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Original Article / Özgün Araştırma

Evaluation of ventricular arrhythmia in children with Wilson's disease; cardiac electrophysiological balance index (iCEB)

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Abstract

Aim: To evaluate cardiac involvement in Wilson's disease (WD) noninvasively by electrocardiography and to analyze it with the cardiac electrophysiological balance index (iCEB).

Method: Eighteen Wilson patients and 18 healthy child patients who were followed up in the Pediatric Gastroenterology department between 2022-2023 were included in the study.

Results: Wilson disease patients had normal ventricular and autonomic functions. QT-dispersion (QT-d) (22.61 (±11.47), p=0.000) and Tpe (66.50 (40-78), p=0.02) were found to be significantly higher in the WD group. QRS, QRS-dispersion (QRS-d), QT, QTc, Tpe/QT ratio, Tpe/QTc ratio, QT/QRS ratio, QTc/QRS ratio, Tpe/QRS ratio, Tpe/(QT*QRS) ratio both had similar values in the groups. Heart rate variability parameters (SDNN, SDNN-i, SDANN, rMSSD, pNN50, LF/HF ratio) were at similar values in both groups. rMSSD, pNN50, which indicates parasympathetic activity, was lower in Wilson patients than in the control group, but no statistical difference was detected. LF/HF ratio was significantly higher in WD patients.

Conclusions: Despite normal ventricular function and autonomic function in WD patients, they have an increased risk of ventricular arrhythmia. Although the cardiac electrophysiological balance index (iCEB) can provide useful information in the follow-up of WD patients, we recommend that depolarization, repolarization times, and repolarization dispersion times be evaluated separately in addition to iCEB.

Key words: Wilson disease; index of cardiac electrophysiological balance; autonomic dysfunction; Heart rate variability; repolarization dispersion

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Wilson hastalığı olan çocuklarda ventriküler aritminin değerlendirilmesi; kardiyak elektrofizyolojik denge indeksi (iCEB)

Öz

Amaç: Wilson hastalığında (WH) kalp tutulumunu, noninvaziv olarak elektrokardiyografi ile değerlendirmek ve kardiyak elektrofizyolojik denge indeksi (iCEB) ile analiz etmek amaçlandı.

Yöntemler: Çalışmaya 2022-2023 yılları arasında Çocuk Gastroenteroloji bölümünde takip edilen 18 Wilson hastası ve 18 sağlıklı çocuk hasta dahil edildi.

Bulgular: Wilson Hastalığı olan çocuklarda ventriküler ve otonomik fonksiyonlar normaldi. QT dispersiyonu (QT-d) (22,61 (±11,47), p=0,000) ve Tpe (66,50 (40-78), p=0,02) WH grubunda anlamlı olarak yüksek bulundu. QRS, QRS dispersiyonu (QRS-d), QT, QTc, Tpe/QT oranı, Tpe/QTc oranı, QT/QRS oranı, QTc/QRS oranı, Tpe/QRS oranı, Tpe/(QT*QRS) oranları her iki grupta benzerdi. Kalp hızı değişkenliği parametreleri (SDNN, SDNN-i, SDANN, rMSSD, pNN50, LF/HF oranı) her iki grupta da benzer değerlerdeydi. Parasempatik aktiviteyi gösteren rMSSD, pNN50 Wilson hastalarında kontrol grubuna göre daha düşüktü ancak istatistiksel olarak fark saptanmadı. LF/HF oranı Wilson hastalarında anlamlı derecede yüksekti.

Sonuç: Wilson hastalarında ventriküler fonksiyon ve otonom fonksiyon normal olmasına rağmen ventriküler aritmi riski yüksektir. Kardiyak elektrofizyolojik denge indeksi (iCEB) WH hastalarının takibinde faydalı bilgiler sağlayabilse de iCEB'e ek olarak depolarizasyon, repolarizasyon süreleri ve repolarizasyon dispersiyon sürelerinin de ayrı ayrı değerlendirilmesini öneriyoruz.

Anahtar kelimeler: Wilson hastalığı; kardiyak elektrofizyoloji denge indeksi; otonomik disfonksiyon; kalp hızı değişkenliği; repolarizasyon dispersiyonu.

INTRODUCTION

Wilson's disease (WD) is an autosomal recessive disease in which copper accumulates in organs as a result of a defect in the protein (ATP7B) that provides transport in copper metabolism and excretion of copper from the liver[1].

Damage to organs in WD occurs mainly because of copper accumulation and damage as a result of free oxygen radicals[2,3]. Cardiomyopathy and arrhythmias may develop because of cardiovascular system involvement[4].

In postmortem studies of WDs; Histopathological findings, such as myocardial hypertrophy, fibrosis, and sclerotic changes, were detected. Cases of sudden death have been reported in WD[4,5].

There is very little evidence in the literature of cardiac arrhythmias in WD. In our study, we aimed to objectively evaluate the ventricular electrophysiological characteristics in WD using the index of cardiac electrophysiological balance (iCEB) in addition to conventional measurements.

METHOD

This was a prospective, case-control study. Approval was obtained from the local ethics committee (decision no: 558/2023).

Eighteen Wilson patients and 18 healthy pediatric patients followed up from the pediatric gastroenterology department were included in the study.

Wilson disease patients diagnosed according to Wilson's disease diagnostic criteria[6] were patients with hepatic involvement without central nervous system involvement and normal liver function tests at the time of evaluation. All Wilson disease patients underwent penicillamine therapy.

The body weight of all patients was expressed in

kilograms and the height in centimeters. Blood pressure was measured after the patients had rested for 15 minutes.

After a detailed examination of all patients, they were evaluated by transthoracic echocardiography (ECHO) (Vivid S60, General Electric Healthcare, GE, Vingmed, Norway)[7].

Left ventricular wall thickness and diameter, systolic and diastolic function parameters were measured in accordance with the recommended guidelines[7,8].

All patients were evaluated using 12-lead electrocardiography (ECG) (Econet Cardio M Plus, Germany; filtered 0.5–150 Hz, 25 mm/s, and 10 mm/mV). Holter ECG and ECG are commonly used to assess symptoms that may be associated with intermittent arrhythmias, such as syncope, dizziness, chest pain, palpitations. ECG recordings were evaluated offline by magnifying them 200 times on a computer.

Mean values were obtained by measuring at least five consecutive waves or intervals in lead DII or V5. QRS, QT, and Tpe were measured. A correction was made for the heart rate[9,10,11,12]. Thus, the QTc was obtained[13]. The difference between the maximum and minimum values of the measured time was defined as the dispersion value. Thus, QRS-d, QT-d, and QTc-d were obtained[9,14]. The cardiac electrophysiological balance index (iCEB), QT/QRS ratio, Tpe/QRS ratio, cardiac electrophysiological balance index corrected for heart rate (iCEB-c) QTc/QRS ratio were measured[15,16,17].

24-hour electrocardiography recording and heart rate variability (HRV) software based on Holter ECG recordings (Delmar Reynolds lifeCard CF; Impresario Software). Parameters derived from the time interval (SDNN (the standard deviation of all normal R-R intervals), SDNN index, rMSSD (the square root of the mean of the sum of the squares of differences between adjacent RR intervals), mean heart rate, pNN50 (the proportion of adjacent R-R intervals that differ by more than 50 ms in the 24-h recording)) and parameters derived from frequency (LF (low frequency), HF (high frequency), LF/HF ratio) were obtained[18,19].

Statistical analyses were performed using IBM SPSS (SPSS, Chicago, IL, USA). Continuous variables are expressed as mean±standard deviation or median (minimum-maximum), and categorical variables are expressed as percentages (%). Appropriate tests were selected on the basis of their distribution characteristics. Statistical significance was set at $p < 0.05$significant.

RESULTS

Wilson disease and control groups mean age values were 12.44 (± 4.15), and 12.49 (± 4.17) years, respectively. In this study, 44% of the Wilson and control group patients were male.

As a result of comparing the WD group and the control group, the values of age, sex, weight, height, SBP and DBP were similar in both groups and there was no statistical difference.

LVEDd, EF, FS, Tapse, mitral E and A velocities, E/A ratio, and DT values in the WD and control groups were similar in both groups, and no statistical difference was found (Table I).

Table I: Comparison of demographic data and transthoracic echocardiographic data of Wilson patients and control group

Parameter	Wilson group (n=18)	Control group (n=18)	p value
Gender (M/F, %)	44,56	44,56	1.00[3]
Age (year)	12.44 (± 4.15)	12.49 (± 4.17)	0.97[1]
Weight (kg)	40.39 (± 16.37)	41.88 (± 15.49)	0.32[1]
Height (cm)	144.88 (± 19.35)	151.22 (± 18.53)	0.25[1]
SBP (mmHg)	110.83 (± 13.23)	113.22 (± 11.11)	0.56[1]
DBP (mmHg)	62.88 (± 9.06)	65.44 (± 9.24)	0.40[1]
LVEDd (mm)	42.21 (± 5.47)	43.52 (± 5.40)	0.21[1]
EF (%)	66.88 (± 6.67)	69.94 (± 5.59)	0.14[1]
FS (%)	35.50 (30-52)	38.50 (32-49)	0.10[2]
TAPSE (mm)	22.40 (± 2.24)	22.71 (± 2.97)	0.70[1]
E wave (m/s)	0.92 (± 0.14)	0.99 (± 0.214)	0.15[1]
A wave (m/s)	0.62 (± 0.16)	0.58 (± 0.13)	0.50[1]
E/A ratio	1.56 (± 0.46)	1.74 (± 0.35)	0.18[1]
DT (ms)	126.27 (± 21.06)	140.27 (± 28.27)	0.11[1]

1: student T test, 2: Mann-Whitney U test, 3: chi-square test

The ECGs of all patients were in sinus rhythm, and none had a ventricular branch block. QRS duration, QRS-d, QT, QTc, QTc-d, Tpe/QT ratio, Tpe/QTc ratio, QT/QRS ratio, Tpe/QRS ratio, QTc/QRS ratio values in the WD and control groups were similar in both groups (Table II).

QTd and Tpe values were significantly higher in the WD group compared to the control group (p = 0.001 and 0.02, respectively) (Table II).

Table II: Wilson disease group and control group electrocardiography parameters comparison table

Parameter	Wilson group (n=18)	Control group (n=18)	p value
QRS (ms)	80 (79-100)	80 (80-86)	0.18[2]
QRS-d (ms)	0 (0-20)	0 (0-10)	0.23[2]
QT	357.80 (296-408)	322.00 (289-386)	0.05[2]
QT-d	22.61 (± 11.47)	11.66 (± 8.22)	**0.001[1]**
QTc	408.9 (± 32.66)	392.39 (± 22.87)	0.08[1]
QTc-d	41.59 (± 21.68)	35.19 (± 19.30)	0.35[1]
Tpe (ms)	66.50 (40-78)	57 (40-92)	**0.02[2]**
Tpe/QT ratio	0.18 (0.13-0.22)	0.16 (0.12-0.24)	0.18[2]
Tpe/QTc ratio	0.16 (± 0.02)	0.14 (± 0.03)	0.28[1]
QT/QRS ratio	4.16 (± 0.49)	4.07 (± 0.30)	0.49[1]
QTc/QRS ratio	4.88 (± 0.61)	4.86 (± 0.31)	0.93[1]
Tpe/QRS ratio	0.76 (± 0.11)	0.71 (± 0.14)	0.23[1]
Tpe/(QT*QRS)	0.0022 (± 0.0003)	0.0022 (± 0.0003)	0.79[1]

HR, SDNN, SDNN-i, SDANN, rMSSD, pNN50 and LF/HF ratio values were similar in both groups and no statistical differences were observed (Table III).

Table III: Comparison of heart rate variability parameters of WD group and control group

Parameter	Wilson group (n=18)	Control group (n=18)	p value
HR (beat/min)	89.88 (± 10.37)	88.80 (± 8.10)	0.77[1]
SDNN (ms)	163.53 (± 46.27)	151.31 (± 38.13)	0.53[1]
SDNN index (ms)	79.28 (± 29.33)	80.20 (± 32.00)	0.93[1]
SDANN (ms)	134.62 (± 44.04)	122.05 (± 32.42)	0.37[1]
rMSSD (ms)	76.35 (± 46.81)	81.05 (± 37.63)	0.75[1]
pNN50 (%)	22.70 (± 13.66)	28.36 (± 10.84)	0.19[1]
LF/HF	2.05 (± 0.82)	1.64 (± 0.51)	0.11[1]

1: student T test, 2: Mann-Whitney U test, HR: heart rate, SDNN: the standard deviation of all normal R–R intervals in the 24-h electrocardiogram (ECG) recording, SDNNindex: the mean of the

standard deviation of all R–R intervals for all 5-min segments of the 24-h ECG recording, SDANN: the standard deviation of the mean of R–R intervals in all 5-min segments of the 24-h ECG recording, rMSSD: the square root of the mean of the sum of the squares of differences between adjacent RR intervals, pNN50: the proportion of adjacent R–R intervals that differ by more than 50 ms in the 24-h recording, LF low frequency (0.04–0.15 Hz), HF high frequency (0.15–0.40 Hz)

DISCUSSION

Although ventricular and autonomic functions were normal in our study, the repolarization time and dispersion times were high in the WD group. The WD group was high-risk for ventricular arrhythmias.

The basic myocardial damage mechanism in WD is thought to result from copper deposition[3], damage from free oxygen radicals[2] and autonomic dysfunction[20,21]. Sudden cardiac death has been reported in WD in the literature[5] but there is insufficient evidence for ventricular arrhythmia.

In our study, ventricular electrophysiological features, depolarization properties, repolarization properties and repolarization-depolarization balance were examined under headings.

For depolarization analysis, QRS duration and QRS dispersion analysis were used. QRS interval ECG representation of ventricular depolarization. QRS dispersion is the difference between QRS-max and QRS-min according to the change in the cardiac rate[9]. The QRS dispersion is the reciprocal of spatial distribution of conduction velocity. An increased QRS dispersion may cause one-way block and reentry[22]. In one study, a high QRS duration was reported in Wilson disease patients[23]. In our study, in parallel with the literature[24-26], ventricular depolarization and dispersion of depolarization were found to be similar. Thus, in our study, copper did not cause conduction abnormalities due to myocardial accumulation during the early stages of WD.

The myocardium consists of three layers with different electrophysiological properties: endocardium, mid-myocardium (M cell), and epicardium. The epicardium had the shortest and the m cell had the longest duration of electrical activity. The electrical gradient between these layers increases the risk of arrhythmia[27].

In our study, in parallel with the literature[24,25], QT and QTc values were similar in both groups, but the QT dispersion was greater. In one study, QT and QTc interval values were higher in the WD group[26]. In this study, WD with high QT/QTc intervals had neurological involvement and this relationship was associated with autonomic dysfunction. In our study, autonomic function parameters were similar in Wilson disease patients without neurological involvement, which supports the results of the study. However, the fact that QT dispersion in this study had similar values in both neurological and non-neurological WDs, unlike in our study, makes the mechanism underlying QT dispersion prolongation controversial. Studies have shown that central[21,22] and peripheral involvement[28,29] occur in the development of autonomic dysfunction in WD. In our study, none of the patients had neurological involvement, and autonomic function parameters were normal.

An increase in the repolarisation dispersion time or transmural repolarisation dispersion time creates a predisposing condition for reentry. Sudden cardiac death (SCD) may ocur[11,12,30]. In our study, Tpe, a transmural repolarization dispersion parameter, was higher in the WD group than in previous studies[24,26]. This supports the existence of a ventricular transmural gradient difference in the early period and that they are especially risky for reentry ventricular arrhythmias.

The electrical wavelength (λ) is important for arrhythmia formation. An increase or decrease in the wavelength creates a predisposing condition for reentrant arrhythmias. Wavelength measurements (λ) can be

noninvasively displayed on an ECG. Lu et al. developed iCEB parameters to represent wavelength[15]. iCEB is a parameter based on (repolarization time/depolarization time). The QT/QRS ratio[15] can be used as an iCEB parameter and the QTc/QRS ratio[17] can be used as a corrected iCEB (iCEB-c) parameter. Tse et al. showed that the Tpe/QRS and Tpe/(QT*QRS) ratios can be used in addition to these parameters in iCEB evaluation[16].

The iCEB value should be within a certain range. An increase in iCEB causes torsadogenic, and a decrease causes non-torsadogenic ventricular tachyarrhythmias[1]. In our study, the iCEB and iCEB-c parameters were similar in both the groups. When the literature is reviewed, our study is the first to evaluate iCEB in Wilson's patients. The main reason for the normal iCEB in our study may be the long repolarization time in the WD group as well as the long depolarization time. In the study, mean QRS durations in the WD group and control group were 84.50 (±7.12) and 80.68 (±1.57), respectively. The use of iCEB in diseases that are likely to cause depolarization abnormalities, such as Wilson disease, is controversial. Mathematically considering the iCEB value, prolongation of the QRS period in the denominator may stabilize the iCEB ratio.

CONCLUSIONS

In our study, the risk of ventricular arrhythmia was high in Wilson disease patients with normal ventricular and autonomic function. This may be associated with myocardial copper accumulation, damage by free oxygen radicals, and altered ion channels in Wilson's disease independent of autonomic dysfunction mechanisms.

Although iCEB evaluation provides useful information for Wilson's patients, we recommend separately evaluating the depolarization and repolarization time and repolarization dispersion times in addition to iCEB.

The main limitations of our study are the limited number of patients and the lack of cardiac magnetic resonance imaging.

Ethics Committee Approval: This was a prospective, case-control study. Approval was obtained from the local ethics committee (decision no: 558/2023).

Conflict of Interest: The authors declared noconflicts of interest.

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Original Article / Özgün Araştırma

Effectiveness of inactivated and mRNA COVID-19 vaccines on sperm parameters

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Abstract

Objective: The mRNA SARS-CoV-2 vaccine has been shown to have no adverse effects on semen parameters. However, it is yet unknown whether the inactivated vaccinations have the same effect. Thus, our objective was to evaluate the parameters of sperm prior and following the administration of mRNA and inactivated COVID-19 vaccinations.

Methods: In this study, the sperm quality was evaluated both before and after receiving the COVID-19 mRNA and inactivated vaccines. Of the participants, 28 received two doses of CoronaVac vaccine and 152 received two doses of mRNA BNT162b2 mRNA vaccine (Pfizer-BioNTech). Semen analyses were repeated 72 (57-145) days after the same individuals had received their second dose of COVID-19 vaccination.

Results: No significant differences were found in the parameters of sperm before and after administration of two doses of BNT162b2 vaccine. Prior to and following administration of the two doses of the inactivated vaccine, there was no appreciable variation in the volume of semen, sperm concentration, progressive motility, total motility, immotility, or morphologically normal sperm features. Following CoronaVac vaccination before and after two doses, only the total sperm count was shown to statistically decrease (p=0.03).

Conclusion: As a result, while there was no significant difference in the sperm parameters of the mRNA vaccine, it was determined that there was a statistical decrease in the total sperm count before and after two doses of CoronaVac vaccine. Since the semen volume of all patients is within normal limits, the first issue to be used here as a number is sperm per ml, which is important in terms of infertility, is the number.

Key words: Inactivated vaccine, mRNA vaccine, male fertility, sperm parameters

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İnaktive ve mRNA COVID-19 aşılarının sperm parametreleri üzerine etkinliği

Öz

Amaç: mRNA SARS-CoV-2 aşısı olmanın semen parametreleri üzerinde herhangi bir olumsuz etkisinin olmadığı gösterilmiş olmasına rağmen, inaktive aşıların aynı etkiye sahip olup olmadığı henüz netlik kazanmamıştır. Bu nedenle bu çalışmadaki amacımız, mRNA ve inaktive COVID-19 aşılarının uygulama öncesi ve sonrası sperm parametrelerini karşılaştırmaktı.

Yöntemler: Bu çalışmada sperm kalitesi, COVID-19 mRNA ve inaktif aşıların hem öncesi hem de sonrasında değerlendirildi. Katılımcılardan 28'i iki doz CoronaVac aşısı ve 152'si iki doz mRNA BNT162b2 BNT162b2 mRNA (Pfizer-BioNTech) aşısı aldı. Aynı kişilere ikinci doz COVID-19 aşısı yapıldıktan 72 (57-145) gün sonra semen analizleri tekrarlandı.

Bulgular: BNT162b2 aşısının iki dozundan önce ve sonra sperm parametrelerinde anlamlı fark yoktu. İnaktif aşının iki dozunun uygulanmasından önce ve sonra, semen hacminde, sperm konsantrasyonunda, progresif motilite, toplam motilite, immotilite veya morfolojik olarak normal sperm özelliklerinde kayda değer bir değişiklik olmamıştır. CoronaVac aşılamasının ardından iki doz öncesi ve sonrasında sadece toplam sperm sayısında istatistiksel olarak azalma olduğu görüldü (p=0,03).

Sonuç: Sonuç olarak mRNA aşısının sperm parametrelerinde anlamlı bir fark bulunmazken, iki doz CoronaVac aşısı öncesi ve sonrasında toplam sperm sayısında istatistiksel olarak azalma olduğu belirlenmekle beraber tüm hastaların meni hacmi normal sınırlar içerisinde olduğundan burada sayı olarak kullanılacak ilk husus, kısırlık açısından önemli olan, ml başına sperm sayısıdır.

Anahtar kelimeler: İnaktive aşı, mRNA aşısı, erkek fertilitesi, sperm parametreleri

INTRODUCTION

The first case of coronavirus disease 2019 (Covid-19), which has now spread to other parts of the world, was reported in Wuhan, China, in December 2019. Covid-19, an infectious disease, has been caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. In March 2020, the World Health Organization (WHO) announced it to be a pandemic, and tens of millions of people have subsequently been impact worldwide[2]. There is debate right now about whether SARS-CoV-2 could affect male fertility by breaching the blood-testicular barrier[3]. Local expression of the proteins transmembrane protease serine 2 (TMPRSS2) and angiotensin converting enzyme 2 (ACE2) in Leydig cells increased the likelihood of virus presence in the testicles[4,5]. It was established that SARS-CoV-2 enters host cells through ACE2 receptors and that TMPRSS2 is also crucial for SARS-CoV-2 cell entrance[6]. Although everyone intends to gain from the vaccination's protection, some individuals are troubled, and there have been concerns regarding the adverse effects of the vaccine[7]. Concerns have been expressed about the coronavirus's effect on male fertility[8]. Among COVID-19 patients, impaired spermatogenesis was reported; this finding may be explained by an augmented immune response and cytokine storm in testis tissue or by autoimmune orchitis, which was monitored in pathological samples from the male COVID-19 patients who had deceased[9,10]. The SARS-CoV-2 vaccine, which could result in a similar immunological response, might, in theory, have a similar impact[8].According to several research, some semen parameters, for instance total sperm count, the concentration of semen, and total motile count, can be significantly decreased by COVID-19 infection or its adverse impacts, for example fever[11-13]. Even though there has been

still a lack of knowledge on how the COVID-19 vaccines affect the male fertility, these findings make it understandable that couples and donors have reservations about their decision.

The COVID19 vaccination campaign in Turkey began on January[14], 2021. In the world, several COVID19 vaccines are being developed at various stages. The inactivated virus vaccine Sinovac, the mRNA-based COVID19 vaccine

developed by Pfizer-BioNTech, the adenovirus viral vector vaccine Sputnik V, and the inactivated virus vaccination TurkoVac are all now being used in Turkey[14]. In Diyarbakr, vaccinations made by Pfizer-BioNTech and CoronaVac are now being given. Understanding how the COVID-19 vaccine impacts the quality of men's sperm is crucial for medical study as well as decision-making by donors and couples. There was no discernible variation in any semen parameter values between the pre- and post-vaccination levels, according to a recent study on the mRNA COVID-19 vaccine[15]. Yet, there is limited information on how the inactivated COVID-19 vaccine impacts semen quality[7]. In this study, we evaluated the semen's quality both before and after those who received the inactivated COVID-19 vaccination and the mRNA vaccine. The results of this study may serve as a guide for future suggestions regarding fertility, which may aid donors and recipients of assisted reproductive technology (ART) in making wiser choices.

