA	UA	Deep branch of ulnar artery		PPA	RIA	Dorsal metacarpal artery I	DPA (Origin)	DPA (Termination)	Number of palmar metacarpal arteries
						Proximal	Distal						
1	1	M	L	2.82	2.4	*	0.15	1.47	1.55	*	1.38	0.96	3
	2	M	R	3.57	2.55	*	1.5	2.37	1.46	*	1.1	0.9	4
2	3	M	L	3	3.5	*	0.17	1.8	1.8	*	1.7	1.7	3
	4	M	R	4.6	3.1	*	0.20	2.0	2.3	0.95	1.8	1.0	3
3	5	M	R	2.05	1.57	0.6	1.13	1.0	0.35	0.26	0.96	1.08	3
	6	M	L	2.1	1.9	*	1.22	1.2	0.9	*	1.21	0.9	3
4	7	F	R	2.59	1.75	0.63	1.14	1.31	1.11	*	1.02	0.4	4
	8	F	L	2.91	2.45	*	0.69	1.62	1.45	*	1.3	1.0	3
5	9	M	R	2.35	2.38	*	1.24	1.35	1.37	0.85	0.98	0.95	4
	10	M	L	2.85	2.33	*	0.99	1.5	1.2	*	1.22	0.96	3
6	11	M	R	2.82	2.34	*	1.1	1.45	1.38	*	1.13	1.07	3
	12	M	L	2.94	2.44	*	0.58	1.67	1.32	*	1.39	0.89	3
	Total Average	n=12	Right=6 Left=6	**2.88 mm**	**2.39 mm**		**0.84 mm**	**1.56mm**	**1.35mm**		**1.26 mm**	**0.98 mm**	**0.3-0.6 mm**
	Avarage males	n=10		**2.91 mm**	**2.45 mm**		**0.82 mm**	**1.58 mm**	**1.36 mm**		**1.28 mm**	**1.04 mm**	**0.53 mm**
	Avarage females	n=2		**2.75 mm**	**2.1 mm**		**0.91 mm**	**1.46 mm**	**1.28 mm**		**1.16 mm**	**0.7 mm**	**0.49 mm**

*(**RA:** the diameter of the radial artery before giving the superficial palmar branch; **UA:** Ulnar artery; **PPA:** princeps pollicis artery; **RIA:** radialis indicis artery; **DPA:** deep palmar arch; *: not observed)*

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Marmara Med J 2023;36(2): 223-229

To obtain a clear field of visualization of the DPA, the lateral head of the interosseus dorsalis-I and the oblique head of the adductor pollicis were removed, as well as the skin and fascia. Changes in all dissected regions were observed, recorded and visualized. When possible, we measured the diameters of RA, UA, proximal and distal deep palmar branch of the ulnar artery, PP, RI, dorsal metacarpal artery I as well as the distance from the origin of the DPA to its termination. The average diameters of the vessels are presented in Table I.

The experimental protocols were approved by the Clinical Research Ethics Committee (approval no: 02-280097).

Statistical Analysis

Data analysis was performed using SPSS 23.0 program. Measurements were considered to be significant when $p< 0.05$.

3. RESULTS

The DPA was found as a completed arch in all 12 hands (100%). At least one of the DPBUA (proximal or distal) was present in all samples. It was determined that the terminal part of the RA was anastomosed with distal DPBUA in all cases (100%). Proximal DPBUA emerged in only two cases (case-5 and case-7) (16%), but were not involved in any form of the DPA (Figure 5).

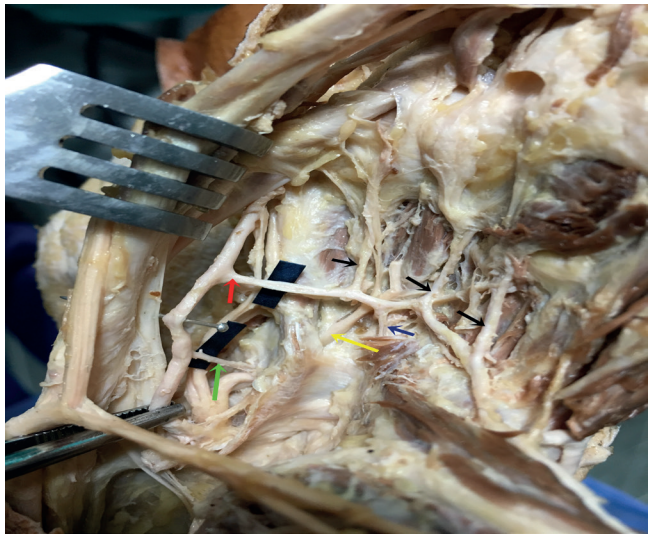

Figure 5. *Proximal and distal deep palmar branch emerged from the ulnar artery and formation of deep palmar arch with distal deep palmar branch of the ulnar artery (case-5). (Green arrow: Proximal deep palmar branch of the ulnar artery; Red arrow: Distal deep palmar branch of the ulnar artery; Blue arrow: perforating branches; Black arrow: palmar metacarpal arteries; Yellow arrow: deep palmar branch of ulnar nerve)*

The MPAs branching from the DPA numbered four (25%) in three cases and three (75%) in nine cases (Table I). In 11 cases, the MPAs were found to originate separately from the DPA. In contrast, in one case (case-4), it was observed that palmar metacarpal II and III, which were closer to DPBUA, first emerged as a common trunk after a short course, bifurcated into two, and then continued the course of these two branches. These branches were then anastomosed with the common palmar digital arteries originating from the SPA (Figure 6). In addition to the MPAs, perforating branches (cases-1, – 2, – 4, and – 5) emerged from the DPA proximally. In some cases, the perforating branches (case-1 and case-4) were involved in this arch formation (Figures 6,7).

Figure 6. *Extraction of the second and third palmar metacarpal arteries from the arcus palmaris profundus in a common trunk and atypical course of the deep branch of the ulnar nerve (case-2). (Blue arrow: perforating branches; Yellow arrow: perforating branches; Black arrow: palmar metacarpal arteries; Green arrow: distal deep palmar branch of the ulnar artery; Red arrow: deep branch of ulnar nerve)*

Figure 7. *Four palmar metacarpal arteries originating from the deep palmar arch and atypical course of radialis indicis artery at hand and anastomosis with common palmar digital arteries (A: case-1; B: case-4). (Black arrow: palmar metacarpal arteries; Yellow arrow: perforating branches originating from the deep palmar arch; Red arrow: radialis indicis artery; Blue arrow: perforating branches; Green asterix: bifurcation of radialis indicis artery and anastomosis with common palmar digital arteries; Blue asterix: deep branch of ulnar nerve)*

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Marmara Med J 2023;36(2): 223-229

The dorsal metacarpal artery-I appeared in only three of the 12 upper extremities (Figures 3,6). It was observed that the RA often made perforating branches in the absence of the dorsal metacarpal artery-I (Figure 4). In one case, the princeps pollicis artery (PPA) emerged from the RA after the radialis indicis artery (RIA). RIA was observed to take a different course in two cases (case-1 and case-2). After the RIA originated from the RA, it was observed to be oblique in the distal direction. Before reaching the second metacarpophalangeal joint, it directed toward the medial side and was bifurcated in two. One branch continued its course distally to the medial depth of the second finger and was soon bifurcated again; and the other branch was found to anastomose with the common palmar digital arteries, which is one of the branches of the SPA (Figure 7).

It was determined that the deep palmar branch of the ulnar nerve (DPBUN), which was adjacent to the DPA, proceeded along a different course in the right hand of two different cadavers and reached the adductor pollicis. In one cadaver (case-4), the DPBUN was observed to pass through the dorsal of MPA II and III, where they first separated from the DPA as a common trunk and bifurcated, and then crossed the first MPA in a palmar direction to reach the adductor pollicis (Figure 6).

After explaining and correlating the vascular patterns of the DPA and its branches, as well as other vessels, the diameters of the vessels were measured with a digital compass. The mean diameters of the vessels are presented in Table I. In a comparison of the arterial diameters, the terminal branch of the RA was found to be more dominant than the DPBUA.

Figure 8. *Four palmar metacarpal arteries originating from the deep palmar arch and the course of deep branch of ulnar nerve (case-6) (Blue arrow: distal deep branch of the ulnar artery; black arrow: palmar metacarpal arteries; red arrow: radialis indicis artery; green arrow: deep branch of ulnar nerve)*

4. DISCUSSION

In modern surgical and anatomical procedures, the recognition of morphometric data and anatomic vascular variations in the branching pattern of the DPA has become of considerable importance. In the present study, the proximal DPBUA was observed in two cases (16.6%), whereas the most common variant observed was the distal DPBUA together with the terminal branch of the RA, which was noticed in all cases. It was further noted that in no case did the proximal and distal branches contribute to DPA formation at the same time. In addition to the contribution of these branches to the formation of the arch, we found that the perforating branches, as well as the MPAs, originated from the DPA. Another important finding was that, in addition to MPAs, RIA was directed toward the medial side, and anastomosed with the common palmar digital arteries before reaching the second metacarpophalangeal joint in two cases.

The classification of the DPA has been demonstrated by many researchers. The most commonly described variant of the DPA is the radio-ulnar type in literature [15]. The few noteworthy studies are as follows. Loukas et al., studied the palmar arterial arch on 120 specimens, and found the DPA to be formed by the RA in all extremities, and classified the DPA into three groups in their study [15]. Type D-I (60%) referred to anastomosis of the DPBRA and the distal DPBUA. Gellman et al., reported that the DPA was formed by the DPBRA in 100% of the samples, and divided the DPA into three groups in their study [18]. They found the DPA to be an anastomosis between the DPBRA and the distal DPBUA in Type-A (44.4%). Coleman and Anson, identified a completed arch in 97% of the cases, which they divided into four groups in their study, among which they observed the anastomosis between the RA and the distal DPBUA to the most common type, accounting for 49% of the total (Type-B) [11]. Bigler et al., determined that one patient had an incomplete SPA, whereby only the SPBRA and a complete DPA were present [22]. In the present study, the DPA was defined as an anastomosis between the RA and distal DPBUA in all cases (100%). RA was found to be more dominant than the DPBUA in most cases. It can be concluded from these results that the distal DPBUA plays a more significant role in arch formation than the other branches. The results of the current study concur with those of previously published studies [11,15,18,22], however it was a remarkable finding that the second and third MPAs first emerged as a common trunk and then bifurcated. No other studies have been found identifying MPAs originated from the DPA as a common trunk. There are some minor differences in the frequency of the anastomotic patterns when compared to previous studies, which may be due to the relatively small sample size (the sample in the current study was only 12 specimens) or the techniques used.

In some studies, it has been determined that the DPA is more variable than the SPA [7,16]. Patnaik et al., reported that the DPA was observed to be completed in all dissections, while also being more variable [16]. The authors found that the UA yielded two deep palmar branches (proximal and distal) in all cases, but both contributed to the formation of DPA by only

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Marmara Med J 2023;36(2): 223-229

10%. They determined that the distal DPBUA formed the DPA in 52% of the samples. Singh et al., also found that DPA showed more variability than the SPA, and classified the DPA into five types depending on its formation in their study [7]. They went on to report that the DPA consisted of anastomosis between the DPBUA and the DPBRA in 72% (36/50) of the samples, and that only one of the proximal and distal DPBUA had anastomosed with the DPBRA in 4% (2/50) of the samples. Dhar and Lall, however, reported that the DPA was less variant than the SPA, and that the predominant type of DPA was formed by anastomosis (60%) of the DPBRA and the distal DPBUA [23]. Zarzecki et al., found the DPA to be a complete arch in 95.2% of cases [5]. They determined that the DPA was less variable than the SPA in 12 studies (n=1093) included in this meta-analysis, but mentioned that it was impossible to carry out a detailed meta-analysis on this trait due to the scarcity of studies reporting its specific variations. In the present study, the proximal DPBUA was observed in two cases (16.6%), while only the distal DPBUA was involved in the arch formation. This study was carried out to in response to the scarcity of macroscopic and morphometric studies on the DPA.

The present cadaveric study makes a morphometric assessment of the DPA and its main branches. The external diameters, branches and corresponding vessels of the DPA have rarely been described in literature. Bilge et al. [24] measured the mean diameter of the introduced terminal portion of the RA, and the proximal and distal DPBUA as 2.60 (± 0.47) mm, 1.77 (± 0.44) mm and 1.63 (± 0.52) mm, respectively. Evaluating the diameter of the MPAs individually, they reported that the mean diameter of the first MPA was 1.50 (± 0.38) mm, the second was 1.41 (± 0.35) mm and the third was 1.45 (± 0.33) mm. Gellman et al. [18] stated that the mean diameter of the MPAs was 1.20 mm, while Gokhroo et al. [1] measured the mean diameter of MPAs as 1.44 (±0.39) mm. In the present study, the mean diameters of the RA and UA before joining the arch were 2.88 (±0.78) mm and 2.39 (±0.64) mm, respectively. Concerning the external diameter of the DPA, the mean diameter of the terminal branch of the RA (at its origin) and the distal DPBUA (at its termination) were 1.26 (±0.65) and 0.98 (±0.73) mm, respectively, while the mean diameter of the MPAs at the point of origin at the DPA was between 0.3 and 0.6 (±0.59) mm. There were no statistically significant differences between group means for right and left sides and for gender ($p>0.05$). A cadaveric assessment of the DPA diameters in the present study revealed comparable figures. A few compatible results have been reported previously [1,18,24]. Based on the findings of the present study, it could be said that the RA is the dominant artery in the arch formation, although the average diameter of the MPAs is thinner than in other studies. The morphometric diameters on the left hand were measured thicker than in the right hand in many cases (Table I). As a result of our study, we clearly stated the importance of keeping the palmar arch and its variations in mind due to the risk of ischemic hand complications before clinical applications.

In the present study, the position of the DPBUN was evaluated. Bilge et al., evaluated the position of the DPBUN as a structure adjacent to the DPA, and reported that the DPBUN passed through the DPA obliquely and dorsally in 38 (76%) specimens, and through the palmar direction in 12 (24%) specimens [24]. Olave and Prates, reported the dorsal neighborhood of the DPBUN in 50% of the cases [25]. Bini and Leclercq, determined that the DPBUN crossed the DPA superficially in 43% of cases, and deeply in 57% cases in 21 hands [26}. In the present study, it was worthy of note that the DPBUN passed through both the dorsal and palmar aspects of the MPAs in the same case (Figure 6, Figure 8). The DPBUN was observed obliquely in the dorsal direction of the DPA and its branches, and reached the muscle in all other cases. It is very important for surgeons engaged in surgeries in this region that the DPBUN and DPA be well known in terms of their interrelations and variations. The DPBUN lies in an area that is not often approached by hand surgeons [27,28], where the anatomy is complex, and where the anatomical studies of the ulnar nerve are often restricted to Guyon's canal [26,29].

Conclusion

Surgical interventions to the hand require more detailed information on the complex anatomical structures of the hand and the upper extremity every day to fulfill the need to verify the validity of the various procedures in practice, and to make new definitions [2,30]. This considerable dissection-based study has presented some significant findings regarding the branching pattern and formation of the DPA, and has added new information on the diameter of these vessels to correlate the findings with literature. These variations can be detected in a modified Allen's test, a Doppler ultrasonography, a pulse oximetry and an arterial angiography before the clinical implications can be understood. A comprehensive understanding of the DPA branching diameters in the hand is important in clinical procedures to support the lack of knowledge in literature. It is clear that this study will contribute to hand surgery and radiological anatomy in the future.

Acknowledgments

We would like to express our intimate gratitude to those who donated their bodies to science for the furthering of understanding and treatment of the living.

Compliance with Ethical Standards

Ethical Approval: The experimental protocols were approved by the Istanbul University, Cerrahpasa Medical School Clinical Research Ethics Committee (approval no: 02-280097). All authors were well versed in the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects – and confirmed that the present study was in full compliance with the declaration.

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

Authors' Contribution: RH: Literature search, experimental studies, data analysis, STP and MY: Concept, experimental studies, data analysis, manuscript editing.

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Is subclinical hypothyroidism a risk factor for gestational diabetes mellitus?

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ABSTRACT

Objective: Gestational diabetes mellitus is characterized by increased blood sugar that first appears during pregnancy. Multiple articles have described a relationship between hypothyroidism/subclinical hypothyroidism (SCH) and a rise in the risk of concomitant pregnancy complications, including gestational diabetes mellitus (GDM), but the effect of SCH on pregnancy is uncertain in the literature. We clarified the contribution of SCH to GDM development.

Patients and Methods: We conducted a retrospective study. From the patient records, the first 250 pregnant women who applied to our hospital for screening at 20-24 weeks and had glucose tolerance tests were included in our study. Retrospectively, all these pregnant women's first-trimester thyroid-stimulating hormone (TSH) levels were recorded. We created two groups according to the oral glucose tolerance test (OGTT) results: a case group diagnosed with GDM and a control group with average blood glucose. Their first-trimester TSH levels were compared between the two groups and defined whether they had euthyroid, subclinical hypothyroidism (TSH=2.5-5.5mIU/L) or overt hypothyroidism (TSH >5.5).

Results: We diagnosed 37 of 191 patients (19.4%) with GDM. When we checked the case and control groups, the mean TSH of the GDM group was 1.8 mIU/L, and the control group was 1.7 mIU/L, but the difference was not statistically significant (p=0.121). 24.32% (n=9) of 37 pregnant women with GDM were diagnosed with subclinical hypothyroidism/hypothyroidism; this rate was as low as 14.93% (n=28) in the non-GDM group, but no statistical difference was found (p=0.21).

Conclusion: It can be predicted that other accompanying factors may be the primary determinant in the development of GDM rather than subclinical hypothyroidism. Risk scales that include the first trimester TSH level should be established for the development of GDM.

Keywords: Gestational diabetes mellitus, Subclinical hypothyroidism, Thyroid Stimulating Hormone, Pregnancy complications

1. INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized by increased blood sugar that first appears during pregnancy [1]. Furthermore, it is the most common nonsurgical disease accompanying pregnancy and has severe implications for both mother and baby [2].

Several risk factors are defined, such as older mothers, GDM history, large for gestational age (LGA) baby, race/ethnicity, smoking, and excess body mass index [3]. On the other hand, multiple articles have described a relationship between hypothyroidism/subclinical hypothyroidism (SCH) and a rise in the risk of concomitant pregnancy complications, which include gestational diabetes, but the effect of SCH on pregnancy is uncertain [4-6].

As we know, hypothyroidism is an insufficiency of thyroid hormones; If this condition is associated with decreased thyroxine (T4) hormone (with average, high, or less TSH level), it is defined as overt hypothyroidism (OH); If the increased TSH level can keep T4 at normal ranges, it is called subclinical hypothyroidism (SCH). In fact, subclinical hypothyroidism is a compensated version of thyroid dysfunction, so its effects are limited.

Oppositely, the studies defined the relationship between SCH and GDM; some studies declared that SCH is not related to develop GDM [7,8].

Our study aims to identify how subclinical hypothyroidism contributes to the development of GDM.

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2. PATIENTS and METHODS

We conducted a retrospective study after the Ethics Committee of Izmir Katip Celebi University Atatürk Training and Research Hospital granted ethical approval for our study (No. 676). We retrospectively scanned patient records between November 1st, 2022, and January 30th, 2022 250 pregnant women who applied to our hospital for screening at 20-24 weeks and had glucose tolerance tests were included in our study.

Retrospectively, all these pregnant women's first-trimester thyroid-stimulating hormone (TSH) levels were recorded for those who had been treated according to their TSH levels.

We could not reach the TSH level records of the first trimester for 38 pregnancies, we saw that 21 patients did not come for the follow-up to our hospital after the 20-24th week of screening. Therefore, 59 patients were not included.

We evaluated 75 g oral glucose tolerance test (OGTT) results of patients according to American Diabetes Association (ADA) criteria. Patients with 1st-hour fasting blood glucose, and 2nd-hour fasting blood glucose results ≥92 mg/dL, ≥180 mg/dL, ≥153 mg/dL, respectively, were diagnosed as GDM [9].

We created two groups according to OGTT results: a case group diagnosed with GDM and a control group with average glucose levels. Their first-trimester TSH levels were compared and diagnosed as having euthyroid subclinical hypothyroidism (TSH=2.5-5.5mIU/L with normal T4) or overt hypothyroidism (TSH >5.5 mIU/L).

Statistical Analysis

IBM SPSS Statistics version 20 was used for statistical analysis. While evaluating the study data, descriptive statistics (mean, standard deviation, and frequency) were used. A students t test was used for the parameters that showed a normal distribution between the two groups. Results were evaluated with 95% confidence intervals, and significance was set at p <0.01.

3. RESULTS

The mean age of our patients was 29 years. The medium crown to rumps length (CRL) at which serum TFT samples were taken was 58.1 mm. The average TSH values was 1.8 ± 1.0 mIU/L (0.35-5.34 mIU/L) (Table I).

Table I. *Demographic variables and follow-up findings in pregnancy*

Variables	Days
Demographic Variables	
Age, median (IQR)	29 (8)
Gravida median (IQR)	2 (1)
Parity, median (IQR)	1 (2)
Variables of the follow-up findings in pregnancy	
First-trimester fasting blood glucose (mg/dL) median (IQR)	80.0 (13.0)
First-trimester (TSHmU/L) , median (IQR)	1.76 (1.0)
CRL, mm, median (IQR)	58.1 (12.5)

IQR: Interquartile range, GDM: Gestational diabetes mellitus, CRL: Crown to rump length, THS: Thyroid stimulating hormone

We have diagnosed 37 of 191 patients (19.4%) with GDM according to OGTT test results. (Figure 1).

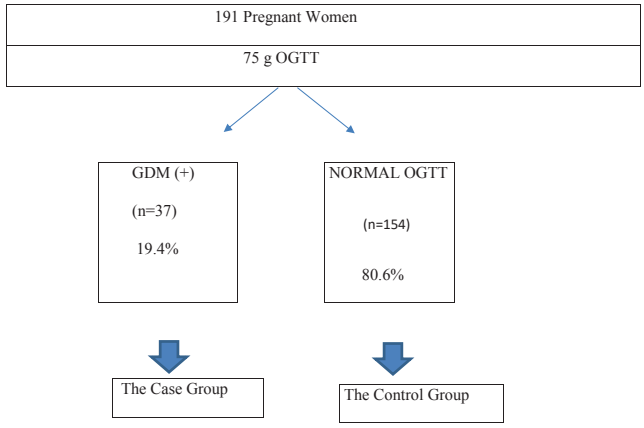

Figure 1*. Flowchart of the study*

When we compared the case and the control groups, the TSH of the GDM group was 1.8 mIU/L and that of the control group was 1.7 mIU/L, but the difference was not statistically significant. (p=0.121) (Table II). All demographic variables and follow-up findings of case and control groups are shown in Table II.

Table II. *Demographic variables and follow-up findings of case and control groups*

Variables	The non-GDM group (n=154)	The GDM group (n=37)	Univariate P value
Age. median (IQR)	27 (8)	31 (8)	0.06
Gravida median (IQR)	2 (1)	2 (1)	0.320
Parity, median (IQR)	1 (2)	1 (1)	0.257
First-trimester TSHmIU/L) , median (IQR)	1.7 (1.0)	1.8 (1.2)	0.121
CRL date, days, median (IQR)	86 (7)	86 (5)	0.902
CRL, mm, median (IQR)	59.0 (13.5)	57.0 (9.3)	0.708

IQR: Interquartile range, GDM: Gestational diabetes mellitus, CRL Crown to rump length, TSH: Thyroid stimulating hormone

Four pregnancies were diagnosed with overt hypothyroidism (TSH >5.5 mIU/L), and GDM developed in 1 (25%) of them.

Twenty-seven pregnant women were diagnosed with subclinical hypothyroidism (TSH=2.5-5.5 mIU/L), and 8 (29.62%) of them developed GDM.

One hundred sixty pregnant women were euthyroid (TSH ≤ 2.5 mIU/L) and 28 of them (17.5%) developed GDM (Table III).

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Table III. *Thyroid hormone status of the case group*

	Euthyroid TSH ≤ 2.5 mIU/L group (n=160)	Hypothyroid(SCH+OH) TSH>2.5 mIU/L group (n=31)	P value
GDM (+)	17.5% (n=28)	29.03% (n=9)	0.14

SCH: subclinical hypothyroidism, OH: overt hypothyroidism, TSH: Thyroid stimulating hormone

When all pregnant women with TSH >2.5 mIU/L and euthyroid pregnant women were compared, the lowest incidence of GDM development was in euthyroid pregnant women (29.03% vs. 17.5% p=0.14), but there was no statistically significant difference.

24.32% (n=9) of 37 pregnant with GDM were diagnosed with subclinical hypothyroidism/hypothyroidism; this rate was as low as 14.93% (n=28) in the non-GDM group, but no statistical difference was found (p=0.21) (Table IV).

Table IV*. Hypothyroidism incidence of case and control groups*

	The GDM group	The non-GDM group	P value
TSH>2.5 mIU/L	24.32% (n=9)	14.93% (n=28)	0.21

TSH: Thyroid stimulating hormone, DGM: Gestational diabetes mellitus

4. DISCUSSION

There is a consensus in the literature about the complications of overt hypothyroidism in pregnancy, but the effects of subclinical hypothyroidism are still unclear. There is much confusion about pregnancy outcomes of subclinical hypothyroidism and diagnosis of SCH in the literature.

In a study by Goldman et al. in which they evaluated 10990 pregnant women, they found that subclinical hypothyroidism in both the first and second trimesters did not increase the risk of developing gestational diabetes, on the contrary, they found a higher incidence of gestational diabetes in the euthyroid group in both trimesters [(1^{st} trim.:3.0% vs 2.6%; OR:0.86 95% CI :0.37–1.96) and 2^{nd} trim.:3.0% vs 1.7%; OR:0.63 95% CI:0.23–1.73)] [10].

In our study, twenty-seven pregnant women were diagnosed with subclinical hypothyroidism and 8 (29.62%) developed GDM. One hundred sixty pregnant women were euthyroid (TSH ≤ 2.5 mIU/L) and 28 of them (17.5%) developed GDM.

Although, some studies seem to have found a relationship between GDM and SCH, it was found that there was no statistically significant relationship in risk calculations adjusted for maternal age, weight, and parity in these studies [8]. Similarly, in our study, 24.32% (n=9) of 37 pregnant women with GDM were diagnosed with subclinical hypothyroidism/hypothyroidism; this rate was as low as 14.93% (n=28) in the non-GDM group, but no statistical difference was found (p=0.21).

Moreover, the diagnosis of subclinical hypothyroidism continues to be discussed and revised with new recommendations. First of all, the 2011 American Thyroid Association (ATA) guidelines recommended 2.5 mIU/L of the first trimester TSH upper limit during pregnancy [11]. Then, they increased the upper limit to 4 mIU/L in 2017 [12]. Otherwise, many studies indicate that if we accept the reference limit in this way, we will miss the diagnosis of many subclinical hypothyroidism [13-16]. In our study, we accepted the first trimester TSH upper reference limit as 2.5 mIU/L and defined subclinical hypothyroidism according to this. We found the incidence of GDM in the subclinical hypothyroidism group more than in the euthyroid group, however, it was not statistically significant. If we had accepted the upper limit of TSH as 4 mIU/L, we could have obtained statistically significant results.

On the other hand, many studies have shown that subclinical hypothyroidism may be associated with diabetes mellitus, and that thyroid hormones are effective in insulin resistance [17]. Increased insulin resistance in a normal pregnancy may be complicated by the additional effect of SCH [18].

When we retrospectively studied 1. trimester TSH values of the patients diagnosed with GDM, we found that the non-GDM group had similar TSH values. [1.8 mIU/L vs 1.7 mIU/L, (p=0.121)]. Similarly, in the study of Mukesh et al., no statistically significant difference was found between 80 (26.6%) women with GDM and 221 (73.4%) women without GDM for any of the thyroid function tests [19].

Additionally, Shahbaziant et al., studied thyroid functions of 61 diabetic pregnant women and compared the results with that of 35 healthy pregnant women. Higher thyroid dysfunction was detected in the GDM group, but the difference was not statistically significant [18% vs. 8.6% (P = 0.2)], also, thyroid dysfunction in GDM and the pregestational group did not have a significant difference with the control group (p =0.99, 0.054 respectively) [20].

Our study revealed that although there was no statistically significant difference, the mean TSH value was generally higher in the GDM groups. Similarly, a prospective study in which Ying et al., examined 7084 pregnant women found that subclinical hypothyroidism in early pregnancy was related to a raised risk of GDM [21].

If we classified the patients according to their TSH levels into two groups, euthyroid and subclinical hypothyroidism/hypothyroidism, the lowest incidence of GDM development was in euthyroid pregnant women (29.03% vs. 17.5% p=0.14). Although, we could not find any statistical differences in our work, similar to our study, the review of Li-Li Gong et al., reported that the relative risk of gestational diabetes was also increased in subclinical hypothyroidism with an OR of 1.558 (95% CI 1.292-1.877, p < 0.001) [22].

Furthermore, Yang et al., underlined that thyroid hormone insufficiency in the first trimester raises the risk of GDM development; for that reason, they advised that thyroid hormone levels should be defined in the early months [23]. Li-Li Gong et al., stated that hypothyroidism induces insulin resistance, disrupts glucose metabolism and increases the risk of gestational diabetes [22].

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In a recently published review, Lee et al., stated that the effects of maternal subclinical hypothyroidism on obstetric outcomes have still been debated [24]. In another review, Gietka-Czernel et al., highlighted the controversies in the diagnosis and treatment of TSH in pregnant women [25].

We scanned the patient records retrospectively for their first trimester TSH values, this could be a limitation. On the other hand, since our study group was small, the number of patients diagnosed with overt hypothyroidism was low. All of these are our limitations for making a final generalizable judgment.