METHODS

Male individuals who had semen analysis for suspected infertility at the University of Health Sciences Gazi Yaşargil Training and Research Hospital Gynecology Clinics Andrology Laboratory were included in this research. Vaccine application information was determined with the AŞILA mobile application developed by the Ministry of Health (Turkey), whether 1648 people who had semen analysis performed in the Andrology laboratory between September 2020 and March 2022 were inactive or mRNA COVID-19 vaccine. In terms of the treatment protocol, semen analyzes are routinely requested from the spouses of female patients who receive long-term ovulation induction treatment for infertility treatment in the Infertility Polyclinic of our hospital. The study was initiated with 188 people aged 18 to 54 years old, whose sperm analyses were recorded in the hospital information management system (HIMS) prior to and following two doses of COVID-19 vaccine. However, 8 people were not included in the study because they were diagnosed with azoospermia as a result of semen analysis. In addition, those who did not complete at least 2 doses of the same vaccine and those who had positive Reverse Transcription-Polymerase Chain Reaction (RT-PCR) test in the hospital information management system were excluded from the study. The study included the same people who had a semen analysis before the COVID-19 vaccine, then received at least two doses of the COVID-19 vaccine and had another semen analysis. The trial comprised 152 participants who received two doses of the mRNA BNT162b2 vaccination from Pfizer-BioNTech and 28 participants who received two doses of the CoronaVac (inactivated-Sinovac) vaccine. Before the participants in the research received their first dose of the COVID-19 vaccination, semen analyses were conducted on average 113 (28-307) days earlier. The same participants underwent a second round of semen testing on average 72 (57–145) days following the second COVID-19 vaccination dose. The sperm parameters of sperm samples collected both before and after the COVID-19 immunization were assessed in accordance with the WHO's instructions. After 2-7 days of sexual abstinence from individuals who applied to the andrology laboratory for semen analysis for suspected infertility, semen samples obtained by masturbation without using any lubricant were taken into sterile disposable plastic containers. The collected semen was incubated for 30-60 min in a CO_2 incubator at 37 °C after semen was liquefied, all semen parameters were evaluated macroscopically and microscopically in light microscopy (Olympus CX31) according to 2010 WHO guidelines by an experienced Histology-Embryology specialist with a Makler counting chamber. Sperm parameters such as semen volume, color, pH, viscosity, leukocyte count,

total sperm count, sperm concentration, motility, total motility and immotility were evaluated. Using a pipette, about 10 µl of semen was inserted into the Makler camera to be tested for number and motility. Sperm cells in 10 frames were counted in light microscope with X20 objective and the result was expressed as million (10^6). Sperm count, forward fast and slow motile sperm, respectively, then motile and immobile sperm cells in situ were counted and their percentages were calculated.

According to Kruger's exact morphology standards, the spermatozoon morphology was assessed using the Spermac staining method. One stain fixative and three stains (A, B, and C) are included in the kit of spermac staining (Ref. no. SPS050, Ferti Pro NV Industrie park Noord 32 8730 Beernem, Belgium). After loading the slide with 10 µl of sperm, it was allowed to sit at room temperature for 20 minutes before being cleaned in a container with distilled water for 8-10 times after the fixative solution was added, which took about 10-15 minutes. The slides were once more washed with distilled water 8–10 times after each staining procedure, and then, in the proper order, incubated with the A, B, and C stains for 1 minute each. The slides were air dried for at least 20 minutes before being subjected to morphological examination with an Olympus CX31 light microscope with immersion oil at x100 magnification. The percentage of spermatozoa with a normal morphology was calculated after counting 100 spermatozoa on each slide in the succeeding stage. There were at least five separate fields where the sperm count was done. Teratozoospermia has been described as the existence of fewer than five spermatozoa with a normal morphology.

SPSS 21 for Windows analysis (IBM SPSS Inc., Armonk, NY, USA) was used to conduct the statistical. Using the Shapiro Wilk tests, the normality of the data distribution was evaluated. For pairwise comparisons when the data did not fit the normal distribution, Wilcoxon tests were applied. The means of the two groups were compared to determine whether there was a statistically significant difference between before and after vaccination data. The significance threshold was set at p 0.05.

RESULTS

This study included male individuals who underwent semen analysis and 2 doses of COVID-19 vaccine for infertility research in the andrology laboratory. Of the men included in the study, 152 had received two doses of mRNA BNT162b2 vaccine and 28 had received two doses of CoronaVac vaccine. All individuals receiving infertility treatments had their semen analyzed both before and after the vaccine. Semen analyzes were performed on average 113 (28-307) days before the people included in the study received the first dose of COVID-19 vaccine. Semen analyzes were performed again on average 72 (57-145) days after the second dose of COVID-19 vaccine was given to the same individuals. Table 1 displays the age and vaccination information of the study participants.

Table I: Vaccination information of the people included in the study

Characteristics	Number (%) or median (min-max)
Number of cases	180
Age	33.03 ± 6.86
BNT162b2 (Pfizer-BioNTech)	152 (84.5%)
CoronaVac (Sinovac)	28 (15.5%)
Time between first COVID-19 vaccine dose and pre-vaccine semen analysis median (min-max), days	113 (28-307)
Time between second COVID-19 vaccine dose and post-vaccine semen analysis median (min-max), days	72 (57-145)

COVID-19, Coronavirus Disease 2019; Min-Max: Minimum-Maximum.

When the results of the semen analysis of the people included in the study were examined, 54 people were diagnosed with oligozoospermia before the COVID-19 vaccine and 58 people after the COVID-19 vaccine. Similarly, 28 people were diagnosed with asthenozoospermia before the COVID-19 vaccine, while 25 people after the COVID-19 vaccine. In the morphological evaluation made according to Kruger Strict criteria, 105 people were diagnosed with teratozoospermia before vaccination, and 112 people were diagnosed after vaccination. Comparisons of semen parameters of 152 individuals before and after 2 doses of BNT162b2 vaccine are given in Table 2. In our investigation, there was no difference between the pre- and post-administration of the BNT162b2 vaccine in terms of the concentration of sperm, semen volume, total sperm count, the motility of progressive, total motility, immotility, and morphologically normal sperm parameters (p>0.05). Table 3 also compares the sperm parameters of 28 people prior to and following two doses of the CoronaVac vaccine. In the evaluation of semen analysis before and after 2 doses of Corona Vac vaccine, there hasn't been observed significant difference in terms of the volume of semen, the concentration of sperm, progressive motility, total motility, ımmotility and morphologically normal forms sperm parameters (p>0.05). It was determined that only total sperm count decreased statistically after vaccination before and after 2 doses of CoronaVac vaccine (p=0.03 In the individuals included in the study, there was no serious deterioration in semen parameters after both types of vaccination. However, the number of people diagnosed with oligozoospermia, asthenozoospermia and teratozoospermia increased when compared before and after vaccination. The number of people diagnosed with azoospermia before and after vaccination did not change.

Table II: Correlation between parameters of sperm before and after mRNA COVID-19 vaccination (BNT162b2 - Pfizer-BioNTech)

Sperm parameters	**Pre-vaccination** (n:152)	**Post-vaccination** (n: 152)	**p Value**
Abstinence time (days)	3.27 ± 0.46(3-5)	3.54 ± 0.51 (2-5)	0.000
Semen Volume (ml)Mean ± SD; (Min- Max)	3.05 ± 1.62 (0.2-10.3)	3.07 ± 1.36 (0.2-7)	0.99
Sperm concentration ($x10^6$/ ml) Mean ± SD; (Min- Max)	29.41 ± 29.16 (0.02-112)	27.68 ± 32.50 (0.01-257)	0.07
Total sperm count ($x10^6$) Mean ± SD; (Min- Max)	85.43 ± 98.16 (0.06-525)	81.91 ± 111.12 (0.01-1028)	0.12
Progressive motility (%) Mean ± SD; (Min- Max)	45.62 ± 21.24 (0-90)	46.34 ± 18.69 (0-85)	0.70
Total motility (%) Mean ± SD; (Min- Max)	53.47 ± 21.35 (0-93)	54.77 ± 18.22 (0-90)	0.38
Immotility (%) Mean ± SD; (Min- Max)	46.13 ± 21.31 (7-100)	45.42 ±18.56 (10-100)	0.55
Morphologically normal forms (%); (Min- Max)	4.26 ± 2.58 (0-14)	4.19 ± 2.48 (0-12)	0.80

SD, Standart Deviation; Min-Max: Minimum-Maximum.

Table III: Correlation between parameters of sperm before and after inactivated COVID-19 vaccination (CoronaVac-Sinovac).

Sperm parameters	**Pre-vaccination** (n: 28)	**Post-vaccination** (n: 28)	**p Value**
Abstinence time (days); (Min- Max)	3.21 ± 0.41(3-5)	3.46 ± 0.5 (3-4)	0.05
Semen volume (ml)Mean ± SD; (Min- Max)	2.62 ± 1.32(0.7-5.5)	2.33 ± 0.93 (1-4.8)	0.08
Sperm concentration ($x10^6$/ ml) Mean ± SD; (Min-Max)	36.69 ± 33.2 (0.05-100)	30.01 ± 25.07 (0.02-78)	0.12
Total sperm count ($x10^6$) Mean ± SD; (Min- Max)	86.20 ± 90.26 (0.18-385)	61.74 ± 59.54 (0.07-273)	**0.03**
Progressive motility (%) Mean ± SD; (Min- Max)	48.57 ± 22.86 (4-85)	46.17 ± 20.46 (3-80)	0.53
Total motility (%) Mean ± SD; (Min- Max)	54.5 ± 22.39 (5-90)	56.57 ± 20.36 (5-86)	0.52
Immotility (%) Mean ± SD; (Min- Max)	45.5 ± 22.39 (10-95)	43.42 ± 20.36 (14-95)	0.52
Morphologically normal forms (%); (Min- Max)	3.85 ± 1.79 (2-8)	4.14 ± 2.79(0-11)	0.68

SD, Standart Deviation; Min-Max: Minimum-Maximum. The results statistically significant was shown in bold.

DISCUSSION

Because a COVID-19 vaccine had to be rapidly approved for use in the general population, many safety concerns were raised[8]. While some of the worries had a medical basis, others were voiced by members of the public who were not connected to the medical community[16], among them were worries about how the vaccine would affect both male and female fertility[8]. In this investigation, sperm quality was evaluated prior to and after administration of the mRNA and inactivated COVID-19 vaccines. This included measuring sperm volume, concentration, progressive motility (PR), and total progressive motile count (TPMC).

Men's reproductive health is negatively impacted by COVID-19 infection, according to earlier research. Serum hormone levels revealed a direct correlation between the presence of secondary hypogonadism and SARS-CoV-2 infection, with lower testosterone levels indicating the worst possible clinical prognosis[17]. More than 50% of men who have survived Covid-19 have reported the presence of circulating testosterone levels suggestive of hypogonadism several months later, as well[6]. It is commonly acknowledged that SARS-CoV-2 penetrates the cells of host via ACE2 receptors, and that TMPRSS2 also significantly facilitates SARS-CoV-2 entry into the cell[18]. Given that spermatogonia, Sertoli and Leydig cells have been shown to express ACE2 and TMPRSS substantially, an infection of SARS-CoV-2 may harm the testis and could have an adverse effect on spermatogenesis[5]. In a previous study, Li et al.[10] reported that6 (15.8%) of 38 patients had SARS-CoV-2 PCR-positive semen. They also found that testicular samples from the six deceased patients of SARS-CoV-2 showed histological evidence of localized elevated immune response and the damage of germ cell. Although this is the case, the vast majority of studies that have been published to date have not discovered any viral evidence in the semen of either men who are still ill or those who have recovered. According to a recent assessment, the likelihood of discovering SARS-CoV-2 in the COVID-19 patients' semen is exceedingly low[11,19-22].

There is yet very little proof that the COVID-19 mRNA vaccination affects human fertility[23]. Thankfully, some studies have demonstrated that neither the BNT162b2 nor the mRNA-1273 vaccination affects sperm parameters, for instance the volume of sperm semen,

concentration, the volume of sperm, the motility of sperm, and the total amount of motile sperm[15,24]. In another related study, 75 fertile men's sperm samples were collected 1-2 months after they received the second dose of Pfizer's COVID-19 vaccination, and the semen parameters were compared with the WHO reference ranges[3]. Remarkably, only one patient (1.3%) was reported to display sperm parameters consistent with oligozoospermia and asthenozoospermia. Another study evaluating the efficacy of the mRNA SARS-CoV-2 vaccine compared pre- and post-vaccination data in 36 ART treated couples and found no differences in the quantity of mature oocytes collected, the rate of fertilization, or the pregnancy rate (30% per transfer). Additionally, it was reported that the male partner's sperm parameters were unchanged following vaccination[25]. Reschini et al. 18 concluded that mRNA or viral vector vaccinations should be regarded as safe for men's reproductive health because they had no effect on the quality of sperm and fertilization capacity. These earlier findings are supported by our study. In the current study, which evaluated the sperm parameters in this small cohort of healthy males prior to and after receiving two doses of the COVID-19 mRNA vaccine, none of the sperm parameters revealed a significant decrease. The mRNA vaccine is unlikely to have an impact on sperm parameters because it only contains mRNA and not live virus.

In the current study, we did not detect any significant differences between the pre- and post-administration of two doses of the inactivated vaccinations in terms of the concentration of sperm, the volume of semen, total motility, progressive motility, immobility, and morphologically normal sperm features ($p>0.05$). Only the total sperm count was found to statistically change following CoronaVac vaccine before and after two doses ($p=0.03$). In our study, the only value with a statistical difference was total sperm count, but this value is not as important as the number per ml for infertility. The semen volume of all patients is within normal limits, so the first consideration to use as a number here, which is important for infertility, is the number of sperm per ml.

The SARS-CoV-2 vaccines are effective in preventing infection[26]. CoronaVac is the one of the most commonly used vaccine in Turkey[14]. It contains live virus antigens and has demonstrated good immunogenicity in animal studies[27]. and phase 3 clinical trials[28,29] by inducing significant systemic inflammation and the development of SARS-CoV-2 neutralizing antibodies. CoronaVac-induced immune reactions, however, may be comparable to those caused by live viruses given the features of inactivated vaccines, which may damage human fertility[26]. In recent study, Wang et al.[26] assessed the effects of CoronaVac on male fertility. They reported a slight increase in semen volume and sperm concentration was detected following vaccination, and the total sperm count was similar when compared between cohorts ($p<0.001$). Additionally, they noted that although progressive and total motility declined following vaccination, there was no discernible difference in the number of progressive and total motile sperm. Similar to our study, they also reported that before vaccination, there were more sperm with normal morphology both in terms of percentage and total number. In another study, Xia et al.[30] reported that the groups that received the inactivated vaccination and the unvaccinated groups had similar sperm parameters

This study had some limitations. First, long-term semen analysis results have not yet been reported, as the participants were followed for 3-4 months after the second dose of the vaccine. Second, the sex-related hormone levels (FSH, LH, Testosterone, and Estradiol) were not looked at before and after the COVID-19

vaccine. Third, since male individuals included in this study were routinely asked for semen analyzes in terms of treatment protocol from the spouses of female patients who received ovulation induction treatment in the infertility outpatient clinic, the results obtained may not include individuals from the entire population. Finally, information about the side effects of the SARS-CoV-2 mRNA BNT162b2 and CoronaVac vaccines they used could not be obtained from the participants.

CONCLUSION

In conclusion, there hasn't been found significant difference in sperm parameters before and after 2 doses of BNT162b2-Pfizer vaccine. Since the mRNA vaccine contains only mRNA and no live virus, it is unlikely that this vaccine will have an effect on sperm parameters. According to the study's findings, only the total sperm count was found to statistically decrease following CoronaVac vaccination before and after two doses (p=0.03). However, the semen volume of all patients was found to be within normal limits. For clinicians, sperm count per ml is seen as a more valuable parameter than total sperm count in the evaluation of male infertility.

Ethics Committee Approval: Ethics committee approval for the study was obtained from University of Health Sciences Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee with the date of 03.12.2021 and number 941. This study was conducted in accordance with the current Declaration of Helsinki. All participants provided written informed consent for participation

Conflict of Interest: The authors declared no conflicts of interest.

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Original Article / Özgün Araştırma

How well do we recognise gout disease?

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Abstract

Objective: The clinical burden of gouty arthritis has historically been well recognized; however, gout is often misdiagnosed and mismanaged. In this study, we aimed to evaluate the diagnoses and treatments given to gout patients admitted to different specialties.

Methods: Patients who were diagnosed with gout attacks and treated by a rheumatologist were included, while patients with other non-gout rheumatic diseases (connective tissue diseases, rheumatoid arthritis, spondyloarthropathies, calcium pyrophosphate disease, etc.) were excluded. The branches the patients applied to during the attack, the treatments and diagnoses they received drugs, the number of attacks they had, demographic data, comorbidities, and laboratory data were recorded retrospectively.

Results: 424 gout patients were included. Patients were mostly male (70.7%). The mean age was 62.4± 12.4 years, and women were older than men (67.9±10 vs 62.4±12 years, p<0.001). Hypertension was the most common comorbidity, observed in 230 patients (54.2%). Among the patients who applied, 86 (20.2%) had previously been diagnosed, while 338 (79.7%) were diagnosed for the first time. The number of patients who had their first attack was 210 (49.5%), the number of patients who had their second attack was 88 (20.7%), and the number of patients who had ≥3 attacks was 126 (29.7%). The most commonly involved joint was the 1st metatarsophalangeal joint (MTF) and the second most commonly involved joint was the ankle joint. The rate of gout diagnosis was higher in patients presenting with podogra. The initial departments consulted during the incident were the emergency department first, followed by orthopedics and infectious diseases. Gout was the most common diagnosis, followed by trauma and injury, cellulitis, septic arthritis, and soft tissue infection. Nonsteroidal anti-inflammatory drugs(NSAIDs) were the most frequently prescribed drugs, followed by antibiotics and colchicine.

Conclusion: Gout is still not sufficiently recognized. Different diagnoses and treatments other than gout are made in applications to different branches. All physicians, regardless of their specialties, may be the first to see patients with gout attacks and therefore play a critical role in the diagnosis and treatment of these patients. With correct diagnosis and treatment, many visits to the doctor can be reduced.

Keywords: Gout, Attack, Gout mimics

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Gut Hastalığını ne kadar tanıyoruz?

Öz

Amaç: Gut artritinin klinik yükü tarihsel olarak iyi bilinmektedir; ancak gut hastalığı sıklıkla yanlış teşhis edilmekte ve yanlış yönetilmektedir. Bu çalışmada, farklı uzmanlık alanlarına başvuran gut hastalarına konulan tanıları ve uygulanan tedavileri değerlendirmeyi amaçladık.

Yöntemler: Gut atağı tanısı alan ve romatoloji uzmanı tarafından tedavi edilen hastalar çalışmaya dahil edilirken, gut dışı diğer romatizmal hastalıkları (bağ dokusu hastalıkları, romatoid artrit, spondiloartropatiler, kalsiyum pirofosfat hastalığı vb) olan hastalar çalışma dışı bırakıldı. Hastaların atak sırasında başvurdukları branşlar, aldıkları tedaviler ve tanılar, geçirdikleri atak sayısı, demografik verileri, eşlik eden hastalıkları, laboratuvar verileri retrospektif olarak kaydedildi.

Bulgular: 424 gut hastası çalışmaya dahil edilmiştir. Hastalar çoğunlukla erkekti (%70,7). Ortalama yaş 62,4±12,4 yıldı ve kadınlar erkeklerden daha yaşlıydı (67,9±10'a karşı 62,4±12 yıl, p<0,001). Hipertansiyon 230 (%54,2) hastada görülen en yaygın komorbidite idi. Başvuran hastaların 86' sına (%20,2) daha önce tanı konmuşken, 338' ine (%79,7) ilk kez tanı konulmuştu. İlk atağını geçiren hasta sayısı 210 (%49,5), ikinci atağını geçiren hasta sayısı 88 (%20,7) ve ≥3 atak geçiren hasta sayısı 126 (%29,7) idi. En sık tutulan eklem 1. metatarsofalangial (MTF), ikinci sırada ayak bileği eklemiydi. Podogra ile başvuran hastalarda gut tanısı konulma oranı daha yüksekti. Atak esnasında ilk başvurulan bölümleri ilk sırada acil servis, ikinci sırada ortopedi ve üçüncü sırada enfeksiyon hastalıkları oluşturmaktaydı. Tanılardan ilk sırada gut varken, daha sonra en sık görülenler sırayla travma ve yaralanma, sellülit, septik artrit ve yumuşak doku enfeksiyonu oluşturmaktaydı. Nonsteroid antiinflamatuvar ilaçlar (NSAİD) en sık yazılanken, ikinci sırada antibiyotikler, üçüncü sırada kolşisin yer almaktaydı.

Sonuç: Gut hastalığı hala yeterli düzeyde tanınmamakta. Farklı branşlara başvurularda gut dışı farklı tanılar ve tedaviler yapılmakta. Uzmanlık alanları ne olursa olsun tüm hekimler gut atağını ilk gören kişiler olabilir ve bu nedenle bu hastaların teşhis ve tedavisinde kritik bir rol oynarlar. Doğru teşhis ve tedavi ile birçok doktor ziyareti azaltılabilir.

Anahtar kelimeler: Gut, Atak, Gut Taklitçileri.

INTRODUCTION

Gout is a common and curable disease caused by the deposition of monosodium urate crystals in tissue. Gout, historically known to as “the unwalkable disease” and the “disease of kings,” was first recognized by the Egyptians in 2640 B.C. and later by Hippocrates in the fifth century B.C1. Gout was defined by podagra, which is discomfort in the first metatarsophalangeal joint[1]. The clinical impact of gouty arthritis has long been known; nonetheless, gout is frequently misdiagnosed and mistreated. The most important risk factor for the development of gout is a high blood urate content. Hyperuricemia (blood urate concentration over the saturation threshold) is commonly recorded in clinical practice and research when serum urate is greater than or equal to 7 mg/dL[2]. When deposited monosodium urate crystals trigger gout flare-ups, it manifests as intermittent, severely painful attacks of arthritis[2]. Over the 20th century, gout became more common, most likely as a result of shifting demographics in terms of age and the rise in the frequency of metabolic syndrome and related diseases[2]. There is no one number that accurately represents the frequency of gout worldwide because it varies greatly throughout ethnic groups and geographical areas[3]. Gout typically first manifests as an acute flare-up of inflammation that affects the foot or ankle4. It is self-limiting during 1–2 weeks, the so-called intercritical period, with total remission of joint inflammation signs and symptoms[2]. The first flare occurs after an asymptomatic period of hyperuricemia. Persistent hyperuricemia may lead to recurring flare-ups, known as polyarticular flares, that affect several joints, including the joints in the upper limbs, and grow

more frequent and persistent[4]. Acute arthritic symptoms, such as pain, edema, heat, redness, and difficulty moving the inflamed joint, are prevalent during gout flare-ups[5]. The first MTF joint is the most frequently involved site, while other foot and ankle locations are also frequently impacted, which can make difficulty with walking and other activities[6]. Flares frequently happen at night, when the patient wakes up with severe joint pain[7]. One could characterize the discomfort as throbbing, burning, gnawing, or stabbing[8]. The flare is accompanied by variable degrees of erythema, warmth, and swelling[9]. Fever and other signs of systemic inflammation may also exist, especially in the event of a polyarticular flare[3]. Patients seek the first available physician due to these symptoms. The consulted branches usually make differential diagnoses according to their areas of interest. Septic bursitis, septic arthritis, erythema nodosum, paronychia, fight bite, cellulitis, necrotizing fasciitis, tenosynovitis, and abscess are among the conditions that mimic gouty arthritis[2].

This study aimed to analyze the differential diagnoses and treatment approaches of various specialties in acute gouty arthritis attack.