Conclusion

Considering these results, although hypothyroidism is a risk parameter for the development of GDM, it can be predicted that other accompanying factors such as maternal age, weight, and parity and previous/family history may be the primary determinants in the development of GDM.

In conclusion, risk scales should be established for the development of GDM, and the first trimester TSH level should be included as an essential factor in the scale.

On the other hand, thyroid hormone-level status must be determined unconditionally in the first trimester as part of pregnancy follow-up. If there is overt or subclinical hypothyroidism in the first-trimester screening, it should be treated, and these patients should be followed closely for developing GDM.

Compliance with Ethical Standards

Ethical Approval: The Ethics Committee of Izmir Katip Celebi University Atatürk Training and Research Hospital granted ethical approval for this study (No. 676). Informed consent was obtained from all patients.

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

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Assessments of energy, macro and micronutrient intakes in children and adolescents with type 1 diabetes mellitus

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ABSTRACT

Objective: This study aims at examining dietary intake in children and adolescents with type 1 diabetes mellitus (DM) and comparing the results with national dietary intake recommendations.

Patients and Methods: One hundred fifty children and adolescents (52.7% female) with an average age of 12.2±3.1 years and with type 1 DM who were followed by the Pediatric Endocrinology Polyclinic participated in the study. Three-day food intake records and clinical information regarding the type 1 DM condition of the participants were obtained.

Results: No gender-related significant difference was found among the participants regarding food intake. The percentage of energy derived from fat (average 39.6%) and saturated fat (16.1%) were higher than the recommended levels in both gender groups. The percentage of energy derived from carbohydrates (female 44.1±5.7%, male 43.0±6.8%) was below the recommended levels. The dietary fiber intake in children aged 6-10 years with type 1 DM met recommendations, whereas, it was below the recommended levels in other age groups. Micronutrient inadequacy was common in children and adolescents with type 1 DM.

Conclusions: The authors believe that guidelines and programs are needed for children and adolescents with type 1 DM to reduce total fat and saturated fat intake, increase carbohydrate and dietary fiber intake up to the recommended levels, and prevent multiple micronutrient inadequacies.

Keywords: Type 1 Diabetes, Children, Adolescents, Nutritional Status, Dietary Intake, Macronutrient Distribution

1. INTRODUCTION

Type 1 diabetes mellitus (type 1 DM) is an autoimmune illness that results from insulin deficiency due to the autoimmune destruction of insulin-producing pancreatic β-cells characterized by hyperglycemia [1]. Some factors such as modern insulin infusions, advanced medical care, strict glycemic control, insulin types, insulin application frequency, the amount of insulin applied, bolus and basal insulin rates, the frequency of hyperglycemia-hypoglycemia and its treatment, more flexible mealtimes, more frequent snacking, and changes in the lifestyle can lead to excessive weight gain in patients with type 1 DM [2,3]. The increase in Body Mass Index (BMI) is associated with poor metabolic control and increased risk for comorbidity [4]. Controlling the amount of energy and macronutrient intake before leading to hypoglycemia is recommended for the treatment of this condition [5].

The main aim of DM treatment is to maintain glucose levels within a normal range. Metabolic control of type 1 DM depends on an effective plan of intensive insulin therapy, follow-up, and lifestyle changes. A child or person with type 1 diabetes needs to take insulin regularly and adjust their food intake and exercise according to the action and dose of insulin. Although, there are significant improvements in the treatment and technology, Medical Nutrition Therapy (MNT) is still an essential part of controlling diabetes [5,6]. This treatment's primary components are to follow the carbohydrate intake at each meal and adjust the insulin dose [7]. Effective dietary intervention may help obtain better clinical and metabolic results in children and adolescents with type 1 DM [8]. While applying the MNT, energy, and macronutrient intakes should be planned according to age, growth rate, and physical activity and these intakes should be sufficient to ensure optimal growth and maintain ideal body

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weight [5,8]. Dietary recommendations for children with diabetes are based on those recommended for healthy children. Dietary recommendations should be planned considering the child's cultural, ethnic, customary, and psychosocial needs [5]. Current nutritional recommendations include limiting intakes of simple carbohydrates, saturated fatty acids, trans-fatty acids, and salt and increasing intakes of complex carbohydrates and unsaturated fatty acids [7]. However, previous studies pointed out that the carbohydrate intake was below and saturated fat intake was above the recommended levels in children and adolescents with type 1 DM [9].

Therefore, the current paper aims to determine the energy and nutrition intakes of children and adolescents with type 1 DM and compare the results with the national dietary intake recommendations.

2. PATIENTS and METHODS

This study was carried out between December 2019-June 2020 on 150 children and adolescents aged 6-18 years with type 1 DM who were followed up at Bursa Uludağ University Health Application and Research Center Pediatric Endocrinology Polyclinic. The sample of this study was selected among the patients who were followed up in the pediatric endocrinology outpatient clinic and voluntarily agreed to participate in this study using the simple random sampling method. The ethics committee approval (no. 2018-14/38) was obtained from Bursa Uludağ Ethics Committee. And permission to conduct the study (no. 73115338-819/38160) was received from Bursa Uludağ University Directorate for Health Application and Research Center. All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patients have given their informed consent for participation in the study.

One hundred fifty children and adolescents aged 6-18 who attended the Pediatric Endocrinology Polyclinic and were diagnosed with type 1 DM (diagnosed at least 1 year ago) were included in the study. Those with chronic diseases (thyroid, celiac, etc.) and using obesity-related drugs were excluded from the study.

The children's body weight and height percentile values were calculated using the World Health Organization (WHO) AnthroPlus software. The obtained percentile values were evaluated using the WHO 2007 BMI-for-age (5-19 years) growth curves [10,11]. Body weight was measured without shoes using a Seca-813 (Kimeks, Istanbul, Turkey) professional weighing scale with a resolution of 0.1 kg. Mesilife–13539 (Mesitaş, Istanbul, Turkey) portable stadiometer with a resolution of 1 mm was used for height measurements. BMI was used to evaluate body weight according to height. BMI values were calculated using the following formula: BMI = Body weight (kg)/height (meter)^2 [12].

Nutrient Analysis

To determine the daily energy and nutrition intakes of the children and adolescents with type 1 DM, the full version of the Nutrition Information System 8.2 (BeBiS 8.2), which was developed on a computer and adapted for Turkey, was used [13]. The nutrition intake data of the children and adolescents with type 1 DM were recorded on three consecutive days (two weekdays and a weekend day). To increase the accuracy of nutrition intake data, training was given to the participants by an expert dietitian. The dietitian reviewed all nutrition intake records. The energy, carbohydrate, protein, fat, and micronutrient contents were calculated using Nutrition Information System 8.2 (BeBiS 8.2). The data obtained from the nutrition intake records were compared with the "Turkey Dietary Guidelines 2015 (TDG)" [13]. While evaluating the daily energy and nutrition intakes, ≤66% was considered poor, 67%-133% was adequate, and >133% was high [14].

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 23. The Kolmogorov-Smirnov test was used to evaluate the normality of data. Independent Samples t-test was used to compare two independent and normally distributed populations. One-way analysis of variance (ANOVA) was used to compare more than two independent populations. The obtained data are presented as mean ± standard deviation ($\bar{x}$ ± ss). Mann-Whitney U test was used for the data that are not normally distributed and the Kruskal-Wallis H test was used for three or more independent populations and the results are presented as median (minimum-maximum) values. Pearson's chi-squared test, Fisher's exact test, and Fisher-Freeman-Halton test were used to compare categorical variables and the results are presented as frequency (n, %) values. A significance level of $\alpha = 0.05$ was adopted in this study [15].

3. RESULTS

The baseline characteristics of the participants are summarized in Table I. A total of 150 children and adolescents with an average age of 12.2±3.1 years with type 1 DM were included in the study. A total of 79 participants were female (52.7%) and 71 were male (47.3%), the average type 1 DM diagnosis age was 6.9±3.3 years, and the duration of DM was 5.3±3.3 years. Average body weight, height, and BMI were 46.2±15.1 kg, 148.4±16.6 cm, and 20.3±3.4 kg/m^2, respectively. The mean HbA1c value of the participants was calculated as 9.5%±1.8. Regarding insulin therapy, 30 of the participants (20%) were using insulin pump therapy (IPT) and 120 (80%) were multiple daily injections (MDI).

The energy, macronutrients, and dietary fiber intakes by gender are presented in Table II. Daily energy, carbohydrate (g), protein (g), fat (g), cholesterol, and omega-3 intakes in boys with type 1 DM were higher than in girls ($p<0.05$). The percentages of daily energy derived from fat were similar among genders.

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Table I. *Demographic and Diabetes-Related Characteristics of Children and Adolescents with Type 1 Diabetes*

Characteristic	Amount represented
Demographics	**x̄±SD**
Age (y)	12.2±3.1
Sex	**n (%)**
Female	79 (52.7)
Male	71 (47.3)
Mother's Education	
Secondary school and below	98 (65.7)
High school	36 (24.2)
College degree and higher	15 (10.1)
Father's Education	
Secondary school and below	73 (49.7)
High school	51 (34.7)
College degree and higher	23 (15.6)
Income (TL)	
< 2500	36 (24.0)
2501-5000	73 (48.7)
>5001	41 (27.3)
Diabetes and health-related characteristics	
	x̄±SD
Height (cm)	148.4±16.6
Body Weight (kg)	46.2±15.1
BMI (kg/m²)	20.3±3.4
BMI Z-score	0.41±0.92
Type 1 DM diagnosis age (years)	6.9±3.3
Duration of Type 1 DM (years)	5.3±3.3
Total insulin dose (U/day)	42.8±18.0
Insulin dose (U/kg/day)	0.92±0.21
HbA1c (%)	9.5±1.8
Insulin regimen	n (%)
Multiple daily injections	120 (80.0)
Pump	30 (20.0)

Mean ± standard deviation (x̄±SD)

Table II. *Daily Energy and Nutrition Intakes of Children and Adolescents with Type 1 DM By Gender*

	Girls	Boys
Nutrients	x̄±SD	x̄±SD
Energy (kkal)**	1376.2±328.1	1546.5±360.3
Carbohydrate (g)*	147.8±38.9	162.5±48.6
Carbohydrate (%)	44.1±5.7	43.0±6.8
Protein (g)**	55.3±14.8	65.1±19.2
Protein (%)	16.5±2.1	17.2±2.8
Fat (g)**	61.1±17.5	68.9±18.2
Fat (%)	39.4±5.2	39.8±5.7
Cholesterol (mg)*	267.8±100.4	307.6±130.3
Saturated fat, % of energy	15.9±3.2	16.4±2.8
Monounsaturated fat, % of energy	13.7±2.2	13.9±2.7
Polyunsaturated fat, % of energy	7.4±2.1	7.3±.2.2
Omega 3*	1.2±0.4	1.4±0.6
Omega 6	9.5±3.7	10.4±4.2
Dietary fiber (g)	15.4±4.8	16.9±5.6

*Mean ± standard deviation (x̄±SD). Mann-Whitney U test, * p<0.05, ** p<0.01*

In relation to whether the nutrition recommendations provided in the TDG were met or not, the protein intakes were found to be above recommended amounts for 60.8% of the girls and 73.2% of the boys (Table III). Similarly, daily fat intakes were above recommended amounts in almost half of the boys and girls. Regarding dietary fiber, the ratio of girls with inadequate dietary fiber intake (24.1%) was higher than boys (15.5%). Furthermore, the ratio of girls with inadequate daily thiamine, vitamin B6, calcium, and iron intake was higher than boys (p<0.05). Moreover, inadequate daily iodine intake was higher in girls compared to boys (p<0.05).

Daily nutrition intakes in children and adolescents with type 1 DM and TDG's recommended amounts according to age are presented in Table IV. Daily energy intake was below recommended levels in all age groups. The carbohydrate intakes in adolescents aged 15 years and over with type 1 DM were within the recommended amounts, whereas, the carbohydrate intakes in other age groups were below the recommended doses. Fat intake was above recommended values in all age groups. The cholesterol intake was above recommended values in children aged 6–10 years, while it was within recommended levels in other age groups. On the other hand, the fiber intake was above recommended values in children aged 6–10 years, whereas it was below recommended levels in other age groups.

The daily vitamins A and K, niacin, biotin, sodium, and phosphor intakes of children and adolescents in all age groups were above recommended amounts, while vitamin D, potassium, iron, and selenium intakes were below recommended values (Table V).

Table III. *Information Regarding Meeting the Daily Nutrition Recommendations in Children and Adolescents with Type 1 DM By Gender*

Nutrients	Girls		Boys	
	Inadequate (≤66%) n (%)	Excess (>133%) n (%)	Inadequate (≤66%) n (%)	Excess (>133%) n (%)
Carbohydrates	1 (1.3)	20 (25.3)	-	23 (32.4)
Protein	3 (3.8)	48 (60.8)	-	52 (73.2)
Fat	-	36 (45.6)	-	38 (53.5)
Dietary fiber	19 (24.1)	5 (6.3)	11 (15.5)	11 (15.5)
Vitamin A	-	75 (94.9)	-	68 (95.8)
Vitamin E	35 (44.3)	2 (2.5)	30 (42.3)	6 (8.5)
Vitamin C	15 (19.0)	34 (43.0)	12 (16.9)	27 (38.0)
Thiamin	*29 (36.7)	7 (8.9)	*13 (18.3)	10 (14.1)
Riboflavin	13 (16.5)	29 (36.7)	5 (7.0)	37 (52.1)
Vitamin B_6	*28 (35.4)	16 (20.3)	*11 (15.5)	20 (28.2)
Vitamin B_{12}	18 (22.8)	23 (29.1)	10 (14.1)	29 (40.8)
Niacin	7 (8.9)	40 (50.6)	4 (5.6)	48 (67.6)
Folate	28 (35.4)	8 (10.1)	17 (23.9)	11 (15.5)
Calcium	*45 (57.0)	6 (7.6)	*26 (36.6)	3 (4.2)
Iron	*49 (62.0)	-	*27 (38.0)	3 (4.2)
Zinc	37 (46.8.)	4 (5.1)	23 (32.4)	8 (11.3)
Selenium	70 (88.6)	-	64 (90.1)	2 (2.8)
Iodine	*10 (12.7)	**28 (35.4)	*1 (1.4)	**41 (57.7)

*Inadequate (≤66%), Excess (>133%). chi-squared test, * p<0.05, ** p<0.01*

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Table IV. *Daily Energy and Macronutrients Intake of Children and Adolescents with Type 1 DM by Age*

	6-10 years		11-14 years		15 years and older	
	Recommended	Intake	Recommended	Intake	Recommended	Intake
Energy (Kkal)	1576	1473.8±292.4	1851	1420.3±339.8	2619	1503.6±454.5
Carbohydrate (g)	130	150.1±31.1	130	148.3±39.9	130	174.9±61.1
Carbohydrate (%)	45-60	40.9±5.2	45-60	41.9±5.9	45-60	46.3±6.3
Protein (g)	18.2-28.4	61.7±15.0	31.5-45.0	58.0±17.0	49.8-53.0	61.0±22.5
Protein (%)	5-20	16.7±2.1	8-20	16.3±2.4	9-20	16.1±2.9
Fat (%)	20-35	41.2±5.4	20-35	40.8±5.1	20-35	36.4±5.1
Cholesterol (mg)	<300	305.0±100.0	300	269.9±103.6	300	292.0±158.1
Omega 3 (g)	0.6-1.2	1.3±0.4	0.6-1.2	1.40±0.6	0.6-1.2	1.2±0.5
Omega 6 (g)	5-10	9.8±4.4	5-10	10.0±3.6	5-10	10.1±3.8
Dietary fiber (g)	16	17.5±5.1	19	15.0±4.7	21	16.2±6.1

Table V. *Daily Micronutrients Intake of Children and Adolescents with Type 1 DM by Age*

	6-10 years		11-14 years		15 years and older	
	Recommended	Intake	Recommended	Intake	Recommended	Intake
Vitamins						
Vitamin A (mcg)	400	1155.4±1698.6	600	957.7±787.1	750	829.5±499.4
Vitamin D (mcg)	15	14.8±10.6	15	6.6±6.5	15	7.4±8.9
Vitamin E (mg)	9	12.0±5.5	13	10.8±3.8	13	11.5±4.1
Vitamin K (mcg)	55	106.0±79.2	60	85.3±63.5	75	92.1±76.9
B_1 (mg)	0.6	0.80±0.24	0.9	0.69±0.2	1.2	0.7±0.2
B_2 (mg)	0.6	1.45±0.41	0.9	1.1±0.3	1.3	1.0±0.4
Niacin (mg)	10	10.4±4.4	10	10.1±04.4	10	10.4±5.4
pantothenic acid (mg)	4	4.8±1.2	5	4.1±1.1	5	4.2±1.5
B_6 (mg)	0.6	1.0±0.3	1	0.90±0.2	1.3	0.8±0.3
Biotin (mcg)	25	50.1±17.5	35	37.4±12.6	35	36.6±16.4
Folic acid (mcg)	200	242.5±71.8	270	211.4±61.3	330	217.9±71.7
B_{12} (mcg)	2.5	4.24±1.89	3.5	3.9±2.6	4	3.3±1.7
Vitamin C(mg)	45	103.2±45.1	70	77.4±39.3	100	74.9±37.1
Minerals						
Sodium (mg)	1200	3118.9±818.4	1500	3219.1±1040.7	1500	3411.6±1213.6
Potassium (mg)	3800	2390.5±633.9	4500	1944.3±545.1	4700	1929.2±582.5
Calcium (mg)	800	863.2±258.9	1150	714.6±259.2	1150	660.3±257.2
Magnesium (mg)	230	238.0±59.1	300	208.6±59.4	300	215.5±69.9
Phosphor (mg)	440	1074.4±259.2	640	930.9±258.2	640	937.0±339.4
Iron (mg)	11	7.8±2.0	11	7.4±2.2	11	7.7±2.5
Zinc (mg)	7.4	8.4±2.2	10.7	8.0±2.4	14.2	7.9±2.9
Selenium (mcg)	35	18.5±8.9	55	15.2±12.1	70	15.5±10.3

4. DISCUSSION

Only a few studies have examined the nutrient intake of children and adolescents with type 1 DM in Turkey. Accordingly, the present study aimed to compare the energy and nutrient intakes in children and adolescents with type 1 DM in Turkey with the national nutrition guide (TUBER) [14]. The findings showed that the energy intake in all participants was lower than the recommended amount and the percentage of energy from fat was higher than the recommendation. An examination of dietary fiber intake indicated that only the participants in the 6–10-year-old group took more dietary fiber than recommended. Multiple micronutrient intake deficiencies were observed in all participants. This was particularly pronounced in participants aged 15 years and over.

Reducing carbohydrate intake is used to arrange insulin dosing and balance postprandial glucose fluctuations [9,15]. TDG recommended that the percentage of energy derived from

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Marmara Med J 2023;36(2): 235-241

carbohydrates should be at least 45%. According to the commonly used carbohydrate counting model for calculating insulin dose, the percentage of energy derived from carbohydrates is recommended to be 50%. Our findings indicated that the carbohydrate intake in children and adolescents was below recommended levels. Similar results were reported in previous studies [9,16]. Although, carbohydrate intake (g) in boys was higher than in girls, the contribution of carbohydrates to energy was lower in boys. The analysis of macronutrient intake by age revealed that the lowest carbohydrate intake was found in children aged 6–10 years (40.9±5.2%), whereas, the contribution of carbohydrates to energy was increased at older ages (15 years and over; 46.3±6.3%).

According to the TDG and The International Society for Pediatric and Adolescent Diabetes (ISPAD), the percentage of energy derived from fat and saturated fat should be 30-35% and <10%, respectively [5,14]. Although, carbohydrate intake was below recommended values, fat (g) intake in the diet and the percentage of energy derived from fat (39.6±5.4%) and saturated fat (16.1±3.0%) were above recommended levels in our participants. According to the previous studies that examined the nutrition status of children and adolescents with type 1 DM, the percentage of energy derived from fat and saturated fat was found to be above recommended levels [9,16,17]. Seckold et al., examined nutritional status of children aged <7 years with type 1 DM and found that the energy derived from fat was within recommended amounts (33±5%), whereas, the energy derived from saturated fat was above the recommended amount (15±3%) [18]. Our results indicated that fat intake in 45.6% of the girls and 53.5% of the boys were above recommended levels. Among different age groups, while the highest fat intake was found in children aged 6-10 years (41.2±5.4%), the lowest fat intake was observed in the participants aged 15 years and over (36.4±5.1%). These results suggest that children's eating habits change and they consume more high-carbohydrate foods as they grow older. Mackey et al., stated that a higher proportion of energy derived from fat is associated with poor glycemic control [16]. Moreover, according to the Diabetes Control and Complications Trial, a diet high in total and saturated fat is associated with the worst HbA1c values [19].

Our findings indicated that daily protein intake (g) in boys was higher than in girls. The difference between boys and girls was statistically significant ($p<0.05$). For all participants, the percentage of energy derived from protein (16.8±2.5%) was within the recommended levels (15-20%) reported by both TDG and ISPAD [5,13]. Similarly, previous studies reported that the protein intake in children and adolescents was within recommended levels [17,20,21].

Our results showed that the mean fiber intake was 16.1±5.3 g/day. Although the fiber intake in girls was lower than in boys, the difference was not statistically significant. While the fiber intake in 6-10-year-old children was above recommended amounts, it was below in other age groups. Similarly, Stechova et al., found that fiber intake in participants with type 1 DM was below recommended amounts (median: 42% of recommended intake) [22]. Lamichhane et al., stated that the fiber intake of 908 children and adolescents with type 1 DM was below the amount recommended by the American Diabetes Association (14g/1000kcal/day). Fiber intake is associated with improved daily blood glucose concentration and glycemic control development in the long term [23]. However, Thomson et al., reported that 84% of 8-18-year-old Australian children with type 1 diabetes met their recommended fiber intake [24]. Higher fiber intake reduces inflammation and lowers mortality risk in adults with type 1 DM [16]. Therefore consumption of fresh vegetables and fruits and unprocessed foods with high fiber among children and adolescents with type 1 DM should be encouraged.

According to the analysis of daily micronutrient intake, micronutrient intake in children aged 6-10 years was within recommended levels except for iron and selenium. However, vitamin E, thiamine, vitamin B_6, folic acid, pantothenic acid, iron, zinc, calcium, and selenium intakes (the required amounts increase as children grow older) in adolescents aged 11-14 years and 15 years and above with type 1 DM were below recommended levels. Parthasarathy et al., found that mean riboflavin, β-carotene, zinc, and iron intakes in 70 Indian children and adolescents with type 1 DM were below 50%, and thiamine and calcium intakes in the same population were below 60% of the recommended values. They stated that children and adolescents with type 1 DM may be at risk of inadequate intakes of multiple micronutrients [25]. Koç et al., conducted a study with 52 Turkish individuals aged 2-18 years with type 1 DM and determined that intakes of vitamin A in 71.2% of the participants, vitamin E in 86.5%, folate in 76.9%, vitamin C in 32%, and potassium in 100% of the participants were below the recommended levels [26]. In the present study, intakes of multiple micronutrients in children and adolescents with type 1 DM were found to be inadequate as reported in previous studies [25,27]. Inadequate intakes of thiamine, vitamin B6, calcium, iron, and iodine were higher in girls than boys and the difference was statistically significant. Calcium intake in 57% of the girls and iron intake in 62% were found to be inadequate. These results suggest that girls with Type 1 DM consume fewer foods rich in vitamins and minerals than boys and therefore, are more vulnerable to multiple micronutrient inadequacy.

It is known that there is no specific nutrition guidance for children and adolescents with type 1 DM and their needs are similar to healthy peers. Nutrition suggestions for type 1 DM focus on optimal glycemic control and ensuring growth and development. Our findings indicate that the diets of children and adolescents with type 1 DM include an unbalanced macronutrient proportion and some inadequate micronutrient intakes. Poor adherence to medical nutrition therapy may lead to health complications in the long term. Therefore, the authors suggest that all healthcare professionals involved in the treatment and follow-up of type 1 DM should encourage patients to perform medical nutrition therapy.

The study's cross-sectional design and the random sampling of the participants may have weakened the representativeness of the sample and the generalizability of the findings.

Selecting participants from a single clinic may reduce the representativeness of the results on nutritional status. Therefore, comprehensive studies are needed to evaluate the nutritional intake of children and adolescents with type 1 Diabetes and to establish effective policies.

Compliance with Ethical Standards

Ethical Approval: Ethics committee approval was received before conducting the study (No: 2018-14/38). This study was conducted in accordance with the Declaration of Helsinki. Informed consents were obtained from both children and their parents. All patients provided written informed consent.

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Authors' Contribution: Both authors contributed equally.

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How does an additional insulin dose for a high-fat, high-protein breakfast affect glysemic response in adolescents with type 1 diabetes?

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ABSTRACT

Objective: In this study, it was aimed to evaluate the effects of an additional insulin dose for high-fat and high-protein meal on blood glucose levels in adolescents with type 1 diabetes.

Patients and Methods: This study was single-center, crossover, and randomized. Seven adolescents with type 1 diabetes between the ages of 14 and 17 were given breakfast containing high-fat (45.9% energy) and high-protein (21.9% energy) for two consecutive days, and two different insulin doses were administered. According to the first application dose of carbohydrate/insulin ratio, the second application was given this dose of additional insulin up to 30% in postprandial 180th minute. Blood glucose was monitored for 360 minutes at 30-minute intervals using a continuous glucose monitoring system (CGMS).

Results: The average time spent in the target range (TIR) of participants was 30.6±11.83%, and time spent in hyperglycemia and hypoglycemia (time above range (TAR) and time below range (TBR)) were 67.0±14.31% and 2.4±4.89%, respectively. There was no statistically significant difference between the early (0-120th min), late (120-360th min), and total (0-360th min) glycemic responses of the applications (p>0.05). According to CGMS result, mean blood glucose, glycemic variability, and absolute blood glucose difference median and mean absolute deviation (MAD%) were found to be similar after two applications (p>0.05).

Conclusion: Insulin dose applications should be individually calculated to prevent delayed-prolonged postprandial hyperglycemia caused by high-fat high – protein intake in adolescents with type 1 diabetes.

Keywords: Type 1 diabetes, Preprandial insulin, Fat and protein counting, Meal pattern

1. INTRODUCTION

Type 1 diabetes (diabetes mellitus) is defined as a disease resulting from the autoimmune destruction of insulin-producing pancreatic beta cells [1]. Maintaining good glycemic control is extremely important in preventing microvascular and macrovascular complications associated with diabetes in the management of type 1 diabetes, whose prevalence is increasing today. Glycemic control requires insulin therapy, healthy nutrition, controlled carbohydrate intake, balanced meals in terms of macronutrients, regular exercise, and close monitoring [2]. Postprandial hyperglycemia is an important risk factor in the development of diabetes complications. Therefore, providing postprandial blood glucose control is one of the main treatment goals in reducing the risk of complications in individuals with type 1 diabetes [3].

Carbohydrates are the main nutrients that affect the postprandial blood glucose and determine the prandial insulin requirement [4]. Therefore, in the management of diabetes in individuals with type 1 diabetes who receive intensive insulin therapy, algorithms based on the number of carbohydrates in the meal are used to calculate the pre-prandial insulin dose [5, 6]. However, postprandial glycemic excursions can be seen after the consumption of meals with high-fat and high-protein with

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insulin dose calculated based on carbohydrate counting [7-10]. Because the consumption of meals with high-fat and high-protein in children and adolescents with type 1 diabetes causes delayed hyperglycemia (which can last up to 3-6 hours after meals) and an increase in insulin requirement. In addition, high-fat and high-protein meals may cause hypoglycemia risk in the early period (1-2 hours) due to delaying gastric emptying and digestion [2]. Therefore, in determining the pre-prandial insulin doses, the effect of fat and protein on blood glucose should be considered in addition to the carbohydrate counting [7, 11, 12]. However, there is no accepted algorithm developed based on the fat and protein content of meals in addition to the amount of carbohydrates in determining the pre-prandial insulin doses. Therefore, it was aimed to evaluate the effect of additional doses of insulin on postprandial blood glucose of adolescents with type 1 diabetes after eating breakfast with high-fat and high-protein in the current study.

2. PATIENTS and METHODS

Place, Time and Sample of the Study

In this study which was carried out between April 2018 and June 2018, 7 adolescents (4 males and 3 females) with type 1 diabetes mellitus for more than a year, whose ages ranged from 14 to 17, were followed up in the Child and Adolescent Endocrine Policlinic of Ankara University. These adolescents who received an intensive insulin therapy (3 times rapid-acting and one long-acting insulin per day), who had insulin requirement > 0.5 kg / U / day, who had carbohydrate counting at least for 6 months, and who had a determined carbohydrate/insulin (C/I) rate were included. Diagnosed with celiac, hyperlipidemia, gastric motility problems, and other complications related to diabetes (neuropathy, nephropathy, retinopathy), exercising 24 hours before test meals, having hypoglycemia or ketoacidosis, overweight or obese (BMI z score for age ≥1SD and ≥2SD), individuals with diabetes who were not in the follicular or periovulatory phase of the menstrual cycle were not included in the study. During the study period, adolescents with type 1 diabetes mellitus and their families who applied to the polyclinic were interviewed by the research team. In these interviews, the research team informed the participants about the process of the study and what they expect from them during this study. As a result of sixty interviews, 16 individuals and their families volunteered, while 7 volunteers who met the inclusion criteria attended.

The study was conducted with the Ethics Committee Approvals of Ankara University Clinic Studies Ethical Committee (18-1163-17) and Turkish Republic Ministry of Health Turkey Pharmaceuticals and Medical Devices Agency (93189304-514.04.01-E.245193).

Study Design

In this randomized and crossover study, the effect of two different insulin doses administered for a high-fat and high-protein breakfast on postprandial blood glucose was evaluated.