METHODS

This study includes patients who applied to the rheumatology outpatient clinic with a diagnosis of gout between December 2019 and September 2023. The study included 424 patients aged between 18 and 89 years and diagnosed with gout according to the 2018 EULAR/ACR classification criteria[10]. Patients who were diagnosed with gout attacks and treated by rheumatologists were included, while patients with other non-gout rheumatic diseases (connective tissue diseases, rheumatoid arthritis, spondyloarthropathies, calcium pyrophosphate disease, etc.) were excluded. The specialties consulted during the attack, the treatments and diagnoses received, the number of attacks, demographic data, comorbidities, and laboratory data were recorded retrospectively. Demographic data; age, gender, body mass index, and comorbidities were recorded. The diagnoses made by other specialties in the patients were reviewed repeatedly and the diagnoses were clarified again with further examinations, imaging examinations, and consultations when necessary. Patients with a definite diagnosis of gout were included in the study after the exclusion of trauma, insect bite, paronychia, abscess, deep vein thrombosis (DVT), septic arthritis, erythema nodosum. Patients with gout and these diseases were excluded from the study. Pseudogout was not included in the study because of the potential difficulties in the early stages of differential diagnosis. Laboratory indicators were examined including blood urea nitrogen (BUN), serum creatine (Cr), uric acid(UA), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), total bilirubin (TB), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C); inflammatory indicators, involving erythrocyte sedimentation rate (ESR, normal range: 0–20 mm/H) and C- reactive protein (CRP, normal range: 0–5 mg/L). We defined hyperuricemia as a uric acid level ≥7 mg/dl for men and ≥6 mg/dl for women[11]. Body mass index (BMI) was calculated using the standing height and weight recorded in the medical record at the time of gout diagnosis. BMI was categorized based on the World Health Organization classification12: normal (18.5–24.9 kg/m2), overweight (pre-obese; 25–29.9 kg/m2), class I obesity (30–34.9 kg/m2), class II obesity (35–39.9 kg/m2), class III obesity (≥ 40 kg/m2) Those with a BMI≥30 kg/m2 were considered obese. This study was approved by the local ethics committee (approval date: 22/11/2023, decision no: ESH/GOEK 2023/55). All procedures were carried out according to the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

When evaluating the study data, quantitative variables were determined by mean, standard deviation, median, minimum, and maximum values; qualitative variables were indicated by descriptive statistical methods such as frequency and percentage. The Independent Sample T test was used for two-group comparisons of normally distributed parameters, and the Mann-Whitney U test was used for two-group comparisons of non-normally distributed parameters. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 21. If $P<0.05$, the difference between the means was considered significant.

RESULTS

The study involved 424 patients and all patients were evaluated by a rheumatologist. Characteristics of the study group are listed in Table 1. Patients were mostly male (70.7%). The mean age was 62.4± 12.4 years, and women were older than men (67.9±10 vs 62.4±12 years, $p<0.001$). Of the gout patients, 384 (90.5%) were over 50 years old, while 40 (9.4%) were under 50 years old. The frequency of the comorbidities in the study group was as follows; obesity (n: 226, 53.3%), diabetes (n:100, 26 %), hyperlipidemia (n: 140, 36.4%), hypertension (n: 230, 54.2%), heart failure (n: 65, 16.9%), coronary heart disease (n: 120, 31.2%) and chronic renal failure (n: 132, 34.3%), stroke (n: 25, 6.5%), hepatic disorders (n: 26, 6.7%), malignancy (n: 18, 4.6%), osteoporosis (n: 45, 11.7%), other diseases (n: 20, 5.2%) and any comorbidity (100, 23.5%). The most commonly involved joint was the 1st MTF and ankle joint. The rate of gout diagnosis was higher in patients presenting with podogra. Among the patients who applied, 86 (20.2%) had previously been diagnosed, while 338 (79.7%) were diagnosed for the first time. The number of patients who had their first attack was 210 (49.5%), the number of patients who had their second attack was 88 (20.7%), and the number of patients who had ≥3 attacks was 126 (29.7%).

Table I: Patient characteristics and associated comorbidities

Variables	Overall,*n*(%)	Men, *n*(%)	women, *n*(%)
Sex	424	300 (70.7)	124 (29.2)
Age, mean, years	62.4	60.1	67.9
18-49	40 (9.4)	28 (9.4)	12 (9.6)
50-59	120 (28.3)	87 (27.6)	33 (26.6)
60-69	133 (31.3)	91 (29.8)	42 (33.8)
70-79	85 (20.0)	52 (18.1)	33 (26.6)
80-89	46 (10.8)	42 (14.9)	4 (3.2)
BMI, kg/m^2			
Underweight (<18.5)	6 (1.4)	6 (2.0)	0
Normal (18.5-24.9)	28 (6.6)	19 (6.3)	9 (7.2)
Overweight (25.0-29.9)	146 (34.4)	117 (39.0)	29 (23.3)
Class I Obesity(30.0-34.9)	137 (32.3)	104 (34.6)	33 (26.6)
Class II obesity(35.0-39.9)	50 (11.7)	25 (8.3)	25 (20.1)
Class III obesity (≥ 40)	39 (9.1)	21 (7.0)	18 (14.5)
Unknown	18 (4.2)	8 (2.6)	10 (8.0)
Major associated comorbidities			
			50 (40.3)
Hypertension	230 (54.2)	180 (60.0)	40 (36.6)
Coronary artery disease	125 (29.4)	85 (28.3)	27 (24.7)
Heart failure	65 (16.9)	38 (13.8)	10 (0.9)
Stroke	25 (6.5)	15 (5.4)	35 (32.1)
Diabetes mellitus	100 (26.0)	65 (23.6)	52 (47.7)
Hyperlipidemia	140 (36.4)	88 (32.0)	38 (34.8)
Renal disease	132 (34.3)	94 (34.1)	11 (10.0)
Hepatic disorders	26 (6.7)	15 (5.4)	8 (0.9)
Malignancy	18 (4.6)	10 (3.6)	30 (27.5)
Osteoporosis	45 (11.7)	15 (5.4)	24 (19.3)
Any comorbidity	100 (23.5)	76 (25.3)	4 (3.6)
Other	20 (5.2)	16 (5.8)	
Involved joint			
1.MTF			
Ankle	384 (90.5)	290 (96.6)	94 (75.8)
Knee	75 (17.6)	56 (18.6)	19 (15.3)
Metacarpophalangial	36 (8.4)	31 (10.3)	18 (4.0)
Proximal interphalangeal	12 (2.8)	6 (2.0)	6 (4.8)
Distal interphalangeal	14 (3.3)	12 (4.0)	2 (1.6)
	24 (5.6)	20 (6.6)	4 (3.2)

Table 2 shows the first admissions during the attack. The emergency department was the most common, followed by orthopedics and infectious diseases. While the number of patients admitted to the rheumatology department during the attack period was 58, according to the number of branches to which the patients were referred before coming to

rheumatology, the number of patients with one branch application was 102 (27.8.0%), two branch applications was 109 (29.7%), three branch applications was 124 (33.8%), four branch applications were 22 (6.0%), and the number of patients with five or more branch applications was 9 (2.4%). The number of patients who were laboratory tests checked by the physician to whom they were first applied were 285 (67.2%). The number of patients in whom uric acid level was checked in these tests was 186 (65.2%).

Table II: Distribution of patients according to attacks and branches applied

First applied branches	Total *n*(%):424	1st attack *n*(%): 210	2nd attack *n*(%): 88	≥3. attack *n*(%): 126
Emergency physician	127 (29.9%)	70 (33.3%)	32 (36.3%)	25 (19.8%)
orthopedics	93 (21.9%)	42 (20.0%)	21 (23.8%)	30 (23.8%)
Infectious diseases	44 (10.3%)	18 (8.5%)	14 (15.9%)	12 (9.5%)
Rheumatology	58 (13.6%)	10 (4.7%)	8 (9.0%)	40 (31.7%)
Family physician	23 (5.4%)	16 (7.6%)	3 (3.4%)	4 (3.1%)
Internal Medicine	33 (7.7%)	25 (11.9%)	4 (4.5%)	4 (3.1%)
Physical therapy and rehabilitation	22 (5.1%)	16 (7.6%)	3 (3.4%)	3 (2.3%)
Nephrology	14 (3.3%)	8 (3.8%)	2 (2.2%)	4 (3.1%)
Other branches	10 (2.3%)	5 (2.3%)	1(1.1%)	4 (3.1%)

Table 3, distribution of patients according to attacks and diagnoses. The most common department visited during the attack was the emergency department and the number of admitted patients was 127 and 48(37.7%) of them were diagnosed with gout. The diagnoses made in order of frequency are gout, trauma and injury, cellulitis, septic arthritis, soft tissue infection, deep vein thrombosis, and paronychia. The number of patients whose uric acid levels were checked is 40. All patients with gout were administered NSAIDs, while steroids were initiated in 8 cases and NSAIDs in combination with colchicine in 32 cases. Of the patients who were started on colchicine, 10 were given 2 tablets at 2-hour intervals of 8-12 tablets daily, 6 were given 6 tablets/day, 12 were given 3 tablets/day, and 4 were given 2 tablets/day. Five patients were prescribed allopurinol while 68 patients (53.5%) were started on antibiotics. The number of patients referred to rheumatology from the emergency department was 40, most of whom had elevated uric acid levels. In 12 patients with suspected DVT, Doppler USG was performed, but no evidence of DVT was found.

Orthopaedics was the second most frequent specialty, admitting 93 patients (21.9%). The most common diagnoses are trauma, injury, septic arthritis, tendonitis, and soft tissue infection. Gout was considered in 18 (19.3%) patients. X-rays were taken in 85 of the patients. Five patients underwent arthrocentesis of the knee for suspected septic arthritis. The number of patients whose uric acid levels were checked is 25. All patients were given oral and local NSAIDs. Antibiotics were started in 52 patients. The number of patients who were started on NSAIDs and colchicine in patients with gout was 10, allopurinol was started in 4 patients, and colchicine and antibiotics were started together in 5 patients. Three patients with tophus who had not yet been diagnosed with gout were operated on with the preliminary diagnosis of tumor.

The third most common specialty was infectious diseases and the number of patients admitted was 44 (10.3%). The first diagnoses considered were respectively soft tissue infection, cellulitis, septic arthritis, paronychia, trauma, injury, septic arthritis, and gout.

Gout was considered in 8 (18.1%) of the patients. Number of patients whose uric acid levels were checked[15]. All patients were given antibiotics and NSAIDs. The number of patients who were started on NSAID and colchicine for gout was[2].

Most of the patients admitted to internal medicine and nephrology departments had comorbid factors and were followed up in these departments, so they were also admitted during the attack. The percentage of gout diagnosed in internal medicine and nephrology is 75.7% and 85.7%. Patients diagnosed with gout were started on colchicine, NSAID, steroid, and allopurinol.

Patients presenting to the rheumatology department with a gout attack were examined on the same day. While the number of patients presenting with the first attack was low (4.7%), the rate of referral to rheumatology with recurrent attacks increased with the exclusion of other causes as the number of attacks increased (31.7% with ≥3rd attack). The number of patients on colchicine at admission was 75, allopurinol 28, and febuxostat6. All patients were diagnosed by excluding other causes and observing an acute gouty arthritis attack. Diagnosed patients were treated according to their comorbid factors. The number of patients given colchicine during the attack was 412, the number of patients given steroids was 225, the number of patients given NSAİDs was 140, the number of patients started on uric acid lowering therapy during the attack was 82, while the number of patients started on uric acid lowering therapy after the attack was 302.

Patients also applied to physical therapy, family physicians, dermatology, cardiovascular diseases, and cardiology departments during the attack, and it was observed that each department was examined and treated for their diseases. It has been observed that, like other branches, the differential diagnosis of gout is little considered.

Table III: Distribution of patients according to attacks and diagnoses

Diagnosis at first presentation	Total *n*(%):424	1st attack *n*(%): 210	2nd attack *n*(%): 88	≥3. attack *n*(%): 126
Gout	117 (27.5%)	53 (25.2%)	22 (25.0%)	42 (33.3%)
Trauma and injury	66 (15.5%)	33 (15.7%)	14 (15.9%)	19 (15.0%)
Cellulite	62 (14.6%)	38 (18.0%)	14 (15.9%)	10 (7.9%)
Septic arthritis	54 (12.7%)	25 (11.9%)	11 (12.5%)	18 (14.2%)
Soft tissue infection	48 (11.3%)	20 (9.5%)	12 (13.6%)	16 (12.6%)
Deep vein thrombosis	21 (4.9%)	10 (4.7%)	3 (3.4%)	8 (6.3%)
Erythema nodosum	15 (3.5%)	9 (4.2%)	4 (4.5%)	2 (1.5%)
Paronychia	12 (2.8%)	6 (2.8%)	2 (2.2%)	4 (3.1%)
Tendinitis	8 (1.8%)	6 (2.8%)	0	2 (1.5%)
Insect bite	10 (2.3%)	6 (2.8%)	2 (2.2%)	2 (1.5%)
Abscess	11 (2.5%)	4 (1.9%)	4 (4.5%)	3 (2.4%)

DISCUSSION

The typical first presentation of gout is an intensely painful acute inflammatory arthritis (gout flare) affecting a lower limb joint[13]. In the absence of treatment, the gout flare is typically self-limiting over 7–14 days. After resolution, there is a pain-free asymptomatic period (intercritical gout), until another gout flare occurs. Over time, some people with persistent hyperuricemia also develop tophi, chronic gouty arthritis (persistent joint inflammation induced by monosodium urate crystals), and structural joint damage[2].

Gouty attacks often begin at night. They are characterized by a rapid onset and build-up of pain (usually 2–4 hours). The exquisite pain in acute gout is associated with warmth, redness, swelling, and decreased range of motion of the affected joint. Systemic symptoms and signs may also be present, such as malaise and low-grade fever (usually well tolerated and even unnoticed by the patient). Trauma, alcohol abuse, surgery, dietary excess, hypouricemia

and diuretic therapy, and severe medical illness can precipitate attacks[14].

The diagnostic value of a UA level is limited. A normal UA level does not exclude acute gout. As many as half of patients with acute gout may have normal UA levels during acute gout despite their increased UA pool[15]. Serum urate levels clearly can either increase or decrease with gout attacks and may even be below saturation levels for urate (6.8 mg/dL)[15]. As many as 49% of patients may have normal UA levels during bouts of acute gout[15]. Conversely, an elevated UA level alone does not serve as the sole criterion for gout. Most patients with hyperuricemia will never have an attack of gout.

The presence of local redness (erythema), uncommon in other causes of acute inflammation of articular structures, can be seen in crystal-induced arthritis and infectious (septic) arthritis, typically limited to small peripheral joints or superficial bursae or tendon sheaths. Overall, distinguishing between an infection and acute arthritis (septic or crystal-induced, like gouty arthritis) may be quite challenging. Ultrasound scans of the joints involved together with synovial fluid analysis remain the gold standard exams for the appropriate diagnosis; however, laboratory tests, including urate serum, inflammatory markers, and procalcitonin levels should be performed to provide a global view of the patient. Septic arthritis was considered in 12.7% of our patients, synovial fluid analysis was performed in those with knee swelling and no bacterial growth, synovial fluid analysis was not performed in those with MTF arthritis and antibiotic treatment was started. Septic arthritis was not considered in any of the patients who subsequently presented to rheumatology. Orthopedics and infectious diseases were the most common specialties to make this diagnosis.

Local symptoms and signs accompanying acute inflammation of articular and periarticular joint structures such as soft tissue edema and erythema of overlying skin may vary widely, from mild to severe, with the latter often mimicking cellulitis. Cellulitis causes pain and tenderness, edema, swelling caused by fluid buildup, redness of the skin warm to the touch, and fever. Cellulitis is a potentially serious skin infection caused by different types of bacteria (β-hemolytic streptococci, and generally group A streptococcus, i.e., Streptococcus pyogenes, followed by methicillin-sensitive Staphylococcus aureus[16]) may be clinically similar to a gouty attack, especially when involving lower limbs with concomitant redness and soft tissue swelling. Cellulitis was considered in 14.6% and soft tissue infection in 11.3% of our patients and antibiotics were started in all of them and infectious diseases and orthopedics were the most common specialties to make this diagnosis.

Erythema nodosum is the most common form of panniculitis and is characterized by tender erythematous nodules mainly in the lower limbs on the pretibial area[17]. The clinical course of EN is characterized by an acute onset of sensitive and erythematous nodules and plaques of 1–6 cm in diameter. Lesions are bilateral and symmetric, typically distributed on the distal lower extremities of the pretibial areas, although lesions can also involve the ankles, thighs, and forearms[17]. Lesions in and around the ankle may be confused with gout due to sudden onset and accompanying fever. However, it can be differentiated from gout by being symmetrical and distant from the joint periphery, especially in the pretibial area. It was considered in 3.5% of our patients and emergency physicians were the most common specialty to make this diagnosis.

Several insects, including bees, spiders, fleas, hornets, wasps, and mosquitoes can bite or sting. This is especially common in rural areas. Suspicion of insect bites may be among the preliminary diagnoses in emergency

applications in patients with findings such as redness, swelling, pain, and tenderness in and around the ankle. Insect bites were suspected in 2.3% of our patients at emergency presentation. The most important point in the differential diagnosis is the location of the bite and the accompanying itching and non-articular locations.

Sprains and strains are usually minor injuries that often occur during sports, exercise, or other physical activity. A sprain is an injury to a ligament, the tissue that links bones together at joints. Sprains happen most often in the ankle, knee, elbow, or wrist. Strain or trauma is the initial diagnosis in patients presenting with swelling of the big toe and ankle Although none of our patients had a history of trauma, accident, etc., it was observed that it was associated with a history of long travel, long-standing, and long walking. This diagnosis was especially high in orthopedics and emergency department applicants. Trauma, injury, strain, and sprain were diagnosed in 15.5% of our patients and most of them were treated with rest, cold treatment, and NSAIDs.

There are 3 different types of treatment needed in patients with gout[18]. The treatments include treatment of the acute attack; using urate-lowering drugs to decrease the increased total UA pool, and providing prophylaxis to prevent acute attacks while using urate-lowering drugs. The options available for the treatment of acute gout are NSAIDs, colchicine, and systemic and intraarticular corticosteroids. The most frequently used combinations are NSAIDs with intraarticular or oral corticosteroids and NSAIDs with oral colchicine. The number of patients diagnosed with gout before consulting a rheumatologist was 117 (27.5%) and the number of patients on colchicine at admission was 75 (17.6%). It was observed that the use of colchicine in some of our patients was incorrect. Historical dosing of colchicine followed the "dose to diarrhea" mantra, with a dose administered every 2 hours until any of the following occurred: relief of gouty arthritis, toxicity (diarrhea, nausea, vomiting), or achieving a maximum dose of 4.8 mg[19]. Although colchicine has been used for many years, it has been studied in only two randomized controlled trials for acute gout flares[20]. Low-dose colchicine commenced within 12 h of a flare (1.2 mg immediately followed by 0.6 mg after 1 hour) is as effective as high dose (1.2 mg immediately followed by 0.6 mg hourly for 6 hours) and is associated with substantially fewer adverse effects, particularly gastrointestinal adverse effects[20]. Thus, low-dose colchicine is the preferred option.

Important developments in the clinical management of gouty arthritis have occurred in recent years. The ACR/EULAR gout classification criteria provide clinicians with the ability to more specifically identify patients with acute gouty arthritis[10].

When asked about the reasons why patients applied to the branches during the attack, they stated that they thought it was due to the accessibility of emergency services and being seen without an appointment, for orthopedics, they thought it was due to a cause such as a fracture or sprain due to sudden swelling, limitation of movement, and for infectious diseases, they stated that they applied with signs of infection such as an increase in temperature and fever in that area.

In conclusion, although gout has been known for centuries, it can be confused with different diseases. Gout should always be considered in cases with a gout-like clinic such as septic arthritis, cellulitis, soft tissue infection, trauma, EN, DVT, and paronychia, which start with redness and pain in the lower extremities. With early diagnosis and treatment, participation in social life, work attendance, unnecessary treatment, and admissions can be prevented.

CONCLUSION

The burden of gout is significant. Acute gouty arthritis is a very painful and bothersome condition that is associated with a decreased quality of life. All physicians, regardless of their specialties, may be the first to see patients with gout and therefore play a critical role in the diagnosis and treatment of these patients. With correct diagnosis and treatment, many visits to the doctor can be reduced.

Ethics Committee Approval: This study was approved by the local ethics committee (approval date: 22/11/2023, decision no: ESH/GOEK 2023/55). It complied with the Helsinki Declaration's ethical criteria for human testing (2013).

Conflict of Interest: The authors declared no conflicts of interest.

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Original Article / Özgün Araştırma

Impact of Naples Prognostic Score on the development of surgical reexploration in open heart surgery patients

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Abstract

Background: Significant and minor perioperative bleeding is common in patients undergoing open heart surgery. Perioperative bleeding has become an important prognostic indicator. Naples Prognostic Score (NPS); It is an effective tool that can be used to detect malnutrition, calculated according to serum albumin level, total cholesterol amount, neutrophil-lymphocyte ratio (NLR), and lymphocyte-monocyte ratio (LMR). In our study,we were planned to evaluate malnutrition and show its negative effects by using the NPS score in patients undergoing open heart surgery.

Methods and Results: For the study, a total of 2071 patients who underwent open heart surgery were examined in detail randomly, sequentially and retrospectively. A total of 1825 patients were examined in detail after the patients covering the exclusion criteria were removed. It was determined that a total of 73 patients underwent surgical reexploration. In 53 (4.3%) of the patients with coronary artery bypass surgery, in 11 (3%) of the patients with heart valve surgery, in 8 (4.7%) of the patients with heart valve surgery combined with coronary artery bypass surgery, It was determined that surgical reexploration was performed in 1 (2.8%) of the other procedures. Multivariate regression model; showed that being in the high NPS score group, increasing age, and female gender were independent determinants of the need for surgical reexploration. It was determined that the need for reexploration was observed in the group with higher NPS score (median 3.18 vs 2.69, $p<0.001$).

Conclusion: A high NPS score can provide very important prognostic information. Patients in the high NPS score group required surgical reexploration more frequently.

Keyword: Open Heart Surgey, NPS Score, surgical reexploration

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Açık kalp ameliyatı olan hastalarda NaplesPrognostik Skorunun cerrahi revizyona olan etkisi

Öz

Amaç: Açık kalp ameliyatı geçiren hastalarda önemli ve küçük perioperatif kanamalar yaygındır. Perioperatif kanama önemli bir prognostik gösterge haline gelmiştir. Naples Prognostik Skoru (NPS); Serum albümin düzeyi, toplam kolesterol miktarı, nötrofil-lenfosit oranı (NLR) ve lenfosit-monosit oranına (LMR) göre hesaplanan, malnütrisyonun tespitinde kullanılabilecek etkili bir araçtır. Çalışmamızda açık kalp ameliyatı geçiren hastalarda NPS skoru kullanılarak malnütrisyonun değerlendirilmesi ve olumsuz etkilerinin gösterilmesini planlandık.

Yöntemler ve Sonuçlar: Çalışma için açık kalp ameliyatı geçiren toplam 2071 hasta rastgele, sıralı ve geriye dönük olarak detaylı olarak incelendi. Dışlama kriterlerini karşılayan hastalar çıkarıldıktan sonra toplam 1825 hasta detaylı olarak incelendi. Toplam 73 hastaya cerrahi revizyon uygulandığı belirlendi. Koroner arter bypass ameliyatı olan hastaların 53'ünde (%4,3), kalp kapağı ameliyatı olan hastaların 11'inde (%3), koroner arter bypass ameliyatı ile birlikte kalp kapağı ameliyatı olan hastaların 8'inde (%4,7), diğer işlemlerin 1'inde (%2,8) cerrahi revizyon yapıldığı belirlendi. Çok değişkenli regresyon modeli; yüksek NPS skoru grubunda olmanın, artan yaşın ve kadın cinsiyetin cerrahi revizyon ihtiyacının bağımsız belirleyicileri olduğunu gösterdi. NPS puanı yüksek olan grupta yeniden araştırma ihtiyacının gözlendiği belirlendi (medyan 3,18 vs 2,69, p<0,001).

Sonuç: Yüksek NPS skoru çok önemli prognostik bilgiler sağlayabilmektedir. Yüksek NPS skoru grubundaki hastalar daha sık cerrahi revizyona ihtiyaç duydu.

Anahtar kelimeler: Açık Kalp Ameliyatı, NPS Skor, surgical reexploration.

INTRODUCTION

Significant and minor perioperative bleeding is common in patients undergoing open heart surgery. Perioperative bleeding has become an important prognostic indicator[1]. Strangely enough, there is no standardized definition for perioperative bleeding. Surgical re-exploration can be performed as a rescue in patients who cannot control bleeding related to the procedure.

Malnutrition is a serious health problem that has a bad effect on all systems and is very common in patients hospitalized for any reason. Malnutrition is also very common in the patient population who are candidates for or have a history of cardiovascular surgery and is associated with adverse postoperative outcomes[2]. It is known that a significant portion of patients undergoing cardiac surgery have defined or undefined levels of malnutrition[3].