Test Breakfast and Insulin Regimes

Adolescents with type 1 diabetes mellitus were given a breakfast containing high – fat (35.6 g; 45.9% energy) and high-protein (38.2 g; 21.9% energy) for two consecutive days (Table I). Their blood glucose was followed for 360 minutes with the continuous glucose monitoring system sensor (CGMS®; Medtronic *i*Pro2 system Northridge, CA, USA). In order to eliminate the effect of other meals on blood glucose, insulin dose was intervened at breakfast [9, 13]. A correction dose was not administered at least 4 hours before breakfast.

Applications were sustained by the research team in the Nutrition Laboratory at the University. Breakfast was prepared by the researchers for each participant separately in the kitchen of this Nutrition Laboratory. On the application days, the participants were made available at 08:45 at the latest, and the breakfast was given at 09:00. Participants were asked to finish breakfast within 20 minutes, not consume food unless necessary for hypoglycemia treatment within 6 hours after consumption, and not to do excessive physical activity, and a suitable environment was provided for this. Their meals other than breakfast were not intervened, and they were informed about their routine nutritional treatment.

Table I. *Content of high-fat and high-protein breakfast*

Test Breakfast	Carbohydrate (g)	Protein (g)	Fat (g)	Energy (kcal)
200 mL whole-fat cow milk	9.4	6	6.6	121
2 whole eggs (fried in sunflower oil)	3.2	12.8	7.2	128.8
2 egg white (fried in sunflower oil)*	0.5	5.9	0.1	26.5
10 mL sunflower oil	0	0	10	90
60 g whole-fat white cheese	2.1	8.4	10.2	133.8
75 g white bread	40.9	5.1	1.5	197.5
Total	56.1	38.2	35.6	697.6
Percentage distribution of energy	32.2%	21.9%	45.9%	

**Nutrients were calculated according to the label information of foods. Data of https://fdc.nal.usda.gov/ was used only for egg white.*

In the first application, the pre-prandial insulin dose calculated according to the individual C/I ratio was administered. In the second application, the insulin dose was determined based on the studies in the international literature [11]. Accordingly, 30% of the insulin dose calculated according to the C/I ratio was applied additionally at the 180th minute, considering the recommendations made for the prevention of hyperglycemia experienced in the 3rd hour after a meal containing high-fat and high-protein. Prandial fast-acting insulin (Lispro) was injected into the area on the arm after the first blood sample was taken 10 minutes before the breakfast.

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Postprandial Glycemia and Glycemic Response

Adolescents with type 1 diabetes mellitus were invited the day before the test meal was given and CGMS was implanted subcutaneously in the brachium. The patients were said to apply insulin injection in areas at least 7 cm from CGMS during the study period. In addition, adolescents with type 1 diabetes were asked to record their capillary fasting and pre-sleep blood glucose with their own glucometer for the calibration of CGMS. During the study (6 hours), in addition to the CGMS data, blood glucose measured by the researchers at the beginning and at the 180th minute were recorded with the same glucometer. At the end of the applications, CGMS data were computerized for evaluation. From CGMS data, blood glucose at 13 measurement times between 6 hours every 30 minutes, time in range/TIR (70-180 mg/dL%), time above range (TAR) (>180 mg/dL%), time below range (TBR) (<70 mg/dL%), median absolute relative percent difference (MAD%), and mean blood glucose (mg/dL) were recorded. In addition, using the standard deviation (mg/dL) and standard deviation/mean blood glucose formula, glycemic variability/fluctuation (CV/GV) was calculated.

Statistical Analysis

The quantitative data obtained as a result of the research were expressed as mean ($\bar{x}$), standard deviation (SD), lower and upper values. The compliance of the data to normal distribution was examined with the "Shapiro Wilk test" and the change between the two applications was evaluated with the "Paired-Samples T-test". SPSS 15.0.1 statistical package program was used in the statistical analysis of the data. In the analysis of all hypothesis tests, the level of significance was set as $p<0.05$.

3. RESULTS

A total of 7 adolescents with type 1 diabetes mellitus, 3 girls and 4 boys, with a mean age of 15.3±1.11 years, continuing secondary school (n=3) and high school (n=4) education, participated in the study. Body height and BMI percentiles of adolescents for age were 50.3±15.29 and 63.9±26.69, respectively. Participants level of knowledge about diabetes is shown in Table II. Accordingly, the time in range (TIR) was determined as 30.6±11.83%, and the time spent in hyperglycemia and the time spent in hypoglycemia was 67.0±14.31%, and 2.4±4.89%, respectively during the applications. The mean HbA1c determined in the last outpatient clinic visits of the participants was 8.8±1.06%.

None of the adolescents experienced hypoglycemia during the applications. Mean postprandial blood glucose measured at intervals after the applications are shown in Table III. In both applications, the initial blood glucose was high and the mean blood glucose was above the target range. On the 180th and 210th minutes of the second application, the blood glucose was found to be statistically higher compared to the first application ($p<0.05$). There was no significant difference between the two applications at the other measurement times ($p>0.05$, Table III).

Table II. *Diabetes information of adolescents with type 1 diabetes mellitus*

Characteristics	$\bar{x}$ ±SD (lower-upper)
Age of diagnosis (year)	9.7±3.85 (5.5-14)
Age of diabetes mellitus (year)	5.6±4.36 (1-10.5)
Baseline HbA1c (%)	8.8±1.06 (7.3-10)
TIR (%)	30.6±11.83 (16-50)
TAR (%)	67.0±14.31 (46-84)
TBR (%)	2.4±4.89 (0-13)
Duration of the use of carbohydrate counting method (year)	3.8±2.94 (0.5-9)
Insulin requirement (IU/kg/day)	0.9±0.17 (0.7-1.1)
Proportion of bolus insulin dose in total daily insulin dose (%)	61.3±11.78 (38.9-73.3)
Individual C/I ratio for breakfast	6.8±3.87 (4-15)
Individual insulin sensitivity factor (mg/dL)	37.2±9.14 (29-50)
Insulin dose required for breakfast (IU)	9.7±3.71 (3.7-13.8)

TIR: time-in-range, TAR: time-above-range, TBR: time-below-range, C/I: Carbohydrate insulin ratio.

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Marmara Med J 2023;36(2): 242-248

Table III. *Mean postprandial blood glucose measured at intervals after the applications*

Postprandial Blood Glucose (mg/dL)							
Minute intervals	**First application**	**Second application**	**p***	**Minute intervals**	**First application**	**Second application**	**p***
	$\bar{X}$ ±SD	$\bar{X}$ ±SD			$\bar{X}$ ±SD	$\bar{X}$ ±SD	
Baseline	232.4±37.00	230.4±42.00	0.923	210	185.4±39.93	214.9±45.13	0.040**
30	237.9±25.75	235.0±46.79	0.906	240	179.4±44.49	196.9±47.87	0.118
60	243.4±30.81	238.1±50.34	0.843	270	172.3±49.10	180.6±52.27	0.454
90	231.9±30.47	241.4±44.93	0.687	300	187.43±68.71	167.0±52.92	0.447
120	218.7±22.49	243.6±40.70	0.195	330	169.7±42.77	154.8±41.42	0.277
150	205.0±25.34	237.7±39.77	0.056	360	176.7±37.31	166.0±36.94	0.441
180	193.1±30.85	231.0±42.6	0.023**				

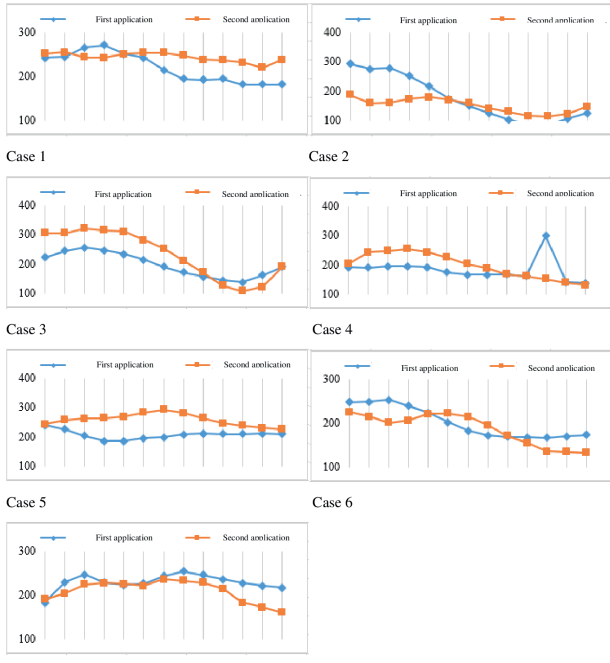
**Paired-Samples T test **p<0.05*

When the glycemic responses of participants to breakfast after different doses of insulin were examined, no significant difference was determined between the two different applications in terms of early, late and total glycemic responses (p>0.05). Mean blood glucose, glycemic variability, and MAD determined, based on CGMS data were found to be similar on the application days (p>0.05, Table IV). However, it was observed that blood glucose responses differ individually after consumption of high-fat and high-protein breakfast, and the additional dose of insulin administered for fat and protein at the 180th minute reduced hyperglycemia in four individuals (Figure 1).

Table IV. *Glycemic responses of adolescents with type 1 diabetes mellitus after two different applications*

Glycemic Responses (mg/dL*min) (AUC)	First application	Second application	p*
	$\bar{X}$ ±SD	$\bar{X}$ ±SD	
Early glycemic response (0-<120 min)	28161.4±3109.54	28545.0±5384.82	0.889
Late glycemic response (120-360 min)	44120.0±8717.92	46430.0±9679.40	0.416
Total glycemic response (0-360 min)	71830.0±7301.48	74765.0±13630.20	0.553
MBG (CGMS) (mg/dL)	195.1±43.13 (116-246)	198.1±41.10 (150-267)	0.841
CV/GV (CGMS) (%)	27.0±11.99 (9.4-44)	28.9±10.13 (13.4-46.7)	0.531
MARD (CGMS) (%)	14.7±10.00 (3.5-33.3)	26.0±26.79 (4.6-83.4)	0.274

**Paired-Samples T test, AUC: area of under the curve, MBG: mean blood glucose, CV/GV: glycemic variability/fluctuation, CGMS: continuous glucose monitoring system, MARD: median absolute relative percent difference*



** Vertical lines indicate 30-minute intervals.*

Figure 1. *Individual blood glucose monitoring results of adolescents with type 1 diabetes*

4. DISCUSSION

It was aimed to evaluate the effect of an additional 30% insulin dose calculated according to the individual C/I ratio for the breakfast with high-fat and high – protein on the postprandial blood glucose. The last measured mean HbA1c of the participants was 8.1±1.11% (Table II). According to the International Society for Pediatric and Adolescent Diabetes Clinical Practice Consensus Guidelines (ISPAD) [2], it is suggested that the

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HbA1c of type 1 diabetes patients should be kept below 7%. In addition, it is stated that with the inclusion of continuous blood glucose measurement systems in diabetes management, the glycemic control should not be evaluated only with the HbA1c, but also with the TIR, TAR, and TBR [14, 15]. Especially, the TIR was found to be associated with chronic complications of diabetes, and in the Diabetes Control and Complications Study (DCCT), a relationship was detected between the percentage of TIR, TAR, and the risk of developing diabetic retinopathy and microalbuminuria [16, 17]. In individuals with type 1 diabetes, the targets are TIR (70-180 mg/dL) >70%, TAR (181-250 mg/dL) <25%, (>250 mg/dL) <5% and TBR (54-70 mg/dL), <4%, (<54 mg/dL) <1% [18]. In this study, the mean TIR, TAR, and TBR of adolescents were determined as 30.6±11.83%, 67.0±14.31%, and 2.4±4.89%, respectively (Table II). These results show that in addition to the HbA1c, TIR and TAR ratios of participants are also high. Especially, the mean TAR was remarkably above the recommended range. Independently from HbA1c, this result points out that the blood glucose levels are above the normal range in times that are not measured from capillary blood. On the other hand, it may be difficult to reach glycemic goals due to reasons such as frequent meals outside the home, missing an insulin injection, skipping meals, not sharing diabetes management with their parents, sleeping until late hours, which are common behaviors in adolescence [2, 19]. The fact that adolescents with good diabetes management could not be reached in this study is the most important limitation of the study.

As a result of the study, it was determined that breakfast with high-fat and high – protein showed individual efficacy in terms of late postprandial glycemia. Blood glucose excursions of adolescents after consuming high-fat and high-protein breakfast were clearly different from each other (Figure I). Studies in both adolescents and adults show that meals with high-fat and high-protein delay postprandial hyperglycemia and reduce early postprandial response [9, 10]. In the study of Smart et al., it was found that when both fat and protein were included in the meal, there was a significant increase and delay in the postprandial glycemic response, and the raise lasted longer [9]. In another study, high-protein meal resulted in hyperglycemia with the peak level at 3.5 hours and continued for 5 hours postprandial, while high-fat meal caused early hyperglycemia that reached the peak at 2 hours then declined within 5 hours. The results showed that the protein and fat contents of meals affect the timing and values of the peak blood glucose as well as the duration of postprandial hyperglycemia [20]. In this study, similar to the blood glucose response of participants towards high-fat, high-protein breakfast, both the peak glucose time and the peak glucose level of the patients were obviously different from each other (Figure I). According to the results of the study by Keating et al., the total mean insulin requirements of adolescents with type 1 diabetes mellitus for the high – protein and high-fat meal were significantly greater almost than for the low protein and low-fat meal [21]. These results, similar to our study, prove that high protein and high fat content meals definitely cause an increase in blood glucose levels and require additional bolus insulin doses in type 1 diabetes. This situation may have various reasons. For instance, dietary fats and free fatty acids cause increased glycemic response and prolonged blood glucose peak time by delaying gastric emptying and digestion, reducing insulin sensitivity, and increasing hepatic glucose levels [2, 9, 22]. Also, protein causes delayed hyperglycemia through increased glucagon secretion and gluconeogenesis [9]. These explain why meals with high – fat and high-protein increase the insulin requirement. However, the biggest problem in this issue is that how many additional insulin doses are required for high-protein high-fat meals, and when should these additional insulin doses be applied.