Many assessment tools have been previously described to identify nutritional conditions that are of prognostic importance. Evaluation of nutritional status with blood parameters has become the latest tool in recent times. However, in order for these assessment tools to provide objective results, it is important that they provide scores that are simple, easily calculated, and whose results do not vary much from person to person or from measurement to measurement[4]. Some tested scoring systems have been shown to provide data with significant prognostic value in patients with cardiovascular disease[5].

NPS; It is an effective tool that can be used to detect malnutrition, calculated according to serum albumin level, total cholesterol amount, neutrophil-lymphocyte ratio (NLR), and lymphocyte-monocyte ratio (LMR). NPS (Naples prognostic score) is a parameter that shows nutritional status by calculating it with laboratory parameters and has been shown to have an effect on prognosis in many diseases[6].

In this study, it was planned to evaluate malnutrition and show its negative effects by

using the NPS score in patients undergoing open heart surgery.

METHODS

Study Design

In our research, we planned to conduct a single-center, observational study examining patients who underwent open heart surgery for any reason. This study was approved by the Dicle University Faculty of Medicine ethics committeedecision number 22/11/2023-301. Patients who underwent open surgery in our clinic between January 2013 and December 2023 were retrospectively screened without random selection. The patients' clinical epicrisis and surgery notes were examined in detail and laboratory parameters were analyzed in detail and a comparison was made.

Patients

For the study, a total of 2071 patients who underwent open heart surgery were examined in detail randomly, sequentially and retrospectively. Patients were excluded from screening due to the presence of diseases that could affect our study results. According to the design of the study, patients who could be monitored for bleeding for at least 48 hours were included in the study in order to test whether there was any bleeding requiring revision. Patients who exited for any reason before the end of the 48-hour follow-up period and whose blood parameters could not be studied were excluded from the study. A total of 1825 patients were examined in detail after the patients covering the exclusion criteria were removed.

Definitions

Patients whose venous blood samples were routinely taken during hospitalization and those who were followed up were included in the study. A complete blood count (CBC) was performed with an automated system and hematological indices were calculated for each patient. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride and other biochemical levels were measured.

Surgical Reexploration

Surgical reexamination may be performed when blood loss cannot be stopped with surgical tubes or blood transfusions. Surgical reexamination may occur early or late. Surgical reexamination is considered an indicator of severe bleeding. In our study, salvage surgical procedures performed after blood loss could not be controlled with surgical techniques and precautions were grouped as surgical reexploration.

Follow-up and study outcomes

Patients were followed up by looking at epicrisis and surgery reports. We used the occurrence of in-hospital bleeding requiring revision that could be attributed to the operation as the main outcome. We analyzed each revision operation separately.

Parameter Assessment

NLR was calculated as the neutrophil count/lymphocyte count ratio. LMR was calculated as lymphocyte count/monocyte count. While calculating the NPS score; If the albumin value was ≥4.0 g/dL, 0 point was given, and if <4.0 g/dL, 1 point was given. While calculating the NPS score; If the total cholesterol value was >180 mg/dL, 0 points were given, and if it was ≤180 g/dL, 1 point was given. While calculating the NPS score; If the NLR value was ≤2.96, 0 point was given, and if >2.96 g/dL, 1 point was given. While calculating the NPS score; If the LMR value was >4.44, 0 point was given, and if it was ≤4.44 g/dL, 1 point was given. The total score was calculated and a comparison was made based on the total score.

Statistical Analysis

We analyzed our data in SPSS for Windows version 25.0. First, we analyzed the normality of

the distribution. For normally distributed data, they were compared using the Student-t test, and for non-normally distributed data, the Mann-Whitney U test was used. Abnormally distributed variables were expressed as median IQR (interquartile range). We calculated the categorical variables we created as percentages (%) and compared them using the Chi-square test/Fischer's Exact. When the number of groups was large, comparison was made using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, as appropriate. P value <0.05 was considered significant.

RESULTS

Baseline Characteristics

The data of 1825 patients were evaluated retrospectively to examine the determined points. Figure 1 shows the flow chart showing the patient acceptance and distribution of our study. The majority of our cases consist of coronary artery bypass surgery. It was determined that a total of 73 patients underwent surgical reexploration. In 53 (4.3%) of the patients with coronary artery bypass surgery, in 11 (3%) of the patients with heart valve surgery, in 8 (4.7%) of the patients with heart valve surgery combined with coronary artery bypass surgery, It was determined that surgical re-examination was performed in 1 (2.8%) of the other procedures.

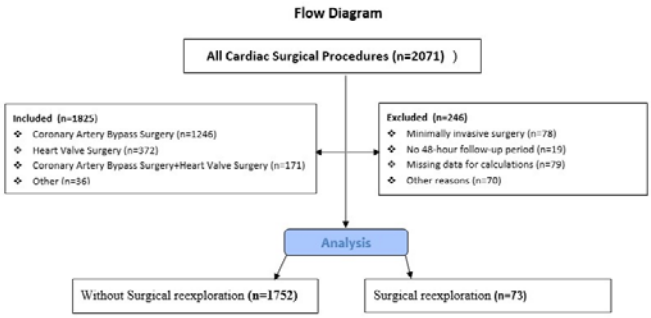

Figure 1. Flow Chart of Trial design and distribution of surgical procedures

Associations between surgical reexploration and clinical variables

Demographic and laboratory parameter variables of patients who underwent and did not undergo surgical reexploration after open heart surgery are given in Table 1.

Table I: General demographic and laboratory characteristics of the patients

Variables	WithoutSurgical Exploration (n=1752)	SurgicalReexploration (n=73)	*p*value
Age (years)	60.1±11.8	64.7±9.7	***0.001***
Femalegender, n (%)	512(29)	39(53)	***<0.001***
Hypertension, n (%)	729(42)	41(56)	***0.014***
Diabetesmellitus, n (%)	509(29)	29(40)	0.050
Smoking, n (%)	563(32)	13(18)	***0.010***
STS score(IQR)	4.20(4.40)	4.32(5.18)	0.796
Euro score(IQR)	17.11 (17.8)	18.95 (22.2)	0.285
LVEF (%)	52.2±6.7	51.5±7.0	0.358
GlomerularFiltration Rate (ml/min)	82.9±23.1	76.4±25	***0.032***
Hemoglobin (g/dl)	13.4±2	12.8±2	***0.014***
Leukocyte (× 10^3 µL)	11.097±5.066	11.213±4.356	*0.825*
Lymphocyte (× 10^3 µL)	2016±1131	1623±765	***<0.001***
Monocytes (× 10^3 µL) (IQR)	687(707)	813(911)	***0.014***
Neutrophil (× 10^3 µL)	8394±4828	8776±4129	*0.444*
Platelet (× 10^3 µL)	250±68	257±71	0.465
Glucose (mg/dl)	154±71	163±60	*0.286*
Serum albumin (g/dl)	3.68±0.48	3.46±0.44	***<0.001***
Total cholesterol (mg/dl)	181±47	174±44	0.201
Triglyceride (mg/dl) (IQR)	153(158)	136(161)	0.186
High DensityLipoprotein (mg/dl)	39±11.4	39±9.3	0.659
LowDensityLipoprotein (mg/dl)	113±38	108±37	0.309
NLR (IQR)	5.64(5.88)	7.64(9.20)	***0.013***
LMR(IQR)	3.53(3.70)	2.37(2.70)	***0.008***
Total NaplesScore (IQR)	2.69(2.74)	3.18(3.37)	***<0.001***

The mean age was significantly higher in the surgical reexploration group (64.7±9.7vs. 60.1±11.8, p=0.001). The need for surgical reexploration was significantly more common in women (53% vs 29%, p<0.001).

It was determined that the history of hypertension was higher in the surgical reexploration group (56% vs. 42%, p = 0.014). Additionally, surgical reexploration revealed less frequent smoking addiction ((18% vs 32%, p=0.010).

GFR, lymphocyte count, albumin level and hemoglobin values were found to be significantly lower in the surgical reexploration group. On the contrary, the number of monocytes was observed to be significantly higher.

In terms of our study hypothesis, it was determined that the need for reexploration was observed in the group with higher NPS score (median 3.18 vs 2.69, p<0.001).

Associations between NPS and clinical variables

The NPS score of the patients was analyzed with the receiver operating characteristic (ROC) to predict the need for surgical reexploration (Figure2). The optimum NPS score cut-off value was determined to be >2.5.

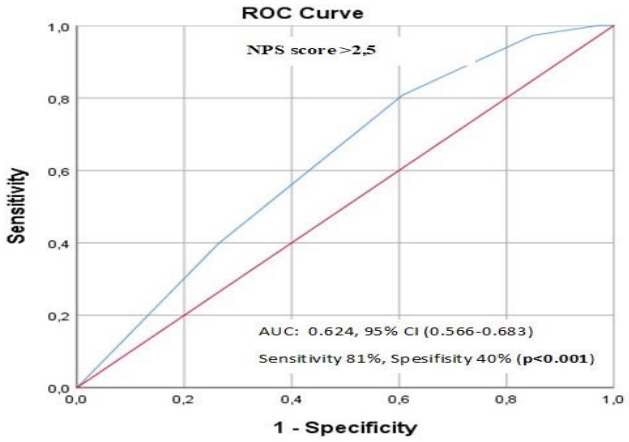

Figure 2. Analysis of NPS score with receiver operating characteristic (ROC) to predict the need for surgical reexploration.

Comparison of clinical and laboratory data when our patients are grouped as those with a score above or below 2.5 according to the NPS score is given in Table 2.

Table II: General demographic and laboratory characteristics of patients according to NPS score group

Variables	NPS score<2,5 (n=703)	NPS score>2,5 (n=1122)	*p*value
Age (years)	58.5±11.4	61.3±11.8	***<0.001***
Femalegender, n (%)	206(29)	345(31)	0.513
Hypertension, n (%)	281(40)	489(44)	0.128
Diabetesmellitus, n (%)	210(30)	328(29)	0.771
Smoking, n (%)	218(31)	358(32)	0.658
LVEF (%)	53±6.9	51.7±6.5	***<0.001***
GlomerularFiltration Rate (ml/min)	85.1±21.5	81.1±24	***<0.001***
Hemoglobin (g/dl)	13.7±1.9	13.1±2.0	***<0.001***
Platelet (× 10^3 µL)	255±63	248±71	***0.025***
Glucose (mg/dl)	156±78	155±66	0.745
SurgicalReexploration, n (%)	14(2)	59(5)	***0.001***

It was observed that the average age was higher in the group with a higher NPS score. In addition, it was observed that glomerular filtration rate, hemoglobin level and platelet level were significantly lower in the group with high NPS score.

There was a significantly higher rate of reexploration requirement in the group with a high NPS score (5% vs. 2% p=0.001).

Prognostic value of NPS

They were evaluated with multivariate regression analyzes in terms of predictors of surgical reexploration in Table 3.

Table III: Determinants of the need for surgical reexploration in multivariate regression analysis

	Multivariateregression		
	OR	**95% CI**	***pvalue***
Age (years)	1.028	1.003-1.053	***0.025***
Femalegender, (%)	2.765	1.692-4.517	***<0.001***
Hypertension,(%)	1.234	0.744-2.047	0.415
Diabetesmellitus, (%)	1.321	0.745-2.344	0.341
Smoking, (%)	1.402	0.737-2.670	0.303
LVEF (%)	0.990	0.955-1.026	0.569
GlomerularFiltration Rate	0.994	0.985-1.004	0.259
Hemoglobin	0.928	0.818-1.054	0.252
Glucose	1.001	0.997-1.005	0.542
High NPS scoregroup	2.222	1.212-4.072	***0.010***

Multivariate regression model; showed that being in the high NPS score group, increasing age, and female gender were independent determinants of the need for surgical reexploration.

DISCUSSION

It is known that low albumin value increases all-cause mortality and the risk of complications in the population undergoing cardiac surgery[7]. In our study, the low albumin value in the group that developed surgical reexploration once again reveals the prognostic importance of albumin. The prognostic importance of low lymphocyte count in patients undergoing cardiovascular intervention has been shown in previous studies[8]. One of the important results of our study is the strong relationship between low lymphocyte count and surgical reexploration. Considering its inflammatory and nutritional importance, the fact that lymphocyte count provides meaningful prognostic data is also important in terms of the power of the NPS score, which is affected by the lymphocyte count, which we examined in our study. A recent study showed that nutritional and inflammatory scoring has prognostic importance in patients undergoing cardiovascular intervention[9]. NPS scoring has been shown in a previous study to provide prognostic prediction after thoracic surgery[10]. Our study is the first to show that NPS scoring provides prognostic information after open heart surgery. The fact that low cholesterol value, one of the NPS score variables, does not provide strong prognostic data in our study is confusing regarding its importance as a nutritional indicator in cardiovascular diseases.

It is known that the prognosis after cardiac surgery is worse in women[11]. Our study shows that female gender is still associated with poor outcome. Considering the average age of our patients, a worse prognosis is expected in female gender in terms of all endpoints in longer follow-up.

Although the average age in our study was relatively young, showing that the frequency of complications increases with age gave a result consistent with the data in the literature[12]. This shows that the passing years not only have a negative impact on human health, but also the benefit of treatment is negatively affected by age.

The importance of renal functions in cardiovascular diseases and patients undergoing open heart surgery has been shown by numerous studies[13]. The relationship between low GFR level and the development of surgical reexploration in our own patient population shows how important preoperative renal function evaluation is.

In a previous study have paradoxically shown that, contrary to expectations, bleeding complications are less common in cardiovascular interventions in smokers[14]. In our study, the demonstration that there is a decrease in the need for surgical reexploration in the smoker population proves that smoking has a positive effect on bleeding parameters.

We reached very important results in our retrospective study involving a large patient population: 1) We found that the frequency of surgical reexploration, which has a very valuable prognostic importance in patients undergoing open heart surgery, is a significant rate, 2) It has been shown that a high NPS score is predictive of the need for surgical reexploration, 3) The need for surgical reexploration has been shown to be more common in women, 4) Low GFR, low hemoglobin and advanced age have been shown to be important determinants for the development of this surgical complication.

Limitations

This study had some limitations. Since the patients could not be followed prospectively, patients who required surgical reexploration but died before the procedure could be performed may have been excluded from the study. Since the number of patients who underwent surgical reexploration is small, more meaningful results may be obtained in a larger group.

CONCLUSION

The need for surgical reexploration is an important prognostic condition. All causes that increase the frequency of encounters can be considered as indirect or direct indicators of morbidity and mortality. It is known that variables such as low albumin value, low lymphocyte count, high monocyte and neutrophil count, which indicate the nutritional and inflammatory status of the patients at the time of admission, already have prognostic importance. The high NPS score, which is formed by the contribution of these at certain rates, can also provide very important prognostic information. Patients in the high NPS score group required surgical reexploration more frequently. In future studies, it may be seen that the frequency of endpoints may decrease after the follow-up NPS values are examined and correction is made.

Ethics Committee Approval: This study was approved by the Dicle University Faculty of Medicine ethics committee decision number 22/11/2023-301.

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Original Article / Özgün Araştırma

The Effect of Cerebral White Matter Lesions on Walking Time & Vascular Risk Factors

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Abstract

Introduction: White matter lesions are common neurological diseases in the elderly. In this study, we aimed to investigate the impact of cerebral white matter lesions on walking time and daily living activities in the elderly population.

Methods: A total of 82 individuals, including 40 healthy volunteers and 42 age and sex-matched patients, were enrolled in this study. Magnetic resonance imaging findings were recorded based on the Fazekas Staging System. Risk factors potentially predisposing individuals to white matter lesions were documented through laboratory testing. Additionally, atherothrombotic plaque formations and stenosis were graded using Carotid and Vertebral Artery Doppler Ultrasonography in patients with white matter lesions. The Lawton Instrumental Activities of Daily Living Scale, Mini-Mental State Examination, and the Timed Up & Go Test were administered to both the patient and control groups to assess daily living activities and cognitive functions.

Results: The results of this research showed that as the percentage of stenosis increased in Carotid and Vertebral Artery Doppler Ultrasonography, there were corresponding decreases in Lawton Instrumental Activities of Daily Living Scale scores, haemoglobin and hematocrit values while fasting blood glucose and homocysteine levels increased as expected. Furthermore, significant differences were observed in the Timed Up & Go Test in advanced stages when evaluated according to the Fazekas Staging System.

Conclusion: Our study indicated that white matter lesions do not significantly affect daily living activities but prolong the walking time in elderly individuals.

Keywords: White Matter, Magnetic Resonance Imaging, Walking, Stroke, Brain, Gait

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Serebral Beyaz Cevher Lezyonlarının Yürüme Süresi ve Vasküler Risk Faktörleri Üzerine Etkisi

Öz

Giriş: Beyaz cevher lezyonları yaşlılarda sık görülen nörolojik radyolojik bulgulardandır. Bu çalışmada, yaşlı popülasyonda serebral beyaz cevher lezyonlarının yürüme süresi ve günlük yaşam aktiviteleri üzerine olan etkisini araştırmayı amaçladık.

Yöntemler: Bu çalışmaya 40 sağlıklı gönüllü ve 42 hasta yaş ve cinsiyet uyumlu toplam 82 birey dahil edildi. Manyetik rezonans görüntüleme bulguları Fazekas sınıflandırmasına göre kaydedildi. Bireyleri beyaz cevher lezyonlarına yatkın hale getirebilecek risk faktörleri ile ilgili laboratuvar testleri kayıt altına alındı. Ayrıca, beyaz cevher lezyonu olan hastalarda aterotrombotik plak oluşumları ve stenoz, Karotis ve Vertebral Arter Doppler Ultrasonografi kullanılarak derecelendirilmiştir. Günlük yaşam aktivitelerini ve bilişsel işlevleri değerlendirmek için hem hasta hem de kontrol gruplarına Lawton Enstrümantal Günlük Yaşam Aktiviteleri Ölçeği, Standardize Mini-Mental Test ve Zamanlı Kalk ve Yürü Testi uygulanmıştır.

Bulgular: Bu araştırmanın sonuçları, Karotis ve Vertebral Arter Doppler Ultrasonografisinde darlık yüzdesi arttıkça Lawton Enstrümantal Günlük Yaşam Aktiviteleri Ölçeği skorlarında, hemoglobin ve hematokrit değerlerinde azalma olduğunu, açlık kan şekeri ve homosistein düzeylerinde ise literatüre uyumlu olarak yüksek olduğunu göstermiştir. Ayrıca, Fazekas sınıflamasına göre değerlendirildiğinde, ileri evrelerde Zamanlı Kalk ve Yürü Testinde anlamlı bulgular saptanmıştır.

Sonuç: Çalışmamız, beyaz cevher lezyonlarının yaşlı bireylerde günlük yaşam aktivitelerini önemli ölçüde etkilemediğini ancak yürüme süresini uzattığını göstermiştir.

Anahtar kelimeler: Beyaz Cevher, Manyetik Rezonans Görüntüleme, Yürüme, İnme, Beyin, Denge.

INTRODUCTION

White matter diseases, also known as leukoencephalopathies, encompass a range of conditions that primarily or exclusively affect the brain's white matter[1].

White matter hyperintensities are prevalent in the ageing population, with up to 80% of healthy individuals aged 60 showing these abnormalities. These hyperintensities are also observed in Alzheimer's Disease (AD), dementia, cognitive impairment, and other conditions[2,3].

However, our understanding of the distinctions between subcortical atrophy and lesions in the periventricular region, the functional implications of lobar region lesions, and the frequency specific to the location of vascular lesions remain limited. Different disorders may exhibit variations in subcortical, juxtacortical, periventricular white matter lesions and vascular lesions in deep brain structures[4].

Consequently, making these distinctions becomes crucial as it can have significant implications. White matter lesions, an irreversible and progressive clinical condition, underscore the importance of early symptom detection and the development of preventive approaches and treatments[5].

Magnetic resonance imaging (MRI) is crucial in diagnosing patients with leukoencephalopathy. It is important to note that each leukoencephalopathy presents diverse patterns of MRI abnormalities, even among patients with the same disease[6].

Detecting clinically silent lesions is of utmost significance in identifying individuals who require treatment and determining appropriate preventive measures.

In this study, our primary objective was to investigate the impact of cerebral white matter lesions on walking time and daily living

activities in the elderly population. By understanding these effects, we aim to contribute to a better understanding of the condition and potentially develop interventions to improve the well-being of affected individuals and their families.

METHOD

A total of 82 individuals, comprising 40 controls (healthy volunteers) and 42 age- and sex-matched patients who were admitted to the outpatient clinic due to headache and myalgia, were included in this study. Patients aged between 40 and 80 years who presented to the outpatient clinic with white matter lesions on cerebral MRI and were diagnosed with headache, myalgia and neuralgia, but without a history of falls and balance disorders were included in the study after a detailed neurological examination by a neurologist. History of additional systemic diseases, systolic and diastolic blood pressure values, body mass index (BMI), The Lawton Instrumental Activities of Daily Living (IADL) Scale, Mini-Mental State Examination (MMSE) and the Timed Up & Go Test (TUG) were administered

by the same neurologist. Individuals with a history of symptomatic stroke or neurodegenerative diseases that may cause gait disturbance were excluded.

All procedures followed the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval was granted by our institution on 09/06/2022 with protocol number 1982, and informed consent was obtained from all participants.

MRI imaging of the patients was performed on a 1.5 Tesla scanner (General Electric Optima 360, Milwaukee - USA, 2014) using 16-channel phased array coils. MRI findings were recorded according to the Fazekas Staging System [Fazekas staging: Fazekas 0: No lesion or single point lesion (white matter hyperintensity), Fazekas 1: Many point lesions, Fazekas 2: Lesions tending to coalesce (bridging), Fazekas 3: Large, confluent lesions)]. The evaluation of MRI white matter lesions according to the Fazekas Staging System was performed by two interventional neurologists in a double blinded manner.

Risk factors that may predispose to white matter lesions (WML) were recorded in laboratory examinations, including the neutrophil-to-lymphocyte ratio, lipid panel, homocysteine levels.

Atherothrombotic plaque formations and stenosis were graded by performing carotid and vertebral Doppler ultrasonography (CVDUSG) in patients with WML.

Statistical Analysis

Statistical analysis was performed using the SPSS software package (Version 25.0, SPSS Inc., Chicago, IL, USA). Normal continuous variables were expressed as the mean ± standard deviation ($p > 0.05$ in the Kolmogorov-Smirnov test or Shapiro-Wilk, $n < 30$), and in case of non-normality, they were described as the median. Comparisons between groups were conducted using the Student's t-test or One-Way analysis of variance (ANOVA) for normally distributed data, and the Mann-Whitney U test or Kruskal-Wallis test was utilized for data that were not normally distributed. A p-value of < 0.05 was considered statistically significant.

The categorical variables between the groups were analyzed using the Chi-square test or Fisher's Exact test. Spearman's correlation coefficient was used to test correlations between parameters.

Correlation coefficients were interpreted as: Strong Correlation $r \geq 0.91 \geq r \geq 0.71$; Medium Correlation; $0.70 \geq r \geq 0.51$; Weak correlation $0.50 \geq r \geq 0.31$; and Very Weak or no correlation $r \leq 0.3$.

RESULTS

The mean age of the patients (n = 42) included in the study was 65.3 ± 10 years, while the mean age in the control group (n = 40) was 56.1 ± 12 years (p = 0.052). The gender distribution was as follows: 65.9% (n = 54) of the included individuals were female, and 34.1% (n = 28) were male. Among the 82 people in both groups, 37.8% (n = 31) had hypertension, 17.1% (n = 14) had diabetes mellitus, 14.6% (n = 12) had coronary artery disease, 24% (n = 12) had psychiatric illness, 2.4% (n = 2) had urinary incontinence, 13.4% (n = 11) had constipation, 25.6% (n = 21) had balance disorder, and 13.4% (n = 11) had a history of falling. Antiaggregant use was reported in 17.1% (n = 11) of the patients (Table 1).