In this study, the test breakfast was given to the participants and blood glucose was monitored for 360 minutes continuously. According to this, it was found that only the blood glucose measured at the 180th and 210th minutes between the two applications was statistically different, and blood glucose was significantly lower on the first application ($p<0.05$) (Table III). This result shows that the additional insulin application did not completely exhibit the expected effect on blood glucose. One of the most important reasons for this result is that additional insulin application was not responsible for the high levels at this minute, since the additional dose of insulin was administered at the 180th minute. The fact that the blood glucose was already high at the 180th minute on the day of the additional dose of insulin may explain the increase in the next measurement, which was the 210th minute measurement. At this point, the closure of the significant difference between the two applications with additional insulin doses, especially between 240-360 minutes, suggests that the additional dose of insulin may have an effect on delayed postprandial hyperglycemia. It also shows that the amount of fat and protein in meals should be taken into account in the calculation of the prandial insulin dose. However, the lack of significant difference between the two insulin applications in early, late, and total glycemic responses, the similar mean CGMS values (blood glucose, glycemic variability, and median absolute relative percent difference) also create a contradiction (Table IV). This may be due to the fact that the participants had high initial blood glucose on the application days and they did not use the continuous subcutaneous insulin infusion, and the additional insulin dose was administered as bolus rather than as spreading. The reason for delayed and prolonged postprandial hyperglycemia after meals with high-fat and high-protein is especially the administration of insufficient doses of insulin [23]. While there is no common recommended consensus regarding the calculation of the pre-prandial insulin dose and the time of administration when a mixed meal is consumed, some researchers give some recommendations based on their clinical experience [9, 10, 24-26]. One of these suggestions is to increase the prandial insulin dose by 30-35% and to give this dose by spreading or dividing in various combinations [24]. In another study, it was stated that for a meal with high-fat and high-protein, up to 65% of the prandial insulin dose can be added [25]. One of the most important recommendations to be taken into account is that the glycemic response to fat and protein may differ individually and therefore the prandial bolus dose should be calculated individually based on postprandial glucose monitoring for up to

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6 hours [10]. Exactly at this point, the results of our study also support these data. Considering the individual variability of the blood glucose response to a high-fat and high – protein breakfast, it indicates that it may be more appropriate to determine the additional dose according to the individual response. Therefore, Figure 1 clearly shows the differences in glycemic response after consumption breakfast with high – fat and high-protein. In new algorithms for excursions in postprandial glucose caused by high fat and high protein-containing meals, it is recommended that the additional insulin dose that is calculated for fat and protein in the meal should be given as spreading [27]. However, the results of the studies conducted and the fact that this application causes clinically significant hypoglycemia is accepted as a limitation of this method [11, 13, 27]. In this study, it should be kept in mind that the effects of different fat and protein types on blood glucose may be different, as may be the reason why the expected effects of additional doses of insulin as a bolus were not observed. There are studies showing that animal protein has a greater effect on postprandial glycemic response [28, 29]. Also, it has been reported that saturated fatty acids increase postprandial blood glucose more than unsaturated fatty acids by decreasing insulin sensitivity and thus increasing HbA1c [30, 31]. Thirteen individuals with type 1 diabetes using insulin pumps participated in the study investigating that the type of fat in meals with high and low glycemic index affects the postprandial blood glucose response. In the randomized controlled study, postprandial blood glucose after consuming a meal with olive oil was significantly lower compared to blood glucose after consuming a low-fat and butter meal ($p<0.0001$) [30]. In this study, sunflower oil rich in unsaturated fatty acids was used as visible oil in test breakfast. Therefore, it may be important and necessary to consider the source of fat and protein as well as the amount of fat and protein in the meal in calculating the prandial insulin dose.

Calculating the prandial insulin dose by considering the amount of fat and protein in addition to the number of carbohydrates in the meal in individuals with type 1 diabetes may affect the postprandial glycemic response. However, postprandial blood glucose should be monitored continuously and individually for at least 6 hours in order to determine the effect of a meal with high-fat and high-protein on blood glucose and increased insulin requirement. For this reason, it is an important requirement that children and adolescents with type 1 diabetes and people responsible for their care be educated at regular intervals by creating a common language with a specialist diabetes team. Within the scope of this training, it should be emphasized that especially the importance of adequate and balanced nutrition and the consumption frequency and number of foods with high fat should be reduced as much as possible. In addition, further studies with more participants are needed to be conducted to determine the additional insulin dose applied for observing effects of high-fat and high-protein meals on glycemia in adolescents with type 1 diabetes mellitus.

Strengths of the study: Participants were followed for 6 hours to observe the long-term effects of high-fat and high-protein breakfast on their blood glucose in the study. During these applications, their blood glucose values were continuously monitored by using the sensor. Depending on the sensor results, not only their blood glucose but also their parameters such as TIR, which have recently become a criterion in the metabolic control of diabetes, were evaluated.

Limitations of the study: There are some limitations to the study. Firstly, in this study, a prandial 30% additional dose was not applied. In order to compare the effects of different additional insulin regimens on blood glucose applied for the high-fat and high-protein, this application should be added. Secondly, data of anthropometric measurements and body composition of the adolescents would have been guided the interpretation of the results. However, these parameters should be questioned in future studies. Finally, we believe that the food consumption and physical activity records of adolescents should be taken and determined. However, in this study, none of the participants were amateur or professional athletes. It was ensured that physical activity was minimized only during the study.

Compliance with Ethical Standards

Ethical Approval: This study was conducted in accordance with the Helsinki Declaration and was approved by Ankara University Clinic Studies Ethical Committee (18-1163-17) and Turkish Republic Ministry of Health Turkey Pharmaceuticals and Medical Devices Agency (93189304-514.04.01-E.245193). All the participants and their parents were informed about the study and their verbal and written consent was obtained.

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

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Authors' Contributions: ABG and AK: Study design, data analysis, writing the article, ABG, AK, and ZS: Data collection, AK, ZS, and MB: Supervision. All authors read the article and approved the final version of the article.

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Review Article

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Crohn's disease: Etiology, pathogenesis and treatment strategies

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ABSTRACT

Crohn's disease (CD), which can be localized in any part of the gastrointestinal tract, is a disease characterized by an irregular immune response to normal and/or abnormal microbial antigens. Recent studies show many extensive data about the roles of genetic and environmental factors, immune function, and gut microbiota in CD. Although, less invasive biomarkers are currently being developed, the diagnosis of the disease is still based on the endoscopy and histological evaluation of biopsy samples. The most common symptoms are diarrhea, abdominal pain, weight loss, and fatigue. Despite the improvements in the treatment methods in the last decade, there is no definitive treatment since the etiology of CD is not known exactly. Therapeutic strategies focus on reducing inflammation and symptoms, maintaining clinical remission, and improving quality of life.

Keywords: Crohn's Disease, Inflammatory bowel disease, Intestinal microbiota, Nutrition therapy

1. INTRODUCTION

Inflammatory bowel disease (IBD) represents a group of intestinal disorders including Crohn's disease (CD) and ulcerative colitis (UC) with clinical, epidemiological and pathological findings. It is characterized by acute and chronic inflammation of the gastrointestinal tract with unknown etiology [1]. Although, it is known as a disease of western societies, the incidence of IBD has increased rapidly in newly industrialized countries since the beginning of the 21st century [2]. It is estimated that currently, approximately nearly 3.9 million females and nearly 3.0 million males are living with IBD worldwide and the number of prevalent cases is on the rise [3]. In Turkey, the incidence of IBD has been reported as 1.4/100000 for CD and 2.6/100000 for UC, while its prevalence has been reported as 130/100000 and 100/100000, respectively [4, 5]. Both forms of IBD share similar clinical features including diarrhea, fever, weight loss, anemia, food intolerance, malnutrition, growth retardation, and extraintestinal situations such as arthritis, dermatologic and hepatic findings [4]. CD is an inflammatory condition of the gastrointestinal tract with focal, asymmetric, and transmural involvement that can occur anywhere between the mouth and the anus. On the other hand, UC is characterized by recurrent inflammation of the colonic sections limited to the mucosa [6]. In this review, the etiology, pathogenesis and treatment strategies of CD will be discussed in detail with updated information.

2. DIAGNOSIS and CLASSIFICATION

The first step in diagnosis of CD requires a detailed history (including family, social and medical) and physical examination. If CD has been potentially diagnosed, laboratory findings such as erythrocyte sedimentary rate, C-reactive protein, and leukocyte and platelet counts are examined [7]. Medical imaging can be used to confirm the diagnosis and to monitor the disease activity. Endoscopy procedure is useful to directly view the affected areas, to determine the degree of disease involvement, and for biopsy [8]. Intestinal biopsy is confirmatory rather than diagnostic [5]. Endoscopic biopsy is important to differentiate the CD from UC and to exclude acute colitis, dysplasia, or cancer [9].

Crohn's disease is clinically divided into 3 phenotypic subclasses; inflammatory, fistulizing, and obstructive. In the inflammatory subtype, diarrhea, abdominal pain, weight loss, and fever are the prominent findings. In the fistulizing subtype, intraabdominal mass, abscess, and fistula are common findings. As a result of immune activation, tissue destruction, sinus canal formation,

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and consequently penetration into neighboring tissue develop. Thus, fistulas are formed between the two segments of the gastrointestinal tract. If the inflammation spreads to the surrounding tissues and organs and remains enclosed, an abscess develops. In the obstructive subtype, as a result of prolonged inflammation, a stenosis due to fibrosis may occur in any segment of the intestine. Postprandial pain, bloating, and vomiting are seen in such patients. Strictures due to fibrostenosis can lead to complete obstruction [10].

3. CLINICAL FINDINGS and SYMPTOMS

Crohn's disease mainly affects the ileum and cecum in more than half of the cases. In patients with CD, involvements are 15%, 20% and 15% in the small intestine, colon and anorectal area, respectively. The characteristic feature of CD is granulomatous intermittent lesions surrounded by sharply demarcated, normal-appearing mucosal tissue. Although, the retained layer is submucosa, all layers of the intestine are involved. The intestinal surface has the characteristic of "cobblestone" appearance arising from clefts and cracks surrounded by submucosal edema areas. Marked inflammatory and fibrotic changes occur in the submucosal layer. Intestinal smooth muscles are relatively less involved. After a while, the intestinal wall thickens and usually loses its flexibility [8].

Crohn's disease is a slowly progressive and aggressive disease with high morbidity [8]. Diarrhea, abdominal pain, rectal bleeding, fever, weight loss, and fatigue are the most common symptoms [11]. Because of the stimulation of pain receptors (located in the serosa and peritoneum) due to transmural bowel involvement, abdominal pain occurs more frequently in patients with CD than patients with UC [5]. Also, perianal ulceration is prevalent due to diarrhea. Since, CD affects the submucosal layer rather than the mucosal layer, bloody diarrhea is less common in patients with CD compared to patients with UC [8].

Although, the course of the disease is different in most patients, perforation, fistula, abscess, and small bowel obstruction are the most common complications of CD [8, 12].Additionally, an increase in cancer incidence is observed in CD patients [13]. Patients may have inflammatory disorders other than the gastrointestinal system such as arthritis (joints), erythema nodosum (skin), uveitis and iritis (eye), epithelial mucosa aphthous ulcers (mucous membrane), sclerosing cholangitis (bile ducts), and cirrhosis (liver) [12, 13]. Renal disorders such as nephrolithiasis due to an increase in oxalate absorption associated with steatorrhea were found in 1/3 of the patients with CD. Amyloidosis and thromboembolic disease which indicate systemic inflammation, are serious complications of CD [12]. Also, malnutrition is frequently observed in patients with CD [8, 12].

4. ETHIOLOGY

Genetic Factors

Crohn's disease is considered as a multifactorial disease resulting from the effect of both environmental and genetic components on the gut microbiome. Differences in occurrence, severity and complications of disease, areas of involvement and differences in treatment response can be explained by the genetic dissimilarities between individuals [4].

Approximately, 200 risk loci for IBD have been identified so far with the development of molecular genetic techniques. However, only a few genes (CARD15, NOD2, ATG16L1, IRGM, LRRK2, PTPN2, IL23R, Il10, Il10RA, Il10RB, CDH1, and HNF4A) have been extensively studied and a strong relationship between these genes and IBD has been idendified [4, 14]. CARD15 and NOD2 gene mutations have shown the strongest association with CD (35-45% of cases) compared to other genes. The CARD15 and NOD2 genes encode a protein (a toll-like receptor) involved in the recognition of gram-negative and gram-positive bacterial wall fragments by intestinal epithelial cells. Therefore, mutations in the CARD15 and NOD2 genes may increase dysbiosis [15]. It is believed that a gene panel including key genes will contribute to the diagnosis and evaluation of IBD in the future [14].

Environmental Factors

Among the CD risk factors, smoking was researched most extensively and it doubles the risk of disease development especially at an early age [16]. It is also associated with increased severity of disease and is thought to cause resistance to treatment [17]. It was observed that the progression of the disease and the need for surgery have been decreased when patients with CD quit smoking [18]. Although the mechanisms underlying the link between smoking and CD are not completely understood, changes in the immune system, abnormal cytokine levels, and changes in intestinal permeability and motility are thought to be involved in the CD development [19].

It has been shown that diets rich in sugar, omega-6 fatty acids, polyunsaturated fatty acids, fat, and meat (except fish) increase the risk of CD, while fiber-rich plant-based diets reduce the risk of CD [20-22]. Antibodies against milk proteins and baker's yeast *Saccharomyces cerevisiae* have been detected in patients with CD [4]. Besides, it has been stated that low intakes of zinc and vitamin D and high iron intake may play a role in the development of CD [23]. However, it is difficult to interpret the findings as nutrition studies generally have poor research methods. Therefore, the role of nutrition in the development of CD has not been fully understood, further studies with high sample sizes are needed.

Oral contraceptive pills have also been associated with the development of CD, especially among smokers [24]. Additionally, non-steroidal anti-inflammatory drugs have been reported to trigger exacerbations in IBD [25].

Hygiene hypothesis associated with the prevalence of autoimmune diseases may also be the cause of IBD. It has been found that living in rural areas, having a large number of siblings, drinking unpasteurized milk, and exposure to domestic animals during in early childhood are inversely proportional to the risk of CD or UC [26].

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Marmara Med J 2023;36(2): 249-254

Although, there are no strong evidences, *Mycobacterium paratuberculosis*, *Pseudomonas* spp. and *Listeria* spp. are all suggested as possible causes for CD [27].

Since, CD is more common in northern latitudes where sun exposure is less, it is thought to be associated with vitamin D deficiency [25].

There is a growing body of research supporting the relationship between psychiatric diseases and development of IBD [28, 29].

5. PATHOGENESIS

In a healthy intestine, a mild inflammatory response is created against microbial agents, on the other hand, this situation is disrupted in CD and leads to uncontrolled inflammation [12]. It has been reported that various disturbances in the immune pathways may cause uncontrolled inflammatory cascades [7]. These disturbances are associated with the intestinal barrier function, innate and adaptive immune responses, and gut microbiota (Figure 1).