Table I: Correlation Analysis for the Patient Group

		CVDUSG	Fazekas Staging	IADL	MMSE	TUG
IADL	r	**-0.32**	-0.23			
	p	**0.041**	0.147			
MMSE	r	-0.301	0.071	**0.60**		
	p	0.053	0.657	**0.0001**		
TUG	r	-0.05	**0.44**	**-0.50**	**-0.56**	
	p	0.737	**0.004**	**0.0001**	**0.000**	
B12(pg/ml)	r	0.22	0.11	0.03	0.06	-0.14
	p	0.157	0.479	0.799	0.603	0.223
Vitamin D(µg /l)	r	0.07	-0.08	0.10	0.14	-0.07
	p	0.654	0.621	0.365	0.215	0.505
WBC(10^3/µl)	r	-0.01	0.26	-0.07	-0.10	0.18
	p	0.960	0.098	0.544	0.367	0.104
HGB(g/dl)	r	**-0.38***	-0.15	0.05	-0.13	0.16
	p	**0.014**	0.339	0.634	0.259	0.143
HTC(%)	r	**-0.42**	-0.21	0.04	-0.16	0.15
	p	**0.006**	0.185	0.728	0.148	0.176
PLT(10^3/µl)	r	0.16	0.18	-0.01	0.02	-0.02
	p	0.311	0.248	0.963	0.839	0.849
FPG(mg/dl)	r	**0.35**	0.01	**-0.23**	**-0.30**	0.17
	p	**0.022**	0.981	**0.034**	**0.006**	0.124
LY(10^3/µl)	r	0.06	0.21	0.03	-0.05	0.03
	p	0.681	0.184	0.754	0.674	0.778
NE(10^3/µl)	r	0.01	0.19	-0.04	-0.07	0.15
	p	0.966	0.216	0.698	0.526	0.179
NLR	r	-0.01	0.02	-0.07	-0.01	0.09
	p	0.939	0.881	0.525	0.899	0.385
Total Cholesterol(mg/dl)	r	-0.03	-0.136	-0.024	-0.048	0.045
	p	0.824	0.390	0.833	0.669	0.687
LDL(mg/dl)	r	-0.172	-0.140	0.048	-0.042	0.060
	p	0.277	0.377	0.666	0.705	0.592
HDL(mg/dl)	r	-0.111	-0.134	0.025	0.142	-.241*
	p	0.486	0.399	0.826	0.204	0.029
Triglyceride(mg/dl)	r	0.031	0.174	-.235*	-.288**	.282*
	p	0.845	0.271	0.034	0.009	0.010
Homocysteine(mmol/l)	r	**0.33**	0.09	**-0.36**	**-0.59**	**0.35**
	p	**0.034**	0.570	**0.001**	**0.0001**	**0.001**
Age(years)	r	-0.04	0.16	**-0.35**	**-0.54**	**0.48**
	p	0.821	0.297	**0.001**	**0.0001**	**0.0001**
BMI(kg/m^2)	r	-0.07	-0.05	-0.13	**-0.43**	**0.31**
	p	0.664	0.747	0.243	**0.0001**	**0.004**

CVDUSG= Carotid and vertebral Doppler ultrasonography, IADL= The Lawton Instrumental Activities of Daily Living, MMSE= Mini-Mental State Examination, TUG= the Timed Up & Go Test, WBC= white blood cell, HGB=haemoglobin, HTC=hematocrit,FPG=fasting blood glucose,LY=lymphocyte, NE= neutrophil, NLR=neutrophil lymphocyte ratio,LDL=low-density lipoprotein, HDL=high-density lipoprotein, BMI= body mass index

In addition, at the beginning of the study, the patient group exhibited higher levels of BMI (p = 0.047), systolic blood pressure (p = 0.004), diastolic blood pressure (p = 0.027), fasting blood glucose (p = 0.006), total cholesterol (p = 0.048), triglycerides (p = 0.009), and homocysteine (p = 0.0001) parameters when compared to the control group. According to the Fazekas System Staging, 31% (n = 13) of the patients were classified as Stage 1, 38.1% (n = 16) as Stage 2, and 31% (n = 13) as Stage 3 (Table 2).

Table II: Comparison of variables according to Fazekas System Staging

	Control Group	Fazekas Stage 1 (n=13)	Fazekas Stage 2 (n=16)	Fazekas Stage 3 (n=13)	P Value	Fazekas Stage 1-2-3 P Value
Age(years)	56.1±12.0	64.5±7.2	65.3±10.7	66.2±12.0	0.051	0.908
BMI(kg/m^2)	26.9±3.9	29.6±4.8	27.4±2.9	30.9±8.7	0.052	0.250
Systolic Blood pressure(mm/hg)	121.5±11.6	129.6±9.9	130.3±15.5	131.5±20.3	**0.042**	0.952
Diastolic Blood pressure(mm/hg)	78.2±8.5	83.4±5.2	80.9±6.4	83.4±13.2	0.130	0.678
WBC((10^3/µl)	6.9±1.5	7(0.0-9.9)	7.2(5.4-11.7)	8.2(5.4-10.4)	0.121	0.190
HGB(g/dl)	12.8±1.7	13.8±1.1	13.2±1.4	13.2±1.1	0.204	0.385
HCT(%)	38.5±4.1	41.4±3.5	39.0±4.1	39.2±2.8	0.147	0.186
PLT(10^3/µl)	259(162-437)	248(177-421)	237(141-376)	293(216-389)	0.377	0.180
FPG(mg/dl)	95(63-112)	100(78-238)	99(73-219)	101(91-340)	**0.016**	0.475
LY(10^3/µl)	2.1±0.6	2.1±0.4	2.2±0.6	2.5±0.6	0.316	0.249
NE(10^3/µl)	3.8(2.1-7.4)	4.3(2.8-64.4)	4.3(2.8-64.4)	5.4(3.0-66)	0.154	0.403
NLR	2.07(0.6-8.2)	1,89(1.1-37.9)	1.96(0.9-4.2)	1.87(1.5-3.1)	0.190	0.363
Total cholesterol(mg/dl)	188(119-271)	222(138-328)	225(125-280)	195(117-302)	0.183	0.703
LDL(mg/dl)	125(67-177)	147(88-241)	148(86-179)	130.615	0.204	0.606
HDL(mg/dl)	45(33-80)	51(31-78)	50(25-76)	46(33.82)	0.841	0.710
Triglyceride(mg/dl)	125(38-271)	149(67-249)	162(75-319)	176(101-248)	**0.039**	0.463
Homocysteine(mmol/l)	8(5-44)	13(10-24)	14(8-17)	16(-24)	**0.0001**	0.284
B12(pg/ml)	258(100-1500)	197(50-1044)	231(123-523)	254(111-1500)	0.116	0.145
Vitamin D(µg /l)	16.0(7.3-25.4)	13.7(7.0-30.9)	16.6(3.1-30.6)	16.7(6.5-38.0)	0.944	0.904
CVDUSG	-	1(0-3)	1(0-3)	1(0-3)	-	0.536
IADL	8(6-8)	8(3-8)	7(5-8)	7(1-8)	**0.011**	0.432
MMSE	30(25-30)	25.1±3.6	25.6±3.4	25.2±3.9	**0.0001**	0.931
TUG	8.2(5.6-14.7)	10.1±1.7	11.1±2.1	15.6±3.1	**0.0001**	**0.008**
Gender *(Female)*	28(70.0)	6(46.2)	12(75.0)	8(61.5)	0.354	0.282
Presence of Urinary incontinence	0(0.0)	3(23.1)	1(6.3)	7(53.8)	**0.0001**	**0.014**

Mean±SD; Medyan (Min-Min); n (%) CVDUSG= Carotid and vertebral Doppler ultrasonography, IADL= The Lawton Instrumental Activities of Daily Living, MMSE= Mini-Mental State Examination, TUG= the Timed Up & Go Test, WBC= white blood cell, HGB=haemoglobin, HTC=hematocrit, FPG=fasting blood glucose, LY=lymphocyte, NE= neutrophil, NLR=neutrophil lymphocyte ratio, LDL=low-density lipoprotein, HDL=high-density lipoprotein, BMI= body mass index

According to laboratory clinical findings, only the TUG-Test showed statistical significance when assessed according to the Fazekas Staging System. No statistically significant correlations were found between gender, hypertension, diabetes mellitus, coronary artery disease, psychiatric diseases, falls, constipation, imbalance, anti-aggregant use, and Fazekas stage. Moreover, based on laboratory clinical findings, only the TUG-Test demonstrated statistical significance when considering Fazekas stages. No difference was observed between Stage 1 and Stage 2 Fazekas concerning the TUG-Test; however, Fazekas Stage 3 exhibited a statistically significant increase.

A positive correlation was identified between the Lawton IADL Scale and MMSE. The Lawton IADL Scale also revealed negative correlations between the TUG-Test, fasting plasma glucose (FPG), homocysteine, and age.

Upon evaluating the correlation table, we observed a statistically significant inverse correlation between CVDUSG and the Lawton IADL Scale, haemoglobin (Hgb), and hematocrit (Htc) variables. Atherosclerotic plaque formation was present in most patient groups (n = 29). Moreover, the correlation table indicated a statistically significant inverse correlation between CVDUSG and the Lawton Brody scale and haemoglobin and hematocrit variables. We expect that as the CVDUSG value increases, the Lawton Brody, Hgb, and Htc values will decrease. A positive correlation was also found between CVDUSG and FPG and homocysteine values (Table 3).

Table III: Distribution of patients according to CVDUSG and Fazekas Staging

	n	%
CVDUSG		
Atherosclerosis	3	7.1
ICA stenosis 0-50%	29	69.0
ICA stenosis 50-70%	7	16.7
ICA stenosis 70-99%	2	4.8
ICA occlusion	1	2.4
Fazekas Staging		
1	13	31.0
2	16	38.1
3	13	31.0

CVDUSG=carotid vertebral doppler ultrasonography, ICA= internal carotid artery

DISCUSSION

WML, also known as leukoaraiosis, are defined as diffuse or localized areas of abnormality in the white matter, often symmetrical, and frequently observed in the periventricular white matter, particularly adjacent to the lateral ventricular horns. Leukoaraiosis is a term used in radiological imaging methods, and its prevalence tends to increase with age, especially after the age of 60. While the average incidence is around 5–8% before the age of 60, this rate rises significantly to 30–40% in individuals exhibiting cerebral white matter changes, as reported in the literature.

Furthermore, leukoaraiosis is found in many patients with certain medical conditions. In Alzheimer's patients, its occurrence ranges from 26 to 70%, while in patients with vascular dementia, the prevalence is between 50 and 80%. Additionally, leukoaraiosis is reported in approximately 44% of patients who have experienced a stroke or a transient ischemic attack[7-9].

These areas of low density can be observed in regions such as the centrum semiovale. This condition, known as leukoaraiosis, is typically associated with small vessel disease, such as lacunar infarction. Leukoaraiosis has been linked to mental deterioration and dementia, and it is believed to result from hemodynamic ischemia of the white matter, which occurs secondary to atherosclerotic thickening of the penetrating arteries that supply the white matter[10].

Various risk factors contribute to the development of leukoaraiosis. While hypertension can be a significant factor in these cases, there is often the involvement of small vessels due to the combination of multiple risk factors[11].

Most patients presenting with headaches, myalgia, and dementia show involvement in the subcortical, periventricular, and basal ganglia regions. Lesions in the cortical and brainstem areas (except for the pons) are infrequent. Additionally, it has been reported that caudate atrophy may be observed, particularly in patients with Parkinson's disease, leading to potential impairments in executive function and the dopaminergic pathway. Tuladhar et al. conducted a study in 2015 that identified a connection between memory and corpus callosum lesions[12].

The presence of WML on MRI does not typically result in a significant change in the activities of daily living in asymptomatic patients with vascular risk factors[13]. However, it should be noted that WML can prolong walking time, and therefore, the underlying cause should be thoroughly investigated. Precautions should be taken due to the higher risk of stroke, dementia, and falls associated with these lesions. In our study, while there was no significant difference observed in phases 1 and 2, it was found that walking time was significantly prolonged in phase 3.

Extensive WML are strongly associated with an increased risk of dementia, cognitive decline, and memory impairment. Research has indicated that a higher burden of diffuse WML elevates the risk of developing dementia[14]. Additionally, considering the risk of dementia associated with microbleeds, it has been found to pose a potential threat. However, it is worth noting that extensive white matter involvement does not necessarily correlate with lacunae, microbleeding, and perivascular spaces.

The incidence of WML tends to rise with advancing age and is commonly observed in various cerebrovascular diseases, such as multi-infarct dementia, amyloid angiopathy, Binswanger's disease, as well as conditions like hydrocephalus and multiple sclerosis[15].

The vascularization of cerebral white matter is more delicate than cerebral cortical areas. This is particularly evident in the subcortical white matter, supplied by deep perforating arteries originating from the pial network on the brain surface, resulting in a relatively weaker capillary structure. Due to this vulnerable vascularization, chronic ischemic microangiopathic changes tend to occur in these areas, and hypoxic regions can form even before the onset of clinical symptoms[16].

The formation of lipo hyalinosis and fibrinoid necrosis in the intima-media layers leads to the degeneration of the muscular layer in the vessels. Consequently, over time, the blood-brain barrier deteriorates in these regions, resulting in increased extravascular fluid collection and gliosis in the glia and oligodendrocytes. This sets the stage for ischemic pathologies, including the formation of lacunas, which can eventually lead to infarction[17].

WML can also be seen as asymptomatic infarct appearances or as a predisposition for infarct formation. They can be distinguished from ischemic infarcts based on certain features, such as lacking well-circumscribed boundaries, being limited to the cerebral white matter without cortical extension, and not causing enlargement of the ventricles and cortical sulci[18].

The current study revealed a positive correlation between the Lawton IADL Scale and MMSE. A negative correlation was found between Lawton Brody, the get-up-walk test, FPG, homocysteine, and age. An increase in the Lawton IADL Scale score was associated with a statistically significant decrease in the results of the get-up-walk test, FPG, homocysteine, and age.

However, due to technical inadequacy, we could not measure the volume of WML in MRI; therefore, an evaluation was conducted based on the Fazekas Staging System. In future studies, obtaining more objective data by performing volumetric measurements of WML in MRI could provide comprehensive insights and a more detailed understanding.

CONCLUSION

In asymptomatic individuals with vascular risk factors, the presence of WML on MRI does not typically result in a significant change in daily living activities. However, it is important to note that these lesions can cause a prolongation of walking time. Due to the potential high risk of stroke, dementia, and falls associated with

WML, a comprehensive investigation of their aetiology is

Ethics Committee Approval: All procedures in this study followed the ethical standards of the responsible committee on human experimentation (both institutional and national) and complied with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been obtained from our institution, and informed consent has been duly obtained from all participants involved in the study.

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Original Article / Özgün Araştırma

Evaluation of the Systemic Immune-Inflammatory Index (SII) and NAPLES Score (NS) in Patients with Non-ST-Elevation Myocardial Infarction (NSTEMI)

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Abstract

Objective: Non-ST elevation myocardial infarction (NSTEMI) is the most common type of acute coronary syndrome and has a poor prognosis. The SII and NS are derived from blood cell counts and reflects the balance between inherited and acquired immunity and the association between the immune system and endothelial dysfunction. This study aimed to compare the prognostic value of two novel inflammatory biomarkers, the systemic immune-inflammatory index (SII) and the Naples score (NS), with that of other inflammatory markers and risk scores in patients with NSTEMI.

Methods: This was a retrospective cohort analysis of 50 NSTEMI patients and 50 controls matched by age and sex who were admitted to our hospital. We calculated the SII and NS scores and other ratios, indices, and risk scores for each patient. We used Pearson's correlation coefficient and receiver operating characteristic (ROC) analysis to examine the correlations and predictive values of the SII index, NS score, and other biometric markers and risk scores.

Results: The SII and NS were significantly greater in the NSTEMI group than in the control group. They had strong positive correlations with the NLR, MHR, PLR, and TC/HDL ratio, and moderate positive correlations with TIMI and HEART scores ($r>0.3$, $p<0.01$ for both). The SII and NS also had higher AUC values than other biometric markers and risk scores ($p<0.05$ for both).

Conclusions: The SII and NS are inexpensive, widely available and easy to measure markers that may have utility for cardiac risk stratification in NSTEMI patients.

Keywords: Non ST-elevation myocardial infarction, NAPLES Score, Systemic immune-inflammatory index, Novel inflammatory biomarkers

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ST Yükselmesi Olmayan Miyokard İnfarktüsü (NSTEMI) Hastalarında Sistemik İmmün İnflamatuar İndeks (SII) ve NAPLES Skorunun (NS) Değerlendirilmesi

Öz

Amaç: Akut koroner sendromun (AKS) en sık görülen tipi olan ve kötü prognoza sahip non-ST elevasyonlu miyokard infarktüsü (NSTEMI) hastalarında, iki yeni inflamatuar biyo belirteç olan sistemik immün-inflamatuar indeks (SII) ve Naples skoru (NS) ile diğer inflamatuar belirteçler ve risk skorları arasındaki ilişki ve prognostik değer karşılaştırılmıştır. SII ve NS, kan hücre sayımlarından türetilen ve doğal ve uyarlanmış bağışıklık sistemi arasındaki dengeyi ve bağışıklık sistemi ile endoteldis fonksiyonu arasındaki ilişkiyi yansıtan indekslerdir.

Yöntemler: Bu çalışmada, hastanemize yatırılan 50 NSTEMI hastası ile cinsiyet ve yaşa göre eşleştirilmiş 50 kontrol grubu retrospektif olarak incelenmiştir. Her hastaya SII ve NS skorları ile diğer oranlar, indeksler ve risk skorları hesaplanmıştır. SII indeksi, NS skoru ve diğer biyo belirteçler ve risk skorlarının korelasyonlarını ve prediktif değerlerini incelemek için Pearson korelasyon katsayısı ve Receiver operating characteristic (ROC) curve analizi kullanılmıştır.

Bulgular: Sonuç olarak, SII indeksi ve NS skoru NSTEMI grubunda kontrol grubuna göre anlamlı derecede yüksek bulunmuştur. NLR, MHR, PLR ve TC/HDL oranı ile güçlü pozitif, TIMI ve HEART skorları ile orta derecede pozitif korelasyon göstermişlerdir ($r>0.3$, $p<0.01$ her ikisi için de). Ayrıca SII indeksi ve NS skoru, diğer biyometrik belirteçler ve risk skorlarından daha yüksek AUC değerlerine sahip olmuştur ($p<0.05$ her ikisi için de).

Sonuç: SII ve NS, ucuz, yaygın olarak kullanılabilir ve kolayca ölçülebilen belirteçler olup, NSTEMI hastalarında kardiyak risk stratifikasyonu için yararlı olabilirler.

Anahtar kelimeler: Non ST-elevasyonlu miyokard infarktüsü, NAPLES Skoru, Sistemik İmmün-İnflamatuar İndeks, yeni inflamatuar biyo belirteçler.

INTRODUCTION

Cardiovascular diseases are among the most important causes of morbidity and mortality worldwide. Among these diseases, acute coronary syndrome (ACS) has various clinical manifestations, such as myocardial infarction (MI) or unstable angina. Non ST elevation myocardial infarction (NSTEMI) is the most prevalent type of ACS and accounts for 61% of MI cases[1,2].

NSTEMI patients have a complex prognosis that depends on multiple factors, such as age, comorbidities, cardiac function and treatment strategies. These patients have lower in-hospital mortality than patients with ST-elevation myocardial infarction (STEMI) but have twice the long-term mortality risk. Thus, it is vital to conduct thorough risk assessments and clinical follow-up of these patients from the time of NSTEMI diagnosis to avoid adverse outcomes. Inflammation is an important factor in the pathogenesis and progression of NSTEMI. It begins with the rupture of atherosclerotic plaques in the coronary arteries, which causes the arterial lumen to be blocked by a thrombogenic environment. This triggers the activation and secretion of various cytokines, chemokines and adhesion molecules by different types of white blood cells, especially lymphocytes, neutrophils, monocytes and platelets. The levels of some of these inflammatory markers, such as interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-α), are increased in NSTEMI patients and are linked to poor prognosis. However, these markers are not specific to NSTEMI and may be influenced by other factors, such as infection, trauma or malignancy[3-8].

Therefore, it is very important to evaluate inflammation with simple and inexpensive biometric markers in NSTEMI patients. These biometric markers include

neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte/HDL ratio (MHR), NS score and total cholesterol/HDL cholesterol ratio (TC/HDL). These biometric markers are calculated as ratios or combinations of parameters obtained from simple blood tests, such as neutrophil, lymphocyte, monocyte, platelet and cholesterol levels. These biometric markers can reflect the relationship between the immune system and endothelial dysfunction and show the impact of inflammation on atherosclerosis. The NLR, MHR, PLR and TC/HDL ratios are biometric markers obtained from simple and inexpensive blood tests that are associated with inflammation and atherosclerosis. These ratios are used to assess the risk of ACS. These ratios are associated with adverse outcomes such as heart failure, myocardial infarction, arrhythmias and mortality in CVD patients. Studies in the literature also support that these ratios have prognostic value in NSTEMI patients. However, some studies also suggest that these ratios are not sufficient or consistent with other risk scores or inflammatory markers[9-13].

The systemic immune-inflammatory index (SII) is calculated as the product of the neutrophil count and platelet count divided by the lymphocyte count and reflects the balance between inherited and acquired immunity. SII is an important indicator of inflammatory processes associated with disease progression, especially in patients with infectious diseases or other conditions. The SII can reflect the relationship between the immune system and endothelial dysfunction associated with chronic inflammatory conditions such as cardiovascular disease (CVD). Studies in the literature support that the SII has prognostic value in NSTEMI patients[14-17]. In particular, it has been shown that the SII is associated with adverse outcomes such as mortality, myocardial infarction, stent thrombosis and heart failure in NSTEMI patients.

The Naples score (NS) score is a score that includes inflammatory markers such as the lymphocyte-monocyte ratio (LMR), NLR, total cholesterol and albumin. The NS score is used to assess the risk of acute coronary syndrome (ACS). It has been shown that the NS score is associated with adverse outcomes such as mortality, myocardial infarction and stent thrombosis in patients with high NS score. Studies in the literature also support that the NS score has prognostic value in ACS patients[18,19]. However, some studies also suggest that the NS score is not sufficient or shows lower performance compared to other risk scores.

Other scores used to assess the cardiovascular risk of NSTEMI patients include HEART (History, ECG, Age, Risk factors, Troponin) and TIMI (Thrombolysis in myocardial infarction) scores. These scores are based on clinical features such as age, ECG findings, angina frequency, cardiac biomarkers, coronary artery disease history, blood pressure and troponin level. Patients with high scores have a greater risk of mortality, myocardial infarction and ischemic complications. In this study, it was also found that these scores are sensitive and specific tests for NSTEMI diagnosis[20,21]. Studies in the literature also support that these scores have prognostic value in NSTEMI patients. However, some studies also suggest that these scores are not sufficient or consistent with each other. Therefore, it is recommended to use these scores together rather than alone.

In this study, aims to compare the SII and NS with the NLR, MHR, PLR and TC/HDL ratios and with other conventional risk scores such as the TIMI and HEART in NSTEMI patients. We hypothesized that the SII and NS would be more suitable independent predictors of in-hospital and long-term mortality than the NLR, MHR, PLR and TC/HDL ratios in this population.

METHODS

The local ethics committee approved this study (Decision Date/No: 20.09.2022/362). This study was a retrospective analysis of 50 patients with non-ST elevation myocardial infarction (NSTEMI) and 50 age and sex-matched controls who were admitted to our hospital from January 2020 to December 2020. We obtained the data from the hospital records. The NSTEMI group included patients who met the fourth universal definition of MI and had complete blood count, lipid profile, troponin level, and electrocardiogram (ECG) data at admission. We excluded patients who had previous MI, coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI), malignancy, chronic kidney disease, or inflammatory or infectious disease. The control group had normal coronary angiography results and no history of cardiovascular disease.

The SII was calculated as follows: SII = neutrophil count x platelet count/lymphocyte count (14). The NS score was calculated as follows: The NS score was based on the levels of NLR, LMR, serum albumin and total cholesterol. According to Galizia et al.'s method (the cutoff values of NLR and LMR were defined by MaxStat analysis), serum total cholesterol level ≤ 180 mg/dL, albumin level < 40 g/L, LMR level ≤ 4 or 44NLR level > 2.96 each was assigned 1 point and otherwise 0 point. The NS score was calculated by adding the scores of the aforementioned parameters[22,23]. The other ratios, indices, and risk scores that were calculated for each patient were: The TC/HDL, PLR, MLR, NLR, the total TIMI risk score, and the HEART score. The TIMI risk score was based on clinical features such as age, ECG findings, angina frequency, cardiac biomarkers, coronary artery disease history, and blood pressure[20]. The HEART score was based on clinical features such as history, ECG findings, age, risk factors, and troponin level[21].

Statistical Analysis

The analysis of the data was conducted utilizing the software SPSS, version 21.0 software (IBM Corp., NY, USA). Continuous variables were expressed as mean±standart deviations. Comparisons between groups were conducted with the independent samples t-test, while the chi-square test was utilized for the analysis of categorical data.

The correlations between the SII, NS and other ratios, indices, and risk scores were analyzed using Pearson's correlation coefficient. A correlation coefficient of more than 0.5 or less than -0.5 was considered strong, while a coefficient of between 0.3 and 0.5 or between -0.3 and -0.5 was considered moderate.

The predictive values of the SII, NS and other ratios, indices, and risk scores were assessed using receiver operating characteristic (ROC) analysis. The optimal cut-off values were determined by maximizing the Youden index. A higher AUC indicated a better predictive performance.

RESULTS

The study population consisted of 50 patients with NSTEMI and 50 age- and sex-matched control subjects. The baseline characteristics of the two groups are shown in Table 1. There were no significant differences between the two groups in terms of hypertension, diabetes mellitus, and left ventricular ejection fraction (LVEF). The NSTEMI group had higher TIMI and HEART scores than the control group ($p<0.001$ for both). The NSTEMI group also had significantly higher values of the SII, NS score, NLR, MHR, PLR, and TC/HDL than the control group ($p<0.05$ for all except LMR and PLR).

Table I: Baseline charesteristics of the study population

	NSTEMI		Control		P
	Mean	SD	Mean	SD	
Age, years	61.74	9.60	60.28	9.50	0.456
Gender, Male (%)	29 (60.4)		19(39.6)		0.036
Hypertansion (%)	33 (48.5)		35(51.5)		0.415
DiabetesMellitus (%)	25 (59.5)		17(40.5)		0.078
LVEF, %	50.18	8.20	58.40	8.21	<0.001
TIMI score	3.24	1.13	1.46	0.76	<0.001
HEART score	7.52	1.16	2.50	1.01	<0.001
SII	827.81	720.91	549.52	347.32	0.016
MHR	0.015	0.009	0.011	0.004	0.007
NLR	3.60	3.43	1.99	0.99	0.002
LMR	4.96	2.50	6.77	2.71	0.117
PLR	124.35	78.17	111.75	42.93	0.321
TC/HDL	4.79	1.37	4.31	1.01	0.053
NS	1.76	1.20	1.24	1.13	0.035

P <0.05 was considered statistical significant. Values are presented as n (%) or mean ± standard deviation depending on the variable distribution. Left ventricular ejection fraction (LVEF), the Systemic immune-inflammatory index (SII) and the NAPLES Score (NS), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), total cholesterol/HDL cholesterol ratio (TC/HDL), lymphocyte-monocyte ratio (LMR), monocyte-HDL ratio (MHR).

Table 2 shows the correlations between the SII, NS with other ratios, indices, and risk scores. The SII and NS had strongly positive correlations with MHR, NLR, PLR, and TC/HDL ratio ($r>0.5$, $p<0.001$ for all). The NS and SII also had moderately positive correlations with TIMI and HEART scores ($r>0.3$, $p<0.01$ for both). These results suggest that the SII and NS reflect the balance between inherited and acquired immunity and the connection between the immune system and endothelial dysfunction better than other biometric markers.

Table II: cross-correlation table between risk scores and indices

		TİMİ	HEART	SII	MHR	NLR	LMR	PLR	TC/HDL	NS
TİMİ	PearsonCorrelation		.853**	.230*	.116	.298**	-.146	.194	.060	.277**
	Sig. (2-tailed)		.000	.021	.251	.003	.147	.053	.552	.005
	N	100	100	100	100	100	100	100	100	100
HEART	PearsonCorrelation	.853**		.287**	.202*	.346**	-.170	.169	.112	.281**
	Sig. (2-tailed)	.000		.004	.043	.000	.091	.092	.265	.005
	N	100	100	100	100	100	100	100	100	100
SII	PearsonCorrelation	.230*	.287**		-.068	.931**	-.164	.863**	-.157	.557**
	Sig. (2-tailed)	.021	.004		.500	.000	.103	.000	.120	.000
	N	100	100	100	100	100	100	100	100	100
MHR	PearsonCorrelation	.116	.202*	-.068		-.061	-.330**	-.301**	.468**	.184
	Sig. (2-tailed)	.251	.043	.500		.550	.001	.002	.000	.067
	N	100	100	100	100	100	100	100	100	100
NLR	PearsonCorrelation	.298**	.346**	.931**	-.061		-.186	.798**	-.155	.572**
	Sig. (2-tailed)	.003	.000	.000	.550		.064	.000	.123	.000
	N	100	100	100	100	100	100	100	100	100
LMR	PearsonCorrelation	-.146	-.170	-.164	-.330**	-.186		-.123	.072	-.321**
	Sig. (2-tailed)	.147	.091	.103	.001	.064		.222	.477	.001
	N	100	100	100	100	100	100	100	100	100
PLR	PearsonCorrelation	.194	.169	.863**	-.301**	.798**	-.123		-.265**	.508**
	Sig. (2-tailed)	.053	.092	.000	.002	.000	.222		.008	.000
	N	100	100	100	100	100	100	100	100	100
TC/HDL	PearsonCorrelation	.060	.112	-.157	.468**	-.155	.072	-.265**		-.336**
	Sig. (2-tailed)	.552	.265	.120	.000	.123	.477	.008		.001
	N	100	100	100	100	100	100	100	100	100
NS	PearsonCorrelation	.277**	.281**	.557**	.184	.572**	-.321**	.508**	-.336**	
	Sig. (2-tailed)	.005	.005	.000	.067	.000	.001	.000	.001	
	N	100	100	100	100	100	100	100	100	100

P <0.05 was considered statistical significant. The Systemic Immune-Inflammatory Index (SII) and the NAPLES Score (NS), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), total cholesterol/HDL cholesterol ratio (TC/HDL), lymphocyte-monocyte ratio (LMR), monocyte-HDL ratio (MHR).

Figure 1 and Table 3 show the receiver operating characteristic (ROC) analysis for the predictive values of NS, SII score, and other ratios, indices, and risk scores for in-hospital and long-term mortality. The area under the curve (AUC) values for NS and SII score were significantly higher than those for other risk scores and biometric markers ($p<0.05$ for both). The optimal cut-off values for the SII, NS score, and NLR were 518.52, 1.5, and 1.94, respectively.

Table III: Receiver-operator characteristic (ROC) curve analysis

	AUC (95% CI)	P	Cutt-off	Sensitivity (%)	Spesifity (%)
SII	0.656 (0.549;0.763)	0.007	518.52	62	62
NS	0.614 (0.504;0.724)	0.050	1.50	64	60
TIMI	0.895 (0.831;0.959)	<0.001	2.50	72	88
HEART	0.990 (0.986;0.995)	<0.001	4.50	94	90
NLR	0.716 (0.616;0.816)	<0.001	1.94	64	64
MHR	0.647 (0.539;0.755)	0.011	0.0127	66	64
PLR	0.502 (0.389;0.616)	0.967	102.79	50	48
TC/HDL	0.606 (0.495;0.716)	0.069	4.46	64	62
LMR	0.374 (0.265;0.484)	0.030	5.04	42	40

Results are presented as area under curve (AUC) with 95% confidence interval (CI). P <0.05 was considered statistical significant. The Systemic immune-inflammatory index (SII) and the NAPLES Score (NS), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), total cholesterol/HDL cholesterol ratio (TC/HDL), lymphocyte-monocyte ratio (LMR), monocyte-HDL ratio (MHR).

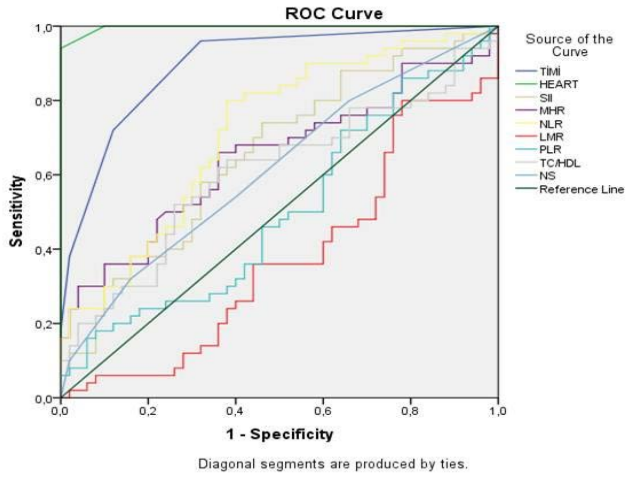

Figure 1. Receiver-operator characteristic (ROC) curve analysis

These results suggest that the SII and NS have better predictive performance than other risk scores and biometric markers in NSTEMI patients.

DISCUSSION

This retrospective cohort analysis found that the SII and NS scores are novel inflammatory biomarkers and independent predictors of mortality in NSTEMI patients. In this study, the association of increased the NS and SII with other inflammatory markers and risk scores in NSTEMI patients was demonstrated for the first time. We also showed that an increased SII and NS score together with the MLR, NLR, PLR, were independent predictors of NSTEMI; and that the SII was significantly correlated with the TIMI and HEART risk scores. In addition to the SII and NS, we studied the PLR, NLR, MLR, TC/HDL ratios which are other indicators of inflammatory status in patients with acute myocardial infarction. Patients with NSTEMI have varying prognoses depending on their characteristics, so it is crucial to stratify them by risk early on to choose the optimal treatment during hospitalization and after discharge. Several risk scores have been proposed to assess the mortality risk in ACS patients, such as the TIMI risk score and the HEART risk score.

The NS is a prognostic scoring model that combines the values of NLR, LMR, albumin and total cholesterol. It has been shown to be useful for predicting mortality in cancer patients and STEMI patients. Therefore, patients with a high NS may benefit from more intensive monitoring and treatment to prevent ischemic events, heart failure, and myocardial infarction. In our study, we found that the NS score was significantly higher in the NSTEMI patient group than in the control group, and that it correlated positively with the TIMI and HEART risk scores. These findings suggest that the NS may be a better predictor of mortality than its individual components in NSTEMI patients[24-27].

In a study conducted with 4,606 patients with heart failure, researchers showed that increased SII predicted short-term mortality[28]. In addition,

in patients with NSTEMI, increased SII level was shown to be an independent predictor of contrast-induced nephropathy[29]. A study by Güzel et al. suggests that the systemic immune inflammation index may be a potential indicator for predicting fractional flow reserve-measured coronary lesion severity[30]. All these studies indicate that increased SII levels are related to poor cardiovascular events in different cardiac pathologies. In our study, similar to these studies, the SII was statistically significantly higher in the NSTEMI patient group compared to the control group. There was also a significant positive correlation with the TIMI and HEART risk scores.

Our study showed that among the various laboratory markers used to prognosticate ACS patients, the SII and NS were more powerful predictors of NSTEMI than ratios such as the MLR, NLR and PLR. Moreover, the SII had a significant correlation between the TIMI and HEART risk scores.

CONCLUSIONS

In conclusion, the SII and NS are inexpensive, widely available and easy to measure markers that may have utility for cardiac risk stratification. Our results may stimulate further research. They may also be incorporated into routine clinical practice for patients with ACS and other cardiovascular conditions. Multicentre and large sample size studies are needed to test the applicability of these findings to a larger population.

Limitations

This study had several limitations, such as the retrospective and single-center design, single geographical location which limits the generalizability of the findings, and the exclusive focus on NSTEMI patients which restricts the applicability to other ACS populations. There may also be uncontrolled confounders that may influence the multivariate regression results. Another limitation was the absence of follow-up values of the variables that constitute the NS, which would have been useful for evaluating the NS of patients over time.

Ethics Committee Approval: The local ethics committee approved this study (Decision Date/No: 20.09.2022/362).

Conflict of Interest: The authors declared no conflicts of interest.

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Original Article / Özgün Araştırma

The Effect of Blood Base Deficit on Neonatal Convulsions and Amplitude Electroencephalography Measurements in Perinatal Asphyxia

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Abstract

Objective: To determine the effect of blood pH levels and base deficit on neonatal convulsions and amplitude electroencephalography measurements in patients with perinatal asphyxia.

Methods: This study included 102 patients monitored in the neonatal intensive care unit for perinatal asphyxia. Amplitude electroencephalography measurements and convulsions were recorded from all patients for 80 hours. Blood samples were taken in the umbilical artery for the pH analysis and calculation of base deficit.

Results: The mean gestational age was 38.13±1.30 weeks with 66/36 (64.7% / 35.3%), male/female ratio. Fifty-seven (55.9%) babies were delivered by normal spontaneous vaginal delivery, while 45 patients (44.1%) had a history of cesarean delivery. There were significant differences between the mean base deficit and amplitude electroencephalography recordings at the first 24th, 48th, and 72nd hours (KW=32.819, p<0.001; KW=23.687, p<0.001, and KW=24.992, p<0.001, respectively). Sixty-five (63.7%) of the patients had neonatal convulsions. The mean base deficit was 20.64±4.70 mmol/L and 17.48±2.92 mmol/L in patients with and without seizures, respectively. The mean base deficit was significantly higher in patients with neonatal seizures (Z=3.912; p=0.001).

Conclusion: Our study showed patients with abnormal amplitude electroencephalography findings and epileptic electrical activity were found to have higher base deficits at the time of diagnosis. It suggests that high base deficit levels may have a negative effect on the neurodevelopmental process in the neonatal period.

Keywords: Amplitude electroencephalography, base deficit, convulsion, perinatal asphyxia

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Perinatal Asfikside Baz Açığının Yenidoğan Konvülzyonları ve Amplitüd Elektroensefalografi Ölçümleri Üzerine Etkisi

Öz

Amaç: Perinatal asfiksi hastalarında kan pH düzeyi ve baz açığının yenidoğan konvülzyonları ve amplitüd elektroensefalografi ölçümleri üzerine etkisini araştırmak.

Yöntemler: Bu çalışmaya yenidoğan yoğun bakım ünitesinde perinatal asfiksi tanısıyla takip edilen 102 hasta dahil edildi. Tüm hastalardan 80 saat boyunca amplitüd elektroensefalografi ölçümleri ve konvülzyonlar kaydedildi. Tüm olgulardan pH ve baz açığı için göbek arterinden kan örnekleri alındı.

Bulgular: Ortalama gebelik yaşı 38,13±1,30 hafta olup erkek/kadın oranı 66/36 (%64,7 / %35,3) idi. Bebeklerin 57'si (%55,9) normal spontan vajinal yolla doğarken, 45'inde (%44,1) sezaryen doğum öyküsü vardı. İlk 24, 48 ve 72. saatlerdeki baz açığı ve amplitüd elektroensefalografi kayıtları ortalamaları arasında anlamlı fark vardı (sırasıyla KW=32,819, p<0,001; KW=23,687, p<0,001 ve KW=24,992, p<0,001). Hastaların 65'inde (%63,7) yenidoğan nöbeti vardı. Ortalama baz açığı, nöbet geçiren ve nöbet geçirmeyen hastalarda sırasıyla 20,64±4,70 mmol/L ve 17,48±2,92 mmol/L idi. Yenidoğan nöbeti geçiren hastalarda baz açığı ortalaması anlamlı derecede yüksekti (Z=3,912; p=0,001).

Sonuç: Çalışmamız anormal amplitüd elektroensefalografi bulguları ve epileptik elektriksel aktivitesi olan hastaların tanı anında baz açığı daha yüksek olduğunu gösterdi. Yüksek baz açığı düzeylerinin yenidoğan döneminde nörogelişimsel süreç üzerinde olumsuz etki yaratabileceğini düşündürmektedir.

Anahtar kelimeler: Amplitüd elektroensefalografi, baz açığı, konvülziyon, perinatal asfiksi.

INTRODUCTION

Perinatal asphyxia(PA) develops with arterial hypoxemia and hypercarbia due to deterioration of oxygen-carbon dioxide exchange as a result of inadequate gas exchange in the placenta or deterioration of ventilation at the pulmonary level as a result of postnatal events[1,2]. Hypoxemia leads to an increase in lactic acid and base deficit (BD) levels and a decrease in pH levels, leading to disruption of normal metabolic activity and the development of metabolic acidosis[3]. These metabolic abnormalities that develop in mature and premature babies are the most important cause of neurological morbidity that may develop later[4]. Neuroimaging and neurophysiological examination are important in predicting prognosis in babies with PA[5,6]. Amplitude electroencephalography (aEEG) allows the evaluation of brain functions. It has been stated that aEEG can be used to predict neurodevelopmental outcomes in asphyxiated infants. It has been reported that mildly distorted and normal traces in amplitude-integrated EEG are associated with better long-term outcomes, while severely distorted traces are associated with worse long-term outcomes[7,8]. In asphyxia babies, aEEG allows the diagnosis of convulsions and monitoring of the effects of anticonvulsant drugs[9]. It has been determined that mortality is increased in newborns suffering from convulsions and that there is an increase in negative neurodevelopmental outcomes in surviving cases. Convulsions in newborns are the hallmark manifestation of neurological diseases and are associated with worse neurodevelopmental outcomes, regardless of the severity of hypoxic-ischemic brain injury[10].

We aim to investigate the effect of pH and BD values at the time of diagnosis on neonatal convulsions and aEEG findings in patients followed by a diagnosis of PA.

METHODS

Design

This case-control study was conducted in the tertiary neonatal intensive care unit between January 2020 and June 2023. 102 cases who were followed up and treated with a diagnosis of perinatal asphyxia were included. Ethics Committee approval was obtained before the study (decision no: 25, session no: 09, date 28.12.2023).

Participants

This study comprises of 102 neonates with perinatal asphyxia. Patients had a gestational age of ≥ 36 weeks, born within ≤6 h, having pH≤7.00 or BD ≥ 16 mmol/L in the fetal cord blood gas obtained within one hour after delivery, 10-minute Apgar scores <5 or requiring constant resuscitation, and demonstrating intermediate or patients who underwent resuscitation and had moderate or severe encephalopathy findings according to Sarnat criteria were included. Babies with gestational age <36 weeks, more than 6-hour time-lapse after birth, birth weight <2000g, having congenital metabolic disease, family history of energy deficiency and other disorders leading to early encephalopathy, severe cranial parenchymal bleeding, fatal coagulopathy, presence of chorioamnionitis in the mother, chromosomal anomalies and multiple organ pathologies were excluded[11,12].

Collection and analysis of blood samples and an aEEG recording

For blood gas analysis, 2-milliliter fetal blood samples were taken into the umbilical artery in all cases under anaerobic conditions. The needle tips were bent and covered with plastic caps after sampling to prevent fetal blood from contacting oxygen. Blood gas parameters were analyzed in the first 30 minutes under cold chain conditions.

aEEG waves were recorded with Olympic CFM Brainz Monitor (Natus Newborn Care, USA) for 80 hours. Needle electrodes sized 12x29mm (C3, C4, P3, P4, and COM) were used for recording. Of the five electrodes, purple and black colored C3 and P3 electrodes were placed in the left parietal area of the scalp, while the purple and black colored C4 and P4 electrodes were placed in the right parietal area, and the white reference electrode (COM) was settled to the middle at the frontal region. aEEG recordings were evaluated according to the "Pattern classification system" (Table 1)[13-15]. In the aEEG, epileptic seizure activities are usually seen as a sudden rise in the minimum amplitudes, usually accompanied by simultaneous elevations in the maximum amplitudes, and often followed by a short period of decreased amplitude. Epileptic seizures include single seizures, recurrent seizures (≥3 seizure patterns in 30 minutes), and status epilepticus (SE) "sawtooth pattern" It was classified as[16].

Table I: Definitions of the aEEG patterns

aEEG pattern	Definition
Continuous Normal Voltage (CNV)	Continuous background activity with minimal amplitude 5–10 μV and maximal amplitude 10–50 μV
Discontinuous Normal Voltage (DNV)	Discontinuous background activity with a minimum amplitude below 5μV and maximum amplitude >10 μV
Burst suppression (BS)	Discontinuous background with the lowest magnitude without variability at 0–2 μV intermixed with bursts of higher amplitude
Low Voltage(LV)	Continuous background with a very low voltage around or below 5 μV
Inactive, flat trace (FT)	Isoelectric background activity <5 μV
Seizure activity SZ	Single or repetitive ictal activity on a CNV/DNV background

Statistical Analysis

Statistical analysis was performed using SPSS version 24.0 software. Kruskal Wallis test was used to compare the normal non-distributed numerical variables of 4 independent groups; Adjusted p values were evaluated for post-hoc analysis. The Friedmann test was used to evaluate the three dependent groups, and the marginal homogeneity method was chosen as the post hoc method. The results were added as frequency, mean, percentage, and standard deviation (SD). Fisher Exact and Mann-Whitney U tests were used for categorical variables in the study. P values <0.05 were considered significant.

RESULTS

The gestational age of the cases was 38.13±1.30 weeks. The mean birth weights were 3187±491 grams, and the male/female ratio was 66/36 (64.7%/35.3%). 57 cases (55.9%) were delivered by the normal spontaneous vaginal route, and 45 (44.1%) by cesarean section (C/S). The blood gas analysis revealed that the mean baseline pH and BD values were 6.89±0.13 and 19.50±4.40 mmol/L, respectively. According to Sarnat's criteria, 47 (%46.1) patients had moderate and 55 (%53.9) cases had severe encephalopathy in the clinical evaluation of the cases. The aEEG recordings at the 24th, 48th, and 72nd hours during the hypothermia treatment periods were significantly different from each other. The number of patients with continuous normal voltage (CNV) and discontinuous normal voltage (DNV) increased, while those having burst suppression (BS) and low voltage (LV) reduced over the period (Figure 1).

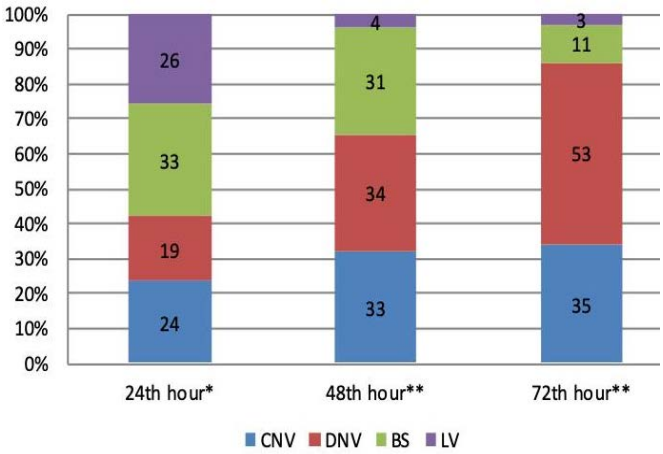

Figure 1: aEEG findings recorded in patients over the 0-72 hour period.

*CNV: Continuous Normal Voltage, DNV: Discontinuous Normal Voltage, BS: Burst suppression, LV: Low Voltage, FT: Inactive, flat trace, MH: Marginal homogeneity. Friedmann Test results:p:<0.001 *24h vs. 48h. MH=6.188; p<0.001. **48h vs. 72h. MH=3.207; p:0.001. ***24h vs. 72h. MH=6.902; p:<0.001*

There was a significant difference in BD values according to aEEG findings in all periods. However, differences in mean pH were only significant in aEEG measurements at 72nd hours (Table II).

Table II: Mean base deficit (BD) and pH values classified with EEG findings at different time points

		n	Mean	SD	95% CI		KW	p-value*
					Lower	Upper		
	24h							
Base deficit	CNV	24	16.83	2.023	15.98	17.68	32.819	**<0.001**
	DNV	19	18.24	3.321	16.64	19.84		
	BS	33	18.86	3.189	17.73	19.99		
	LV	26	23.68	5.174	21.59	25.78		
	Total	102	19.50	4.402	18.63	20.37		
pH	CNV	24	6.93	0.099	6.89	6.97	3.973	0.264
	DNV	19	6.91	0.110	6.86	6.96		
	BS	33	6.90	0.118	6.86	6.94		
	LV	26	6.84	0.176	6.77	6.91		
	Total	102	6.89	0.132	6.87	6.92		
	48h							
Base deficit	CNV	33	17.19	2.284	16.38	18.00	23.687	**<0.001**
	DNV	34	18.96	3.775	17.64	20.27		
	BS	31	22.01	5.013	20.17	23.85		
	LV	4	23.65	6.232	13.73	33.57		
	Total	102	19.50	4.402	18.63	20.36		
pH	CNV	33	6.92	0.106	6.88	6.96	4.482	0.214
	DNV	34	6.89	0.122	6.85	6.94		
	BS	31	6.88	0.150	6.83	6.94		
	LV	4	6.75	0.198	6.43	7.06		
	Total	102	6.89	0.132	6.87	6.92		
	72h							
Base deficit	CNV	35	17.31	2.644	16.40	18.21	24.992	**<0.001**
	DNV	53	19.55	3.554	18.57	20.53		
	BS	11	22.55	4.893	19.26	25.83		
	LV	3	33.00	1.732	28.70	37.30		
	Total	102	19.50	4.402	18.63	20.36		
pH	CNV	35	6.92	0.103	6.88	6.96	9.186	0.027**
	DNV	53	6.90	0.116	6.87	6.94		
	BS	11	6.85	0.185	6.72	6.97		
	LV	3	6.57	0.023	6.52	6.63		
	Total	102	6.89	0.132	6.87	6.92		

**: Kruskal Wallis test was used.*

There was a significant relationship between the level of AEEG abnormality and the frequency of convulsions. It was found that cases with BS and LV at the 24th-hour aEEG record had more frequent convulsions compared to patients with CNV and DNV (Fisher exact test = 67.011; p: <0.001) (Figure 2).