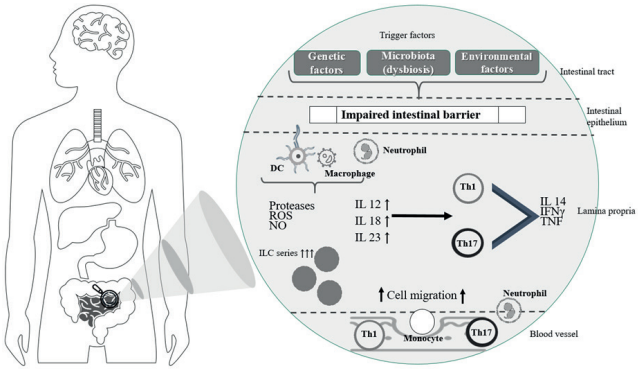

Figure 1. *Pathophysiology of Crohn's Disease.*
DC: Dendritic cell; ILC: Innate lymphoid cell; Th: T helper cell; IL: Interleukin; TNF: Tumor necrosis factor; IFN: Interferon, ROS: Reactive oxygen species; NO: Nitric oxide

Intestinal Barrier Function

The intestinal epithelium which is located between the gut microbiome and the wall of the gastrointestinal tract, prevents entry of bacteria and plays an important role in generating the mucosal immune response. Also, a healthy mucosal barrier includes tight junctions of the epithelial cells. Other defense mechanisms of the gastrointestinal tract include various specialized intestinal epithelial cells such as goblet cells that help to control mucus production, epithelial repair and inflammation, and Paneth cells that secrete antimicrobial peptides [8]. Intestinal epithelial cells are the first line of defense mechanism of the mucosal immune system [10]. Epithelial permeability is increased in patients with CD which allows pathogens to pass through the mucosal layers [7]. Numerous environmental factors, such as microbial pathogens, can trigger the immune response, generate endoplasmic reticulum stress, elicit a misfolded protein response, and initiate the inflammatory cascade [13]. Epithelial cells have inhibitory functions that destroy unwanted cytoplasmic contents by autophagy and prevent spread of invasive bacterial species [30]. Defects in autophagy-dependent genes, such as ATG16L1 and IRGM, have been identified as important risk factors for CD [31]. Defects in intestinal tight junctions are also associated with IBD [13].

Immune Responses

Leukocytes which are located in the intestinal epithelium, have an important role in maintaining homeostasis. T cells, a group of leukocyte members, play a primary role in cell mediated immunity. It has been found that there is an excessive increase in T helper (Th) 1 and Th17 cell responses against proinflammatory cytokines such as interleukin (IL)-12, IL-18 and IL-23 produced by macrophages and antigen presenting intestinal leukocyte cells in CD [31]. Th1 and Th17 cells secrete proinflammatory cytokines such as IL-17, interferon (IFN)-γ ve tumor necrosis factor (TNF)-α, and these cytokines induce production of TNF-α, IL-1, IL-6, IL-8, IL-12 and IL-18 from other cells such as macrophages, monocytes, endothelial and dendritic cells located in lamina propria to sustain inflammation [32, 33]. Thus, mucosal inflammation and damage to the epithelial barrier are accelerated. Furthermore, intestinal damage gradually increases with the release of proteases and reactive oxygen species from leukocytes and with the production of nitric oxide. When increasing numbers of migrated neutrophils to inflammed tissue invade and destroy the intestinal crypts, aphthoid lesions (shallow ulcers) occur [34].

The stimulation of inherited pattern recognition receptors (PRRs) contributes to this inflammatory response in the organism. PRRs recognize microbial pathogen-related molecular patterns and show an immune reaction to substances secreted by the organism itself under stress conditions such as tissue damage and necrotic cell death. PRRs are divided into many subtypes. Among these, the best described receptors are toll-like receptors (TLRs). Ten (TLR1-TLR10) and 12 (TLR1-TLR9, TLR11-TLR13) different TLRs have been identified in humans and mice, respectively [35]. TLR2 recognizes most of the intestinal microbes that make up the natural bacterial flora of the gut. A decrease in the number of TLR3 and TLR5 due to decreased expression was found in patients with CD. This situation can aggressively induce an inflammatory response with the formation of hypersensitivity to commensal exposure [7].

Gut Microbiota

Microbiota is thought to play an important role in the pathogenesis of CD. Dysbiosis is seen in the gut microbiota of IBD patients and is characterized by a decrease in microbial diversity compared to healthy individuals. This reduction in microbial diversity is more pronounced for CD than UC [26]. Dysbiosis is characterized by a decrease in *Bacteroides* and *Firmicutes* bacteria, and an increase in *Gammaproteobacteria* and *Actinobacteria* in patients with CD [36]. Besides, *Faecalibacterium prausnitzii*, which is responsible for the production of an anti-inflammatory protein that inhibits the nuclear factor (NF)-κB pathway, has also been found to be reduced in patients with CD [37].

Patients with CD have a higher number of mucosal surface-associated bacteria (such as adhesive-invasive *Escherichia coli*) due to increased adhesion and invasion compared to healthy controls [38]. These invasive strains cross the mucosal barrier, adhere to the intestinal epithelial cells and trigger the secretion of increased amounts of TNF-α from the macrophages [38, 39].

The intestinal microbiota is diverse and unstable in early childhood. Disruption of this microbiota in early childhood may affect the gut immune response, alter the sensitivity to IBD, and this sensitivity is greater for CD [40]. It has been found that the use of antibiotics, especially in early childhood, increases the risk of CD development more than the use in later periods [41]. Stress, air pollution, hygiene and diet are other factors that affect the composition and functional activity of the gut microbiota. Since the effect of diet on microbial composition is transient, its effect on changed microbial diversity in CD is controversial [13]. As a result, all these factors cause the proliferation of pathogen-specific T cells in addition to the commensal specific effector-T cells that have been temporarily formed in the mucosal barrier [34].

6. TREATMENT STRATEGIES

Despite the advances in treatment methods in the last decade, there is no definitive treatment because the etiology of CD is not completely understood. Therapeutic strategies focus on alleviating and reducing inflammation and symptoms [42]. Smoking cessation is a component of treatment [34].

Medical treatment includes corticosteroids, 5-aminosalicylates, and immunomodulatory agents such as thiopurines and TNF-α blockers [34]. Also, new biological agents are being developed [43]. Options for the treatment of refractory patients include azathioprine, 6-mercaptopurine, methotrexate, and biological therapies [34].

Surgical treatment is usually performed to manage complications such as stenosis, fistula, abscess, and perforation in the gastrointestinal canal or to remove the obstruction. Surgical resection of small bowel segments can reveal complications associated with short bowel syndrome which include malabsorption, diarrhea, and nutrient deficiencies. Symptoms are related to the size and location of resection [34].

Recently, fecal microbiota transplantation (FMT) which is applied to increase microbial diversity, has become a potentially alternative therapeutic strategy for IBD to eliminate dysbiosis of the intestinal microbiota [43, 44]. There are still no definitive findings regarding the timing and frequency of FMT treatment and, the route of administration. Although significant side effects of the FMT treatment were not found in the clinical studies, it has been shown that there are unwanted immunological, physiological or metabolic phenotype transfers in animal studies [6]. In a study, clinical remission was achieved with the FMT treatment at the 12th week in 5 out of 9 patients with the age of 12-19 years [45]. In a case report, clinical remission and endoscopic recovery were achived after a single FMT infusion in a patient with CD who had previously failed biological therapy [44]. Prospective randomized controlled studies are needed to evaluate the safety and efficacy of FMT in IBD patients.

Table I. *The principles of medical nutrition therapy for Crohn's Disease (CD).*

	Ref.
According to the European Society for Clinical Nutrition and Metabolism (ESPEN) practical guideline, the energy requirements of patients with inflammatory bowel disease (IBD) are similar to those of the healthy population.	[48]
Protein consumption of 1.2-1.5 g/kg/day is recommended to prevent catabolism and to meet the protein requirement. The protein requirements in remission are similar (1g/kg/d) to that recommended for the healthy adults.	[48]
Simple carbohydrate consumption should be limited. If the individual has not developed lactose intolerance, lactose consumption does not create a risk. Excessive consumption of fructose or sorbitol can cause abdominal pain, gas, and diarrhea, so that, dietary intake of them should be considered.	[4]
Daily energy of 20-25% and 25-30% for patients should be met from fats in an active period and in a remission period, respectively. Since too much restriction of fat intake can lead to a deficiency of fat-soluble vitamins, attention should be paid to the pattern of fatty acids in the diet rather than fat restriction. High-fat diets can trigger an increase in steatorrhea. In the case of fat malabsorption, the use of medium-chain triglycerides may be beneficial in providing energy and fat-soluble vitamins.	[9]
The European Crohn's and Colitis Organisation recommends a temporary, low-fiber diet for most patients during an exacerbation period of the disease. Organisation recommends consuming as much pulp as needed by a healthy individual in the remission period.	[49]
Patients with IBD should be checked for micronutrient deficiencies on a regular basis and specific deficits should be appropriately supplemented.	[48]
There is no specific 'IBD diet' that can be generally recommended to promote remission. Exclusion diets are not recommended to achieve remission in active CD, even if the patient suffers from individual intolerances. Meals can be tolerated better with less and frequent feeding.	[48]
If oral feeding is not sufficient then EN should be considered as supportive therapy. In CD patients with intestinal strictures or stenosis in combination with obstructive symptoms, a diet with adapted texture, or distal (post-stenosis) EN is advised. EN in CD should be administered via an enteral feeding pump. EN should always take preference over PN, unless it is completely contraindicated.	[48]
No significant benefit for probiotics and prebiotics has been demonstrated in induction and maintenance of remission during the CD exacerbation period. Since probiotic supplements are found to be ineffective in CD, they are not recommended in updated ESPEN guidelines. On the other hand, they may be beneficial in some cases in ulcerative colitis patients.	[48, 50]
Patients with IBD are at risk and therefore should be screened for malnutrition.	[48]

The area and length of involvement in CD, the frequency and duration of the active period of the disease significantly affect the absorption disorder and nutrient deficiency. It causes abdominal pain, nausea, vomiting, diarrhea, and loss of fluid-electrolytes, vitamins and trace elements in exacerbation periods of the disease, and it also accelerates weight loss by reducing food intake and appetite [46]. Therefore, the primary

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goal in the medical nutrition treatment of CD is to improve and maintain the nutritional status and to maintain remission [4]. For this purpose, foods, nutritional supplements, enteral and parenteral nutrition methods can be used according to the period and severity of the disease [47]. IBD needs good nutritional follow-up and treatment to prevent malnutrition, to eliminate micronutrient deficiencies, to prevent osteoporosis. In patients diagnosed with IBD, it is essential to conduct routine screening for iron and folate deficiencies throughout pregnancy, along with nutritional follow-up [48]. The principles of medical nutrition therapy for CD are summarized in Table I.

Conclusion

Crohn's disease is a common disease with high morbidity in the world. Numerous genetic and environmental factors are associated with the development of CD. The importance of genetic factors, especially CARD15 and NOD2 gene mutations, is pointed out. Also, dysbiosis plays a crucial role in the development of the disease and in the effectiveness of treatment. Smoking, antibiotic exposure in early childhood, and western-style eating habits are important environmental factors in the etiology of the disease. In recent years, significant advances have been made in understanding the role of intestinal inflammation in CD pathogenesis. These advances have contributed to the examination of new therapeutic approaches such as FMT that can be preferred instead of current medical and surgical treatment strategies or it can support the existing treatments. Medical nutrition therapy is prominent in preventing symptoms such as diarrhea and abdominal pain which are common in patients with CD, and in maintaining remission. The use of probiotics in medical nutrition therapy has shown conflicting results, further studies are needed on this subject.

Compliance with Ethical Standards

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Review Article

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A growing problem in childhood and adolescence: Metabolic syndrome and its relationship with physical activity and fitness

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ABSTRACT

Metabolic syndrome (MetS); is defined as a life-threatening endocrinopathy in which systemic disorders such as insulin resistance, abdominal obesity, glucose intolerance, diabetes mellitus, dyslipidemia, hypertension, and coronary artery disease are combined. Although, it is generally known as a problem of adults, it emerges as an essential problem in childhood and adolescence. MetS, closely related to obesity, is increasing due to bad eating habits and sedentary lifestyles. The pathophysiology of MetS has yet to be elucidated. Therefore, lifestyle changes, especially diet and physical activity, are the cornerstones of MetS treatment. In general, both physical activity and fitness; appear to be separately and independently associated with metabolic risk factors in children and adolescents. Although, studies show that activities that increase physical activity levels and improve aerobic fitness cause a decrease in the risk of MetS; a definitive prescription for exercise has not been established at this time. This review aimed to review the definition, classification, and factors playing a role in the pathogenesis of MetS, as well as to evaluate the relationship between MetS and physical activity and aerobic fitness in children.

Keywords: Metabolic syndrome, Physical activity, Physical fitness, Pediatrics, Adolescents.

1. INTRODUCTION

Metabolic syndrome (MetS); is an important public health problem that causes severe mortality and morbidity and is increasing all over the world with the realization that metabolic abnormalities such as insulin resistance (IR), obesity, dyslipidemia, and hypertension (HT) cluster in some patients; MetS was first described by Gerald M. Reaven in 1988 as Syndrome X, which is a syndrome that is combining metabolic abnormalities. It was emphasized that Syndrome X is a collection of cardiovascular risk factors, and IR is a factor underlying Syndrome X [1]. Afterward, the diagnostic criteria of MetS were revised many times over time [2]. It is essential to prevent and manage MetS, which causes serious health problems, impairs quality of life, and even threatens life [3]. Increasing physical activity and cardiorespiratory fitness are suggested to be essential in reducing the risk of MetS, but the relationship between MetS and physical activity has yet to be fully elucidated.

MetS and Diagnostic Criteria

Various updates have been made to the MetS diagnostic criteria over time. First, in 1998, the World Health Organization (WHO) determined the diagnostic criteria for the syndrome and accepted its name as MetS. WHO has emphasized the relationship between the main components of MetS and IR. They accepted impaired glucose tolerance, overt diabetes mellutis (DM), or IR as the first condition for diagnosis. Also, they determined that at least two other components, dyslipidemia, abdominal obesity, and microalbuminuria, were associated with IR as diagnostic criteria [4]. International Diabetes Federation (IDF), in addition to absolute abdominal obesity, MetS was defined as

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the combination of two high triglyceride (TG), low high-density lipoprotein (HDL), high blood sugar, and high blood pressure [5]. In contrast, abdominal obesity, an indicator of IR, is the first condition in IDF diagnostic criteria. Turkish Society of Endocrinology and Metabolism published a clinical guideline in 2009. It defined MetS as a fatal endocrinopathy that starts with IR, abdominal obesity, glucose intolerance, or systemic disorders such as DM, dyslipidemia, HT, and coronary artery disease (CAD). The guideline emphasized that no single genetic or environmental factor has yet been defined that explains all the components of MetS. This heterogeneous disease develops based on IR.

Although, MetS is often known as a health problem in adulthood, it has recently emerged as an essential problem in childhood and adolescence. It is known that an increasing number of children and adolescents are affected by MetS [6, 7]. A definite consensus has yet to be reached in the definition of childhood MetS as in adults [8]. Although, the criteria used in the studies are based on national recommendations, there may be various changes in cut-off values according to age, gender, and race [8].

MetS Pathophysiology

The pathophysiology of MetS is complex, and the exact mechanism has not yet been elucidated [9]. However, it is suggested that abdominal obesity and insulin resistance effectively develop MetS [10]. Macrophage infiltration occurs in adipose tissue with intra-abdominal obesity. Various cytokines such as TNF-α and IL6 released from macrophages cause inflammation. This inflammation leads to lipolysis in adipose tissue, increasing circulating free fatty acids, decreased insulin signaling, and decreased glucose transporter (GLUT4) synthesis, resulting in IR [11]. It also leads to increased IL6 caused by obesity, insulin resistance, and acute phase reactants such as C-reactive protein (CRP) synthesis. Various studies have shown a close relationship between high CRP levels and the development of MetS. It is also known that IL6 contributes to the thrombotic pathway by causing an increase in fibrinogen [11].