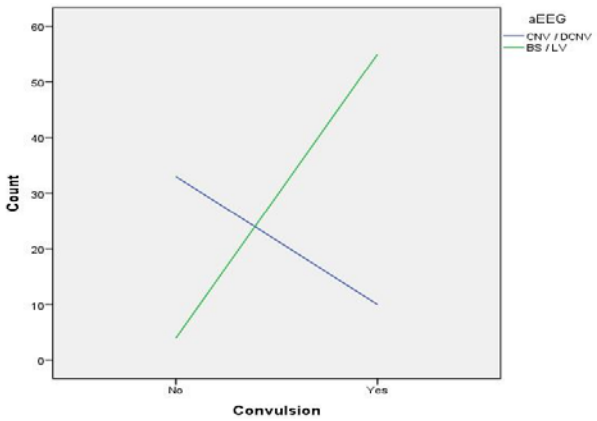

Figure 2: aEEG abnormality and the frequency of convulsions

65 patients (63.7%) experienced convulsion. The mean BD in patients with and without convulsions was 20.64±4.70 and 17.48±2.92, respectively (Z=3.912; p=0.001). The mean pH levels were 6.88±0.145 mmol/L and 6.92±0.103 mmol/L in patients with and without convulsions, respectively and there was no significant difference in terms of the pH levels between them (Z=0.899; p=0.369).

DISCUSSION

Indicators of metabolic acidosis are pH levels and the degree of BD. pH levels < 7.00 mmol/L measured in the fetal cord blood suggests significant fetal acidemia, BD levels BD >16 mmol/L points out in severe hypoxia5. In our study, it was determined that blood gas analysis mean baseline pH: 6.89 ± 0.13 and BD: 19.50 ± 4.40 mmol / L. The cases included in our study were moderate in 46.1% of cases and severe encephalopathy in 53.9% of cases according to Sarnat's criteria. This study revealed that there is a relationship between BD and neonatal convulsion and aEEG findings in patients admitted to the neonatal intensive care unit with a diagnosis of PA.

Amplitude-EEG has a very sensitive early predictive value in estimating prognosis[17]. Amplitude-EEG has been used as a tool for monitoring brain function throughout the treatment period[18,19]. Hallberg et al.20 reported that asphyctic infants treated with hypothermia who had an aEEG abnormality that persisted beyond 24 hours of delivery showed poor neurological outcomes at the end of one year. Chandrasekaran et al.[8] suggested that the effect of six-hour aEEG was inadequate in predicting the prognosis of asphyxia. The authors state that favorable outcomes could be achieved despite an abnormal early aEEG and a persistent abnormal aEEG at 48 hours or later was also associated with a negative neurodevelopmental outcome. Amplitude-EEG has been used as a tool to monitor brain function throughout the treatment period[18,19]. Hallberg et al.[20] reported that asphyxiated infants with aEEG abnormalities lasting more than 24 hours showed poor neurological outcomes at the end of one year. Chandrasekaran et al.[8] suggested that the effect of six-hour aEEG was inadequate in predicting the prognosis of asphyxia. The authors state that favorable outcomes could be achieved despite an abnormal early aEEG and a persistent abnormal aEEG at 48 hours or later was also associated with a negative neurodevelopmental outcome. In our study, a continuous normal voltage (CNV) was observed in 23.3% of the patients and 76.7% of the patients displayed abnormal aEEG findings in the first 24 hours of the hypothermia treatment. On the other hand, abnormal aEEG findings were observed in 68% of the patients for 48 hours.

It has been reported that the hypoxic birth of the baby with BD 12-16 mmol / L, BD> 16 mmol / L is associated with severe hypoxia of the baby, and BD> 20-25 m mol / L is associated with poor prognosis[5,21]. Hellström-Westas L et al.[14] determined that children demonstrating CNV or DNV on an aEEG show a favorable prognosis with a relatively lower risk of sequelae, while patients with BS, CLV, or FT on aEEG have a higher risk of mortality or morbidity. Peliowski A. et al.[22] too, have shown that background abnormalities in aEEG, including BS, LV, and CLV patterns, were associated with a significantly increased morbidity and mortality risk. In our study, we found that patients with LV and BS patterns in AEEG records had higher BD at the time of diagnosis. Qian J et al.[23] In their study, a close correlation was found between brain damage and pH. However, In our study, the differences in the mean pH were significant only for the aEEG measurements at 72nd hours.

Newborn convulsions are the most common symptom of brain injury in newborns and are suggestive of deterioration in brain function. In our study, 65 patients (63.7%) experienced

convulsions. Shellhaas et al.[24] reported that aEEG can provide sufficient information to detect seizures and changes caused by acute events. Besides, Akçay et al.[25] proposed aEEG monitoring in the follow-up of electrophysiological changes and electrographic seizures in newborns with acute brain injury. In the study of Fang Luo et al.[26] a significant relationship was found between epileptic electrical activity and prognosis, as well as aEEG pattern and prognosis. In our study, a relationship was found between aEEG and epileptic electrical activity. High BD levels at the time of diagnosis in PA cases are associated with poor prognosis[4]. In our study, the mean BD in patients with and without convulsions was 20.64 ± 4.70 and 17.48 ± 2.92 mmol / L, respectively. We detected significantly higher BD at the time of diagnosis in patients with convulsions (p = 0.001). There was no significant difference in patients with and without mean pH levels convulsion. Thorp et al; stated that routine umbilical cord pH monitoring is an objective criterion indicating the acid-base balance of the fetus, and it is sufficient to look only at pH from cord blood values. Qian J et al.[23] It was found that there was a close correlation between low brain pH in arterial cord blood between brain damage. In our study, there was no significant difference in patients with and without mean pH levels convulsion.

CONCLUSION

In this study, patients with abnormal aEEG findings and epileptic electrical activity were found to have higher BD at the time of diagnosis. We suggest that healthcare professionals dealing with perinatal asphyxia should be informed that the high BD levels at the time of diagnosis could affect neurodevelopmental prognoses such as abnormal aEEG findings and epileptic electrical activity.

Ethics Committee Approval: This study conformed to the principles of the 2008 Declaration of Helsinki and was approved by the local ethics committee of Harran University, Medical Faculty, Turkey (Approval date/number: 07.01.2019/190102).

Conflict of Interest: The authors declared no conflicts of interest.

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Original Article / Özgün Araştırma

Can topical applicatıons be an alternative to surgery in the treatment of chronic anal fissures?

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Abstract

Introduction: Anal fissure, characterized by a painful ulcer in the anal canal, presents a significant medical challenge. While surgical approaches like lateral internal sphincterotomy (LIS) have been the gold standard for chronic anal fissures, they come with potential complications such as incontinence and abscess formation. In contrast, medical treatments, including topical glyceryl trinitrate and diltiazem, have emerged as alternatives, offering non-invasive options with potentially fewer complications.

Method: In this retrospective study, we evaluated 136 patients treated for chronic anal fissures between June 2019 and December 2022 at Balikesir University Hospital, comparing surgical and medical interventions. The study encompassed demographic analysis, treatment modalities, complete recovery, recurrence rates, and side effects/complications. Statistical analyses, including logistic regression, were performed to assess the efficacy and risks associated with different treatments.

Results: Our findings indicated a higher rate of complete recovery with surgical treatment (86.1%) compared to medical treatments (glyceryl trinitrate: 64.8%, diltiazem: 69.6%). However, no significant difference was observed in recurrence rates between treatment groups. Surgical intervention exhibited a higher incidence of complications such as incontinence and abscess formation, while medical treatments were associated with side effects like headache and gastrointestinal disturbances. Notably, diltiazem therapy showed outcomes comparable to other modalities, indicating its potential as an effective and safer alternative.

Conclusion: Despite the favorable outcomes of surgical treatment, considerations of potential complications underscore the importance of tailored approaches. Prospective, randomized controlled trials with larger cohorts are warranted to further elucidate the efficacy and safety profiles of medical treatments in chronic anal fissures, facilitating informed decision-making in clinical practice.

Key words: Lateral internal sphincterotomy, Fissure in ano, Nitroglycerin, Diltiazem

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Kronik anal fissür tedavisinde topikal uygulamalar cerrahiye bir alternatif olabilir mi?

Öz

Giriş: Anal fissür, anüs kanalında ağrılı bir ülserle karakterize edilen önemli bir tıbbi sorunu temsil etmektedir. Lateral internal sfinkterotomi (LIS) gibi cerrahi yaklaşımlar, kronik anal fissürler için altın standart olmuş olsa da, gaz veya dışkı kaçırma gibi potansiyel komplikasyonlarla ilişkilidir. Buna karşın, topikal gliseril trinitrat ve diltiazem gibi tıbbi tedaviler, daha az invaziv seçenekler sunarak potansiyel olarak daha az komplikasyona sahip alternatifler olarak ortaya çıkmıştır.

Yöntemler: Bu retrospektif çalışmada, Haziran 2019 ile Aralık 2022 tarihleri arasında Balıkesir Üniversitesi Hastanesi'nde kronik anal fissür tedavisi gören 136 hasta değerlendirilmiş, cerrahi ve tıbbi müdahaleler karşılaştırılmıştır. Çalışma demografik analiz, tedavi yöntemleri, tam iyileşme, nüks oranları ve yan etkiler/komplikasyonları içermiştir. Lojistik regresyon da dahil olmak üzere istatistiksel analizler, farklı tedavilerin etkililiğini ve ilişkili riskleri değerlendirmek için yapılmıştır.

Bulgular: Bulgularımız, cerrahi tedavi ile (%86,1) tıbbi tedaviler (gliseril trinitrat: %64,8, diltiazem: %69,6) karşılaştırıldığında, tam iyileşme oranının daha yüksek olduğunu göstermiştir. Ancak, tedavi grupları arasında nüks oranlarında anlamlı farklılık gözlenmemiştir. Cerrahi müdahalenin gaz veya dışkı kaçırma gibi komplikasyonların daha yüksek bir insidansı ile ilişkili olduğu, tıbbi tedavilerin ise baş ağrısı ve gastrointestinal rahatsızlıklar gibi yan etkilerle ilişkilendirildiği belirlenmiştir. Özellikle diltiazem tedavisi, diğer modalitelerle karşılaştırılabilir sonuçlar göstermiş ve etkili ve daha güvenli bir alternatif olarak potansiyelini ortaya koymuştur.

Sonuç: Cerrahi tedavinin olumlu sonuçlarına rağmen, potansiyel komplikasyonların değerlendirilmesi, kişiselleştirilmiş yaklaşımların önemini vurgulamaktadır. Kronik anal fissürlerde tıbbi tedavilerin etkililiği ve güvenlik profillerinin daha iyi anlaşılmasını sağlamak için daha geniş çaplı prospektif, randomize kontrollü çalışmaların yapılması gerekmektedir, bu da klinik uygulamada bilinçli karar verme sürecini kolaylaştıracaktır.

Anahtar kelimeler: Lateral internal sfinkterotomi, Anal fissür, Nitrogliserin, Diltiazem.

INTRODUCTION

Anal fissure is a vertical and painful ulcer in the anal canal extending between the linea dentata and the anal verge. Although it is usually observed in the posterior midline, it can also be seen in the anterior midline[1]. Despite the fact that anal fissure is observed at approximately the same rate in men and women of all age groups, it is is more frequently seen in young adults[2]. Anal fissures have both acute and chronic types according to their manifestations. While acute anal fissure appears as a sharp tear in the anal canal, chronic anal fissure is observed as a triad with the fissure itself, hypertrophied anal papilla in the proximal part and sentinal pili in the distal part. In addition, fissures with symptoms persisting longer than six weeks are considered chronic[3].

Anal fissures are thought to occur due to formation of a tear in the anoderm due to difficult, and traumatic defecation or prolonged diarrhea and increase in internal sphincter pressure due to the tear in anoderm, spasm and decrease in blood flow[4]. In general, severe pain during defecation, fresh blood smeared on feces and strenuous bowel movements are observed in patients with anal fissures[5]. Rectal discharge, tenesmus and itching are other presenting symptoms[4].

The aim in the treatment of chronic anal fissure is to reduce the pressure on the internal sphincter muscle and to weaken sphincter spasm and to increase local blood flow[6]. While the general tendency favours medical treatment in cases of acute anal fissure, surgical treatment is often preferred in the management of chronic anal fissures. Although different surgical techniques have been described, the most commonly performed surgery is lateral internal sphincterotomy (LIS), which is a complete or partial incision of the internal sphincter from the lateral side. LIS is the gold standard method in the treatment of chronic anal fissures[7]. Although surgery has been shown to have a high success rate in many studies, it is also known to cause undesirable results such as gas or fecal incontinence and abscess at the operation site[8]. For this reason, medical treatment approaches in chronic anal fissures have recently become more popular. Various therapeutic agents such as nitrates, calcium channel blockers, botulinum toxin have been proposed for chemical

sphincterotomy[9]. These medical treatment methods may be associated with gastrointestinal side effects such as headache, flushing, abdominal pain, nausea-vomiting, diarrhea and pruritus[10].

In the light of this information, we tried to retrospectively evaluate the surgically or medically treated cases of chronic anal fissures admitted to our clinic and to determine whether medical treatment can be an alternative to surgical treatment.

METHOD

A total of 136 patients who underwent medical or surgical treatment for their chronic anal fissures between June 2019 and December 2022 in the General Surgery Clinic of Balikesir University Hospital were retrospectively investigated. The study was approved by the clinical research ethics committee of Balikesir University (Date: 20.12.2023; Issue No: 2023/192). Preoperatively, informed consent was obtained from all patients who underwent surgical treatment. Patients over the age of 18 years were included in the study. Patients with fissures secondary to diseases such as inflammatory bowel disease, tuberculosis, malignancy, hemorrhoids and fistulas, mentally disabled patients, patients under 18 years of age and patients who had received previous treatment were excluded from the study. A total of 136 patients received either surgical (n:36; 26.5%) or medical (n.100; 73.5%) treatment. As medical treatment, patients received either topical glyceryl trinitrate (n: 54; 54%) or topical diltiazem (n:46; 46%). Patients using diltiazem were instructed to apply 2% of the topical preparation from the fingertip to the first joint of the their fingers for a length of approximately 2 to 2.5 cm twice a day , and insert this ointment about 1.5 cm into the anus. Patients using glyceryl trinitrate were instructed to apply 0.4% ointment twice a day 1-1.5 cm into the anus according to the measurement chart on the box. Topical treatment was applied for 8 weeks and patients were called for monthly controls. Surgically, patients who underwent lateral internal sphincterectomy (LIS) were called for monthly follow-up visits after discharge from the hospital. Complete healing was defined as the complete disappearance of the fissure by inspection in 8 weeks, while recurrence was defined as the re-occurrence of the fissure in patients with complete healing. Patients with complete healing were followed up for 6 months after termination of the treatment to search for the development of recurrence. Patients were evaluated in terms of age, gender, complete healing status, recurrence after complete healing, side effects and complications developed during treatment.

Statistical Analysis

In summarizing the data obtained from the study, descriptive statistics were tabulated as mean ± standard deviation or median, minimum and maximum depending on the distribution of continuous (numerical) variables. Categorical variables were summarized as numbers and percentages. The normality of the numerical variables was checked by Shapiro-Wilk, Kolmogorov-Smirnov and Anderson-Darling tests.

In comparisons of intergroup differences between categorical variables, Pearson chi-square test was used in 2x2 tables with expected value in each cell was ≥5. Fisher's exact and Fisher- Freeman - Halton tests were used in tables, and also RxC tables with expected value in each cell was less than[5]. In this study, logistic regression analyses were applied to determine the factors affecting recovery and recurrence after treatment in patients with chronic anal fissures, and the effect of variables on outcomes was analyzed using odds ratios (ORs), 95% confidence intervals (CI) and P values. These analyses were critical for understanding the efficacy of treatment modalities and the risk of recurrence. Statistical analyses were performed with Jamovi (Version 2.3.28) and JASP (Version 0.17.3) programs and the level of statistical significance level was set at 0.05 (p-value).

RESULTS

A total of 136 participants including 55 (40.4%) male, and 81 (59.6%) female patients with an

overall mean age of 36 years were enrolled in the study. Patients who received treatment were analyzed in three different groups as follows: surgery group (n:36; 26.5%), glyceryl trinitrate group (n:54; 39.7%), and diltiazem group (n: 46; 33.8%). Demographic characteristics, treatment modalities, complete cure/relapse and side effect/complication rates of all patients in all three groups are shown in Table I.

Table I: Demographic and clinical characteristics of all chronic anal fissure patients

	Overall (n=136)
Age (median: range) years	36.0 [18.0 – 73.0]
Gender ‡	
Male (n,%)	55 (40.4)
Female (n,%)	81 (59.6)
Groups ‡	
Surgery (n,%)	36 (26.5)
Topical glyceryl trinitrate (n,%)	54 (39.7)
Topical diltiazem (n,%)	46 (33.8)
Post-treatment status, *recovery* (n,%)	98 (72.1)
Recurrence during 6-month follow-up after complete healing (n,%)	13 (13.3)
Side effects/complications (n,%)	49 (36.0)
Distribution of side effects/complications	
Gas-fecal incontinence (n,%)	2 (4.1)
Perianal abscess (n,%)	2 (4.1)
Headache (n,%)	24 (49.0)
Gastrointestinal side effects (n,%)	18 (36.7)
Other (n,%)	3 (6.1)

When the demographic and clinical characteristics of chronic anal fissure patients were compared according to the treatment groups, no significant difference was observed between the groups in terms of age, gender, and recovery rates. The incidence of side effects and complications was significantly higher in patients receiving topical glyceryl trinitrate treatment ($p<0.001$). Gas/fecal incontinence and perianal abscess rates were significantly higher in the surgery group, and headache was reported at a significantly higher rate in the glyceryl trinitrate group compared to the other groups ($p<0.001$). There was no significant difference between the groups in terms of gastrointestinal system (GIS), and other side effects ($p>0.05$) (Table II). When the recurrence rates were evaluated according to the treatment groups in the 6-month follow-up of patients with chronic anal fissure who had complete recovery, no significant difference was observed between the groups in terms of recurrence rates ($p>0.05$) (Table II).

Table II: Comparison of demographic and clinical characteristics of chronic anal fissure patients according to treatment groups

	Groups			P
	Surgery (n=36)	Glyceryl trinitrate (n=54)	Diltiazem (n=46)	
Age (median, range) years	34.0 20.0 – 73.0]	36.0 [18.0 – 66.0]	36.0 [18.0 –64.0]	0.711**
Gender ‡				
Male (n,%)	15 (41.7)	24 (44.4)	16 (34.8)	0.609*
Female (n,%)	21 (58.3)	30 (55.6)	30 (65.2)	
Complete recovery after termination of the treatment (n,%)	31 (86.1)	35 (64.8)	32 (69.6)	0.079*
Recurrence rates of the patients with complete recovery (n,%)	2/31 (6.5)	7/35(20.0)	4/32 (12.5)	0.300*
Side effects/Complications, (n,%)	4 (11.1)	32 (59.3)	13 (28.3)	<0.001*
Distribution of side effects/complications				
Gas-fecal incontinence (n,%)	2 (5.5)	0 (0,0)	0 (0,0)	<0,001*
Perianal abscess (n,%)	2 (5.5)	0 (0,0)	0 (0,0)	
Headache (n,%)	0 (0,0)	18 (33.3)	6 (13.1)	
GIS side effects (n,%)	0 (0,0)	13 (24.1)	5 (10.8)	
Other (n,%)	0 (0,0)	1 (1.8)	2 (4.3)	

Statistical significance was evaluated using Pearson chi-square, Fisher- Freeman- Halton (*) and Kruskal -Wallis-H (**) tests.

DISCUSSION

Anal fissure is a common and painful disease of the anal canal. Although the etiology of this disease has still not been fully elucidated, it is thought to be caused by hypertrophy of the internal sphincter and increased anal sphincter resting pressure resulting in ischemia of the anoderm[4,11]. Although LIS is the most effective treatment method known in the management of chronic anal fissures, complications such as gas-fecal incontinence and wound infection may be encountered after LIS. Treatment of chronic anal fissures is still a controversial issue. Some studies suggest medical treatment methods as the first treatment option due to the non-invasiveness of these medical therapies and complications of surgical treatment such as gas-fecal incontinence[11]. Calcium channel blockers (diltiazem) and glyceryl trinitrate (nitric oxide derivatives) are commonly used for medical treatment. The these treatments are applied with the aim to reduce sphincter tone, relieve pain and heal the fissure[11,12]. A total 136 participants including 55 (40.4%) male, and 81 (59.6%) female patients with an overall mean age of 36 years participated in our study. Demographic characteristics similar to our study are also observed in the literature. In a meta-analysis of randomized controlled trials, anal fissure was observed more frequently in women with a rate of 54.9%; and in another publication, it was seen more frequently in young and middle-aged patients[6,13].

Many studies have reported varying degrees of efficacy of chemical sphincterotomy using topical glyceryl trinitrate and calcium channel blockers and surgical sphincterotomy. Brown et al. examined 82 patients who received surgical or medical treatments due to chronic anal fissures and found that surgery was superior to applications of glyceryl trinitrate and calcium channel blockers in terms of healing[14]. In a prospective randomized controlled study with a total of 90 patients,Vaithianathan et al. also reported higher healing rates in patients treated with LiS than diltiazem application[3]. Ameta-analysis of 9 randomized controlled studies arrived at a conclusion that topical diltiazem and glyceryl trinitrate had similar healing rates[15]. In our study, similar to the literature data, complete recovery was found at a higher rate in patients who underwent surgery (surgery group: 86.1%; glyceryl trinitrate group: 64.8%; diltiazem group: 69.6%); however, without any statistically significant difference between the treatment groups in terms of complete recovery. We think that this lack of statistically significant intergroup difference stems from lower number of patients who underwent surgery when compared to other treatment groups.

Many side effects and complications may be observed during surgical or topical treatment of chronic anal fissures. In the study by Patel et al. gas incontinence was observed in 3.4% of the patients and fecal incontinence in 3.4% of the patients after LIS[16]. Acar et al. found gas incontinence in 1%, fecal incontinence in 1% and postoperative anal abscess in 1% of the patients who underwent LIS[17]. In our study, postoperatively, gas and fecal incontinence was found in 5.5% and anal abscess in 5.5% of the patients. Gas or fecal incontinence and perianal abscess were not observed in patients who received topical treatment.

In a study comparing the side effects of topical glyceryl trinitrate and diltiazem applications in the treatment of chronic anal fissures, headache and gastrointestinal side effects were observed at a higher rate in the glyceryl trinitrate group[10]. In our study, we observed headache and gastrointestinal side effects at a higher rate in patients receiving topical glyceryl trinitrate than in patients using diltiazem ointment in accordance with the literature data. Gastrointestinal side effects including nausea, vomiting and diarrhea (n:13; 24.1%) and headache (n: 18;33.3.%) were observed in

patients using topical glyceryl trinitrate. Urticaria-like manifestations were observed in one (1.8%) patient. Headache was observed in 6 (13.1%) and gastrointestinal side effects in 5 (10.8%) patients who used diltiazem ointment In 2 patients (4.3%), urticaria-like manifestations and arrhythmia were observed. However, none of the side effects were serious enough to interrupt the treatment. Headache and gastrointestinal side effects were not observed in patients who received surgical treatment.