Increased adipose tissue with obesity; causes a decrease in adiponectin levels and an increase in leptin levels. As a result, pro-inflammatory cytokines released from adipose tissue, increased leptin, decreased adiponectin level and increased free fatty acids in circulation cause atherogenic dyslipidemia, atherosclerosis, and CAD [11].

Another effective pathway in the development of MetS is activating the renin-angiotensin system (RAS). Obesity and IR cause an increase in the production of angiotensin-2 secreted by adipose tissue [11].

Increased lipolysis in insulin resistance causes an elevation of free fatty acids in the circulation. Increased fatty acids in the blood are used to synthesize triglycerides in the liver. Very low-density lipoprotein (VLDL) production increases, leading to dyslipidemia [9].

In conclusion, although the precise mechanisms of MetS have not been fully elucidated, it has been shown that IR and obesity cause MetS by affecting various pathways [12]. Regarding the prevention of abdominal obesity and insulin resistance, the importance of physical activity has been mentioned in previous studies [13, 14].

MetS Prevalence

MetS, which has a 35-40% prevalence in adults, is also an increasing health problem in childhood and adolescence [3, 6]. In the 2016 UNICEF report, it was stated that the frequency of MetS in children and adolescents in Western Europe and the United States (USA) increased from 2% to 25% in the mid-1990s [15]. In the study performed by Ağırbaşlı et al., in Turkey, the frequency of MetS was determined as 2.2% in 1385 healthy children aged 10-17 years [16].

National Health and Nutrition Examination Survey I (NHANES I) study (1988-1994), conducted in the USA, demonstrated that MetS features were found in 3% to 14% of all children and adolescents. These rates were reported as 13% to 37% in obese patients in the study [6]. The prevalence of MetS increased with the severity of obesity and reached up to 50% in severely obese individuals. They also found that increased obesity was associated with increased CRP levels and decreased adiponectin levels.

In previous studies, while the frequency of MetS was between 1-23% in the pediatric population, the frequency of MetS in obese children and adolescents was found in a wide range, such as 3-60% [17]. The main reason for this wide range was reported as criteria used for the MetS in childhood; different criteria used in guidelines and age, gender, race, and ethnicity are the leading causes of difference [18].

Prevention and Treatment of MetS and Obesity

Many studies revealed that MetS has gradually become a public health problem, especially in childhood and adolescence, and obesity is related to an increased risk of MetS [3]. MetS and its components in childhood increase the risk of MetS in adulthood [19]. So, prevention of childhood obesity and MetS is essential [3]. In addition to preventive approaches, effective and permanent treatment options are required regarding the consequences that may occur due to the increasing obesity in the pediatric population. Each syndrome component should be treated as early as possible [20].

Diet and physical activity are the cornerstones of MetS treatment [3]. Evidence on the efficacy and safety of pharmacotherapy in children is scarce [3]. However, in cases where diet and physical activity are insufficient to reduce overweight, dyslipidemia, high blood pressure, and high blood sugar, pharmacotherapy is needed; In resistant cases, surgery is also required [3]. As a result, a multidisciplinary approach and individual treatment are required to treat the components of MetS and obesity [3]. Treatment of obesity aims to reach a healthy weight and to maintain it by ensuring the energy balance between calories taken and spent. All of the components of MetS can be improved by reducing fat mass [3]. Primary approaches and treatments according to the components of MetS are summarized in Table I [3, 21].

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Marmara Med J 2023;36(2): 255-261

Table I. *MetS components and related primary approaches and treatments [3, 21]*

Components	Primary approach	Treatment
Obesity	Lifestyle interventions: 1. Regulation of diet (caloric limitation, personal goals recommended by dietitians) 2. Physical activity (60 min of moderate/vigorous physical activity every day, including vigorous activity three days per week)	1. Pharmacologic treatment, e.g., orlistat 2. Surgical treatment, e.g., bariatric surgery
Dyslipidemia	Lifestyle interventions: 1. Regulation of diet (reducing simple carbohydrate intake, reducing cholesterol intake <300 mg/day and total fat between 25 and 30% of daily calories, possible use of stanol esters or plant sterols) 2. Physical activity	Pharmacologic treatment, e.g., statins
Glucose regulation disorders and type 2 diabetes mellitus	Lifestyle interventions: 1. Regulation of diet 2. Physical activity 3. Sleeping habits	Pharmacologic treatment Although the use of metformin in glucose disorders is uncommon, for type 2 diabetes mellitus, e.g., metformin and/or insulin can be used.
Hypertension	Lifestyle interventions: 1. Regulation of diet (increasing intake of fruits and vegetables, increasing olive oil polyphenols, reducing sodium) 2. Physical activity (30-60 min of moderate/vigorous physical activity at least 3-5 days per week)	Pharmacologic treatment: Starting with a single drug (e.g., angiotensin receptor blocker, ACE inhibitör, long-acting calcium channel blocker, or thiazide diuretics) at the low end of the dosing range.
Non-alcoholic fatty liver disease	1. Lifestyle interventions and weight loss. 2.Omega-3 fatty acids and probiotics may ameliorate the progression of the disease. 3. Vitamin E can improve hepatocellular ballooning	

The Relationship of Physical Activity and Physical Fitness with MetS

Physical activity is any body movement created by skeletal muscles that cause energy consumption. Physical fitness is defined as the ability to create sufficient work in the muscle [3]. The metabolic equivalent of task (MET) is the ratio of a person's metabolic rate at work to his resting metabolic rate. One MET is the amount of oxygen consumed during rest, corresponding to 3.5 ml O_2/kg/min. It also allows the classification of physical activities according to their intensity and frequency. Physical activities below 3 METs, such as slow walking, and daily housework, as "Light-intensity activities"; activities between 3-5.9 METs, such as fast-paced walking, jogging, swimming, and dancing, as "Moderate-intensity aerobic physical activities (MPA)", activities between 6-8 METs such as high-paced running, volleyball, and basketball as "Vigorous-intensity aerobic physical activities (VPA)", and activities above 8 METs are called "Very vigorous-intensity aerobic physical activities" [22]. Maurice et al., reported that adult MET values are unsuitable for children and that the MET value should be taken as a reference as 1.5 to 2 METs for sedentary behavior [22]. Studies have shown that children with high physical fitness who are overweight and children with normal weight and low physical fitness have a similar risk of MetS [23].

There is an inverse relationship between physical activity and the risk of MetS. Increased physical activity in children and adolescents has positive metabolic effects independent of weight. It is also associated with the risk of MetS and its component [3, 24]. For example, physical activity contributes to body composition by reducing fat body mass and the risk of central obesity and its negative consequences [14].

Physical activity decreases insulin resistance and increases insulin sensitivity [1, 6, 25]. Physical activity also decreases the risk of hyperlipidemia by reducing LDL and TG levels and increasing HDL levels. It is also known that high TG leads to an increase in reactive oxygen species, leading to endothelial dysfunction [6]. Mendelson et al., had been performed a study on 20 obese adolescents, and they found a decrease in inflammatory markers after 12 weeks of an exercise program [26]. In a cross-sectional study on questionnaire-based physical activity reported from Brazil, which consists of participants who were not physically active in childhood and adolescence, it has been determined that the risk of high blood pressure, low cardiorespiratory fitness, and MetS is higher than those who are active. In addition, it was evaluated that adolescents who stated that they were only physically active during childhood had better cardiorespiratory compliance than those who were not physically active during childhood; Among interventions to prevent MetS, the importance of early physical activity participation was emphasized [27].

Amiri et al. studied for nine years at 3-year intervals and examined the effects of lifestyle interventions to increase healthy eating patterns and physical activity on MetS [28]. From the beginning, they divided the participants into three groups: fully intervened, partially intervened (interventions were interrupted occasionally), and the control group without any lifestyle intervention. As a result of the study, a significant decrease was observed regarding the prevalence of MetS in the short term in the partial intervention group compared to the control group. However, the decrease was not continued in the long term. A decrease was observed in the prevalence of MetS in both the

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Marmara Med J 2023;36(2): 255-261

long and short-term in the whole intervention group. This shows the importance of the positive effect of the continuity of lifestyle interventions such as physical activity and healthy diet on MetS [28].

Rognvaldsdottir et al. investigated the effects of physical activity and sleep patterns on metabolic profile in 256 adolescents, 146 of whom were girls [29]. Adolescents with variability and irregularity in sleep duration and bedtime were found to have a higher body fat percentage. Those who did less physical activity had higher insulin levels with a higher body fat percentage. As a result, they emphasized that regular sleep and sufficient physical activity positively affect the adolescent metabolic profile [29].

In contrast, some studies in the literature could not find a relationship between physical activity and MetS. For example, when Pan and Pratt examined the relationship between physical activity and MetS according to the NHANES 1999-2002 survey data, they could not find a significant relationship between them [30]. McMurray et al., included these two studies in their meta-analysis [1]. They stated that this might be due to methodological problems, such as assessing physical activity in the pediatric population based chiefly on questionnaires and self-reports.

Physical fitness has been reported to have a positive protective effect on cardiometabolic risk [31]. Anderssen et al., performed a study in a group of 9-15 age years old participants and found that those with a physical fitness level in the lowest quartile were 13 times more at risk for MetS than those in the upper quartile [32]. In another study including 223 girls and 223 boys adolescents, MetS was more common in inactive adolescents and adolescents with low physical fitness [33]. In the study of Martinez-Gomez et al., in which they examined the relationship between physical activity and physical fitness on MetS in 202 adolescents, 99 of whom were girls, a positive correlation was found between high physical fitness alone and low MetS risk, but this relationship could not be found with physical activity alone [34]. However, VPA and moderate-vigorous physical activity (M-VPA) were found to be associated with high physical fitness. Therefore, they emphasized that physical fitness may play a key role in the relationship between physical activity and MetS [34].

Various studies have been conducted on the intensity, duration, and type of physical activity. The relationship between physical activity intensity and MetS is still being determined [35]. Although studies show that high-intensity physical activity for only a few minutes a day positively affects body composition and metabolic risk, how low-intensity physical activity affects body composition has yet to be clarified [6]. Renninger et al., examined the relationship between physical activity duration and MetS in a meta-analysis of 8 studies, 6009 children and adolescents, based on IDF criteria [36]. Even a 10-minute increase in M-VPA and VPA was found to be negatively associated with MetS. On the contrary, Martinez-Gómez et al. found no relationship between physical activity level and MetS score in 202 adolescents aged 13-17 years [34]. Similarly, a study conducted by Dubose et al., with 72 male and female participants found no clear relationship between physical activity levels and MetS; they determined that high physical activity levels were associated with lower diastolic blood pressure [19].

Although, there is no clear information about the level of physical activity required to prevent the risk of MetS in children, there is no definite information about the duration of physical activity, but there are various suggestions. In 2000, the US Department of Agriculture recommended that children and adolescents be on M-VPA for 60 minutes daily [37]. In 2010, this recommendation was re-accepted and approved by the American Dietary Guidelines [38]. In the study of Ekelund et al., an inverse relationship was found between the time spent in light, moderate, and vigorous physical activity and the risk of MetS [39]. Andersen et al., suggested that 90 minutes of physical activity per day is required to prevent the clustering of metabolic risk factors [40]. Unlike other studies examining the MetS and duration of physical activity, in a study conducted by Andaki et al., it has been reported that taking 7872 steps per day in Brazilian boys would be protective against MetS [8]. The study determined that all children classified as MetS remained below 7872 steps per day [8].

Regarding the type of exercise, studies in the literature show that aerobic and resistance exercises reduce the MetS score [41-43]. Dias et al., investigated the effect of resistance exercises on non-diabetic obese adolescents [41]. While the exercise group consisted of 24 non-diabetic obese adolescents, the control group consisted of 20 non-obese adolescents and included the exercise group in a program consisting of 12-week resistance exercises. The groups were evaluated regarding body composition, 24-hour ambulatory blood pressure, adipocytokines, skin endothelial reactivity, metabolic profile, and aerobic and strength fitness before and after the program. They found significant improvements in endothelial function, hemodynamic-metabolic profiles, body composition, and physical fitness, independent of BMI, in non-diabetic obese adolescents with only resistance exercise at the 12th week. Similarly, Son et al., divided 40 obese adolescent girls into two groups control and exercise arms [42]. While those in the control group continued their daily physical activities, the exercise group received a resistance and aerobic exercise program for 12 weeks. At the end of 12 weeks, they observed a significant difference in IR and body composition in the exercise group compared to the control group. As a result, they stated that resistance and aerobic exercise might be beneficial in reducing obesity and MetS risk factors. Marson et al., reviewed the effects of aerobic, resistance, and combined exercises on insulin resistance, fasting glucose, and fasting insulin in overweight, obese children and adolescents in their meta-analysis [43]. They stated that aerobic exercise was associated with decreased fasting insulin levels and HOMA-IR and that MetS and Type 2 DM could be prevented with aerobic exercise.

The last point is that reducing sedentary life is as important as increasing physical activity and physical fitness to reduce the risk of obesity [44]. Cadenas-Sanchez et al., compared participants with overweight and obesity in the Adolescent Nutrition and Lifestyle Study in Europe (n:3528, participation rate: 61.3%) with and without a healthy metabolic profile of physical activity and duration of inactivity [45]. While the duration of inactivity was high in the group with a poor metabolic profile, MPA

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Marmara Med J 2023;36(2): 255-261

and M-VPA were found to be low. Peplies et al., found that a sedentary lifestyle was associated with IR, independent of weight, in a study of the IDEFIC cohort of 3348 children aged 3 to 10.9 years [46]. In the study of Broadney et al., children who interrupted sedentary behavior at regular intervals, with short bouts of physical activity, observed lower insulin resistance than those who did not interrupt. Television (TV) is one of the leading causes of sedentary activity in children [47]. In the review by Carson et al., they found that more prolonged screen exposure and TV viewing time adversely affected body composition in 162 studies in the literature [48]. In 32 studies, it was stated that there is a relationship between longer TV watching time and higher cardiometabolic risk. Finally, studies have shown that the obesity rate increases as screen exposure (TV, computer, telephone, etc.) increases [49].

Family and school environments also play an essential role in increasing childhood physical activity and reducing sedentary life. Families need to be adequately informed, and their awareness has to be increased. In addition, necessary interventions should be taken so that children who spend most of the day at school due to education can engage in physical activity in the school environment [50].

Conclusion

MetS is a severe public health problem that causes serious mortality and morbidity that increasingly affects the pediatric population, where metabolic abnormalities such as IR, obesity, dyslipidemia, and HT are clustered. In this respect, the prevention and treatment of MetS is crucial. In order to increase physical activity in the prevention of MetS, it is necessary to raise awareness of families and make various arrangements in schools. There are still some uncertainties and conflicting results regarding the relationship between MetS and physical activity in the pediatric population. It is necessary to perform new studies on the definition of MetS and its relationship with physical activity in the pediatric population to reduce the risk of MetS, a growing health problem.

Compliance with Ethical Standards

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