The most effective treatment modality with the lowest risk of posttreatment recurrence should be preferred in the management of chronic anal fissures. Many studies cited in the literature have evaluated the development of recurrence in patients who recovered after surgical or topical treatment. Recurrence rates after surgery vary between approximately 3-11% in the literature[18–21]. Shrivastava et al. reported recurrence rates as 33% in the glyceryl trinitrate group and 13.3% in the diltiazem group[22]. Bansal et al. found a recurrence rate of 11.1% in the glyceryl trinitrate group and 15% in the diltiazem group[23]. In our study, recurrence rates were 6.5% (n:2) in the surgical treatment, 20% (n:7) (in the glyceryl trinitrate group and 12.5% (n.4) in the diltiazem group after 6 months of follow-up without any statistically significant intergroup difference.

While the relatively small number of patients in the treatment groups, along with its retrospective nature and single-center design, are limitations of our study, this research has demonstrated that topical treatments may serve as alternatives, particularly in patients who refuse surgical intervention for chronic anal fissures.

CONCLUSION

Our study has revealed that although any statistically significant difference between treatment modalities was not observed, higher recovery, and lower recurrence rates were achieved after surgical treatment when compared to other treatment methods. However, surgery is the most vulnerable method to complications such as incontinence and abscess. Since the side effects or complications that may develop are also decisive in choosing the treatment method, topical diltiazem therapy, which has fewer complications compared to other medical treatment methods, has yielded outcomes similar those obtained with other treatment methods in terms of recovery and recurrence rates, we think that in the treatment of chronic anal fissure, especially in patients who are afraid of complications of surgery such as gas or fecal incontinence, and in patients who do not benefit from this treatment or who cannot adapt to medical treatment, lateral internal sphincterotomy will increase the success and reduce the complication rates. Surgical intervention stands as the foremost efficacious approach for addressing chronic anal fissures; however, in cases where patients decline surgery due to undesirable side effects, topical treatments present themselves as viable alternative

We think that prospective, randomized controlled studies with a higher number of patients will more clearly show the efficacy of topical treatments in the treatment of chronic anal fissures and the relevant rates of improvement and recurrence.

Ethics Committee Approval: The study was approved by the clinical research ethics committee of Balikesir University (Date: 20.12.2023; Issue No: 2023/192).

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Original Article / Özgün Araştırma

Admission Serum Creatinine/Albumin Ratio and its Relationship with 1-Year Mortality in Decompensated Heart Failure Patients

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Abstract

Aim: Despite medical advancements, heart failure (HF) maintains high mortality rates. Our research delves into examining the relationship between the serum creatinine/albumin ratio and one-year mortality in patients with decompensated systolic HF.

Methods: During the period from October 2014 to October 2015, we enrolled 80 patients (comprising 37 females) who had been diagnosed with acute systolic decompensated heart failure and had a left ventricular ejection fraction (LVEF) of ≤ 40%. These patients were divided into two cohorts depending on whether they experienced all-cause mortality within the span of one year.

Results: Among the 80 participants, 31 (39%) experienced mortality within the first year. The average age of the deceased group was 69±14 years, with 38.7% (n=12) being female. In contrast, the surviving group had an average age of 66±12 years, with 51% (n=25) being female. The HF group with mortality exhibited significantly higher levels of serum creatinine-albumin ratio, urea, and creatinine values, along with a higher prevalence of pretibial edema (p<0.01).Furthermore, the deceased HF group exhibited significantly lower LVEF, albumin levels, lymphocyte counts, and systolic and diastolic blood pressure values. Statistical analysis revealed a significant difference in the serum creatinine/albumin ratio between the deceased group (0.68±0.27) and the surviving group (0.38±0.18), with a p-value of less than 0.01. Using a cut-off value of 0.45 for the creatinine/albumin ratio, the sensitivity and specificity for predicting one-year mortality in HF patients were 81% and 78% , respectively.

Conclusion: The conjunction of heightened creatinine levels, diminished albumin levels, and an augmented creatinine/albumin ratio could potentially function as straightforward, economical prognostic indicators for forecasting one-year mortality in systolic decompensated HF patients.

Keywords: Albumin, creatinine/albumin ratio, creatinine, heart failure, mortality

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Dekompanse Kalp Yetmezliği Hastalarında Başvuru Serum Kreatinin/Albümin Oranı ve 1 Yıllık Mortalite ile İlişkisi

Öz

Amaç: Tıbbi gelişmelere rağmen kalp yetmezliği (KY) yüksek mortalite oranlarını korumaktadır. Araştırmamız, dekompensesistolik KY hastalarında serum kreatinin/albumin oranı ile bir yıllık mortalite arasındaki ilişkiyi incelemeye odaklanmaktadır.

Yöntemler: Ekim 2014 ile Ekim 2015 tarihleri arasında akut sistolikdekompanse KY tanısı konmuş ve sol ventrikülejeksiyon fraksiyonu (LVEF) ≤ 40% olan 80 hasta (37 kadın) çalışmamıza dahil edildi. Hastalar, bir yıl içinde tüm nedenlere bağlı mortalite olup olmadığına göre iki gruba ayrıldı.

Bulgular: 80 katılımcının 31'i (%39), birinci yıl içinde ölümle sonuçlandı. Ölen grubun ortalama yaşı 69±14 yıl olup, bunların %38,7'si (n=12) kadındı. Buna karşılık, hayatta kalan grupta ortalama yaş 66±12 yıl olup, bunların %51'i (n=25) kadındı. Mortalite yaşanan KY grubu, serum kreatinin-albümin oranı, üre ve kreatinin değerlerinde anlamlı düzeyde daha yüksek seviyeleri sergiledi ve pretibial ödem prevalansı daha yüksekti (p<0.01). Ayrıca, ölen KY grubu, LVEF, albumin seviyeleri, lenfosit sayısı, sistolik ve diyastolik kan basıncı değerlerini anlamlı derecede düşük gösterdi. İstatistiksel analiz, ölen grup (0.68±0.27) ile sağ kalan grup (0.38±0.18) arasında serum kreatinin/albumin oranı açısından anlamlı bir farklılık olduğunu gösterdi, p<0.01 idi. Serum kreatinin/albumin oranı için 0.45 kesme değeri kullanarak, KY hastalarındaki bir yıl içindeki mortaliteyi tahmin etmek için duyarlılık ve özgüllük sırasıyla %81 ve %78 idi.

Sonuç: Yüksek kreatinin seviyeleri, azalmış albumin seviyeleri ve artmış kreatinin/albumin oranının birleşimi, sistolikdekompenseKY'li hastalarda bir yıllık tüm nedenlere bağlı mortaliteyi öngörmede basit, ekonomik prognostik göstergeler olarak işlev görebilir.

Anahtar kelimeler: Albümin, kreatinin, kreatinin/albümin oranı, kalp yetmezliği, mortalite.

INTRODUCTION

Heart failure (HF) is a multifaceted clinical condition marked by irregularities in structure or function that impede ventricular filling and pumping capabilities, alongside corresponding hemodynamic, renal, and neurohormonal reactions[1]. HF, arising as a consequence of most cardiovascular diseases, continues to be a leading cause of morbidity and mortality[2]. While death rates from coronary artery disease (CAD) and cardiovascular diseases related to hypertension decrease with age, the incidence and prevalence of HF show an increasing trend. Among the primary causes of this increase are the growing elderly population and advancements in the diagnosis and treatment of cardiovascular diseases[3].

HF is characterized by increased mortality, progression of symptoms, and frequent hospitalizations. Approximately 60% of patients cannot survive beyond five years, and 30-40% face either death or the need for readmission within one year. In conclusion, HF represents a complex condition that emerges as a significant consequence of cardiovascular diseases[4]. The disturbances in cardiac functions lead to hemodynamic, renal, and neurohormonal responses. The increasing prevalence with age and high mortality rates underscore the severity of this disease. Therefore, a multidisciplinary approach is essential for the clinical assessment and management of HF patients[5].

HF refers to a clinical condition stemming from acute or chronic dysfunction of the heart's functions, resulting in symptoms when the heart's stroke volume fails to meet the body's demands. HF commonly emerges in the advanced stages of heart disease, affecting quality of life and leading to high mortality rates. Its prevalence varies among countries and tends to increase with age[6]. The rise in HF incidence is associated with increased life expectancy and more effective treatments for heart diseases. Advanced age, diabetes, and kidney insufficiency are predictive of mortality in HF patients. Moreover, indicators such as high creatinine levels, low albumin levels,elevated NYHA clinical class, uric acid, and C-reactive protein are also linked to increased mortality risk[7].

Albumin is the most abundant protein in the body, and reduced levels are associated with malnutrition, inflammation, and other factors. It has been observed that low albumin levels in HF patients increase the risk of mortality in both short and long terms[7]. Additionally, elevated creatinine levels are important in predicting mortality in HF patients. The deterioration of kidney function is a common occurrence in HF patients due to various mechanisms. Reduced cardiac output can lead to impaired renal perfusion, while factors like increased venous congestion, elevated intra-abdominal pressure, and activation of neurohormonal and inflammatory mediators also play a role. Furthermore, drugs like diuretics, spironolactone, and ACE inhibitors can lead to kidney function impairment through different mechanisms[8]. This situation can escalate treatment costs and increase the risk of death[9].

Numerous studies have demonstrated that low albumin levels and high creatinine levels increase the likelihood of unfavorable cardiovascular outcomes in patients with chronic congestive HF[10]. However, there are no sufficient data for patients with acute decompensated HF. Consequently, our aim was to investigate whether the ratio of routinely measured and cost-neutral creatinine and albumin values in acute decompensated HF patients could be used to predict mortality risk.

METHODS

Patient Group Selection and Data Collection

The study comprised individuals diagnosed with decompensated HF based on anamnesis, physical examination, telecardiography, electrocardiography, biochemical parameters, and proBNP levels, who were admitted to the Department of Cardiology, between October 2014 and October 2015. We prospectively enrolled 80 consecutive patients with a left ventricular ejection fraction (LVEF) of 40% or lower and followed them for one year to assess mortality as the endpoint. The research received approval from the Dicle University Ethics Committee (dated 27 November 2015, numbered 221) and consisted of individuals admitted to the hospital with a diagnosis of HF. Necessary permissions were obtained from participants.

Inclusion and Exclusion Criteria for Study Participants

Patient histories were taken, and physical examinations were performed. Patients displaying symptoms of acute decompensated HF were selected. Routine complete blood counts, biochemical tests, electrocardiography, and echocardiography were conducted for participants. The inclusion criteria were as follows: LVEF of 40% or lower and presence of at least one sign of congestion (rale, pretibial edema, venous distension, ascites, pleural effusion, etc.).

Exclusion criteria included pregnancy, end-stage kidney failure (GFR ≤ 30), diseases causing malnutrition, trauma or surgical history within the last month, being under 18 years of age, experiencing acute coronary syndrome in the last month, acute infection, known cancer, connective tissue disease, chronic liver disease.

Within the scope of the study, patients with signs of congestion and LVEF of 40% or lower were considered to have acute decompensated HF[11]. Furthermore, individuals with a blood pressure reading of ≥140/90 mmHg or those prescribed antihypertensive medications were classified as hypertensive, and those with LDL cholesterol levels >130 mg/dL or using lipid-lowering drugs were classified as having hyperlipidemia. All participants received detailed information regarding the study, and upon obtaining their consent, they were enrolled in the research.

Follow-Up and Data Analysis

Patients requiring coronary intensive care unit or clinical hospitalization due to acute

decompensated HF were monitored. Their medical histories were recorded, and physical examinations were performed. These data, along with vital signs and electrocardiographic features, were recorded electronically. Routine test results conducted during hospitalization were extracted from the hospital information system, including hemogram parameters, biochemical parameters, thyroid function tests, liver function tests, serum lipoprotein levels, albumin, blood urea nitrogen, and creatinine values. Serum albumin levels were determined utilizing the Beckman Coulter CX9 device, with the hospital's established normal range set at 3.5-5.5 g/dL. Complete blood count measurements were conducted automatically using the Abbott Cell-Dyn 3700 device. Patients were classified as survivors or deceased based on their vital status at the end of the one-year follow-up period.

Echocardiography

All patients underwent transthoracic echocardiography while positioned in the left lateral supine position using a Vivid S6 machine (Dimension/Vivid S6 Pro, Horten, Norway) equipped with a 3 MHz adult probe. Left ventricular systolic and diastolic functions were evaluated according to the American Society of Echocardiography criteria. LVEF was measured and recorded utilizing the Simpson method based on apical four-chamber and apical two-chamber images.

Statistical Analyses

For all variables, the mean and standard deviation values were provided. Statistical analyses were performed utilizing the SPSS 18 software (Statistical Package for Social Sciences, Chicago, IL, USA). Numerical data were examined for normal distribution using the Kolmogorov-Smirnov test. Student's t-test was utilized for normally distributed numerical data among independent groups, whereas the Mann-Whitney U test was employed for non-normally distributed numerical data. Categorical data between groups were compared using either the Chi-square test or Fisher's Exact Test. Additionally, receiver operating characteristic (ROC) analysis was conducted to assess mortality and identify the optimal cut-off value for the serum albumin/creatinine ratio. A p-value < 0.05 was considered statistically significant.

RESULTS

Eighty HF cases were included in the study [%54 were male (n=43); %46 were female (n=37)]. These patients were followed up for one year and were categorized into two groups: survivors and all-cause mortality.

Comparison of demographic and clinical characteristics between the two groups, including age (p=0.31), gender (p=0.28), hypertension (p=0.16), diabetes mellitus (p=0.82), hyperlipidemia (p=0.93), history of coronary artery disease(p=0.19), and history of bypass surgery (p=0.19), did not reveal any statistically significant differences (p > 0.05). According to physical examination results, the presence of pretibial edema was significantly higher in the not surviving group (p= 0.005, 87% vs. 57%, respectively) (Table I). According to the laboratory results, while albumin (p < 0.001) and lymphocyte levels (p = 0.04) were statistically higher in the surviving group, urea (p < 0.01), creatinine (p < 0.01), and the serum creatinine/albumin ratio (p < 0.01) were statistically higher in the non-surviving group (Table II).

Table I: Demographic and physical examination findings of the groups

Variables	Notsurvivinggroup n=31	Survivorgroup n=49	pvalue
Age, years	69±14	66±12	0.31
Gender, male	19(61%)	24(49%)	0.28
Hypertension	17(55%)	16(39%)	0.16
Diabetesmellitus	16(52%)	24(49%)	0.82
CAD	25(81%)	33(67%)	0.19
CABG	9(29%)	10(20%)	0.19
Hyperlipidemia	4(13%)	6(12%)	0.93
Ral in thelungs	28(93%)	41(85%)	0.28
Pretibialedema	27(87%)	28(57%)	**0.005**
Systolicbloodpressure, mmHg	108±18	117±17	0.02
Diastolicbloodpressure, mmHg	65±6	70±9	0.02

Abbreviations : CABG; Coronary artery bypass grafting , CAD; Coronary artery disease. Mean ± SD for numerical variables, frequencies (%) for categorical variables.

Table II: Laboratory data of groups

Parameters	notsurvivinggroup n=31	survivorgroup n=49	pvalue
Hemoglobin (g/dL)	11.49±2.09	11.64±1.85	0.73
Hematocrit (%)	36.78±6.26	37.18±5.56	0.77
Lymphocyte (NULL)	1.39±0.67	1.79±0.92	0.04
Urea (mg/dL)	101±46	67±40	<0.01
Creatinine (mg/dL)	1.65±0.53	1.15±0.38	<0.01
Albumin (gr/dL)	2.49±0.36	3.11±0.43	<0.01
Creatinine-albuminratio	0.68±0.27	0.38±0.18	<0.01
CRP(mg/dL)	2.09±1.54	1.49±1.43	0.08
ALT(U/L)	25.19±24.14	26.73±17.79	0.74
AST(U/L)	33.32±30.03	33.47±18.57	0.97
Total protein(g/dL)	6.77±0.61	6.74±0.76	0.87
Glucose(mg/dL)	171.26±110.25	167.45±98.39	0.87
HDL cholesterol(mg/dL)	30.26±11.24	35.96±18.97	0.14
LDL cholesterol (mg/dL)	80.42±23.84	87.69±28.29	0.24
Total cholesterol(mg/dL)	140.35±34.07	147.50±39.82	0.41
Triglyceride (mg/dL)	121.19±104.64	115.24±52.11	0.74
Na (mmol/L)	133.55±5.05	135.55±4.50	0.07

Abbreviations : ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, CRP; C- reactive protein, HDL; High-Density Lipoprotein, LDL; Low-Density Lipoprotein, Na; Sodium.Mean ± SD for numerical variables.

In relation to the findings from transthoracic echocardiography, no statistically significant distinctions were noted between the two groups regarding IVS (p=0.81), PW (p=0.23), LVDD (p=0.25), LVDS (p=0.34), LA (p=0.18), RV (p=0.31), and RA (p=0.26) values. However, the deceased HF group had a statistically lower LVEF value (p=0.04). There was no statistically significant difference between the non-survivor and survivor groups in terms of electrocardiographic features such as sinus rhythm (p= 0.44, 15% vs. 28%, respectively) and atrial fibrillation (p= 0.70, 14% vs. 20%, respectively) (Table III). Moreover, there were no notable variances in medication utilization among the patients under study, with no statistically significant differences observed between the two groups (p > 0.05) (Table IV). The sensitivity and specificity of one-year mortality in HF patients with a creatinine-albumin ratio cut-off of 0.45 were 81% and 78%, respectively (Figure 1).

Table III: Echocardiography and electrocardiography parameters of the groups

Parameters	notsurvivinggroup n=31	survivorgroup n=49	pvalue
IVS (cm)	1.12±0.16	1.11±0.17	0.81
PW(cm)	2.58±6.81	1.09±0.20	0.23
LVDD (cm)	7.70±0.56	5.70±0.72	0.25
LVSD (cm)	4.85±1.04	4.51±0.90	0.34
LA (cm)	4.80±0.73	4.59±0.59	0.18
RV (cm)	4.28±0.75	4.05±0.79	0.31
RA (cm)	4.80±0.90	4.60±0.65	0.26
LVEF (%)	29.48±7.44	32.69±6.47	0.04
Sinusrhythm	15(48.3%)	28(57.1%)	0.44
Atrialfibrillation	14(45.1%)	20(40.8%)	0.70

Abbreviations : IVS; Interventricular septum, LA; Left atrium, LVDD; Left ventricular end-diastolic diameter, LVDS; Left ventricular end-systolic diameter, LVEF; Left ventricular ejection fraction, PW; Posterior wall, RA; Right atrium, RV; Right ventricle. Mean ± SD for numerical variables, frequencies (%) for categorical variables.

Table IV: Information on medicines usage

Parameters	notsurvivinggroup n=31	survivorgroup n=49	pvalue
ACE inhibitor	21(68%)	41(85%)	0.06
Beta-blocker	29(93%)	44(90%)	0.56
CCB	3(10%)	6(12%)	0.72
ASA	21(68%)	33(67%)	0.97
Diuretic	29(93%)	41(84%)	0.19
Digoxin	5(16%)	12(25%)	0.37
Spironolactone	16(52%)	25(51%)	0.96
Nitrate	2(7%)	4(8%)	0.78
Oral anticoagulant	10(32%)	12(24%)	0.45

Abbreviations : ACEI; Angiotensin converting enzyme inhibitor, ,ASA; Acetyl salicylic acid, CCB; Calcium channel blocker. Frequencies (%) for categorical variables.

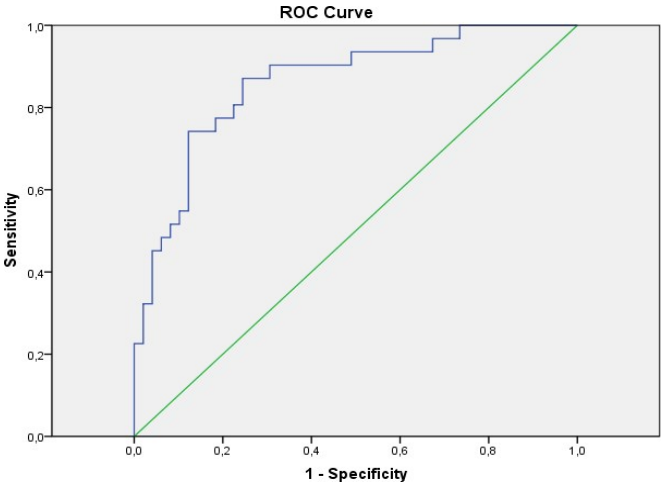

Figure 1. Representation of 1-year mortality due to all causes by serum creatinine-albumin ROC analysis in heart failure

DISCUSSION

Our study demonstrates that elevated creatinine levels and low albumin levels are frequently observed in patients admitted to the hospital due to acute decompensated systolic HF. Furthermore, we found that the creatinine-to-albumin ratio at the time of admission is associated with all-cause mortality within one year after discharge.

HF, which develops at the core of diseases such as coronary artery disease and hypertension and increases in prevalence with aging, remains a significant cause of morbidity and mortality in society[12]. It is emphasized that HF patients are frequently hospitalized within the past year and have high 5-year mortality rates despite treatment. In this study, the one-year mortality rate due to all causes was observed to be 39%. Factors such as advances in medical methods, treatments, and increased life expectancy have been identified as contributing to the increased prevalence of HF, making it a threatening condition to public health. Identifying high-risk individuals among HF patients and predicting mortality risk are crucial for their management. In this context, various parameters such as low LVEF, age, diabetes, renal insufficiency, low albumin, high-sensitivity C-reactive protein, low serum sodium, low hemoglobin level, and low body mass index have been used to predict high-risk HF patients[7,8,13] with increased mortality risk in chronic HF patients, there is insufficient data in the context of acute decompensated HF[14]. This study took a different approach by including only patients with systolic HF and LVEF ≤ 40% in the study group. Within this framework, the study established a notable correlation between elevated creatinine levels, low albumin levels, and reduced LVEF with increased mortality risk. Another similar study conducted in 2014 with similar characteristics also found a relationship between reduced albumin levels and mortality risk in patients with acute decompensated HF[15]. Similarly, this study also showed an association between low albumin levels, elevated creatinine levels, and reduced lymphocyte count with increased mortality risk. Yet, due to the relatively limited sample size in this study, it's possible that certain statistically significant variances might have gone undetected.

Hypoalbuminemia is frequently observed in HF patients, but it is noted that there is not enough epidemiological research in this regard[16,17]. Studies have reported that albumin deficiency is observed in 18% to 89% of HF patients[18]. For instance, in the CHARM study that included 2679 systolic and diastolic HF patients, the prevalence of hypoalbuminemia was determined as 18%[16]. In another study, the prevalence of hypoalbuminemia was found to be 25% in 1726 patients[17]. However, this study observed a prevalence of 81% for albumin deficiency. This high prevalence is attributed to the potential influence of excessive volume load on albumin deficiency among the patients enrolled in the study.

Albumin, the most abundant plasma protein in the body with a weight of 65 kDa, is synthesized, degraded, and distributed based on various factors. Factors such as malnutrition, inadequate production of liver enzymes,

cachexia, chronic inflammation, increased catabolism, hemodilution, protein-losing enteropathy, and nephrotic syndrome can contribute to albumin deficiency. Particularly in decompensated HF patients, hemodilution due to excessive volume load is considered one of the main reasons for albumin deficiency[19].

Kidney dysfunction is common in HF patients and is associated with mortality[20]. For instance, an analysis of 1407 HF patients demonstrated that serum creatinine level is an independent predictor for both 30-day and 1-year mortality[21]. In the same vein, this study revealed a pronounced correlation between elevated creatinine levels and mortality among patients who had deceased. However, this study only examined 1-year mortality. The association of low systolic and diastolic blood pressure with mortality has been emphasized, and comparable results have been noted in other investigations where low systolic and diastolic blood pressure were linked to elevated mortality in HF patients[22]. Low serum sodium levels have also been associated with increased mortality, especially in decompensated HF patients. Anemia has been highlighted as an independent risk factor in decompensated HF patients. The research also noted anemia in 58% of patients. However, despite observing lower hemoglobin levels in the deceased patient group, no statistically significant difference was detected. This result was attributed to the limited number of patients, which could make such analysis challenging. Overall, the study examined parameters that could predict high mortality risk in HF patients and highlighted factors like low albumin levels, elevated creatinine levels, and low lymphocyte counts as associated with increased mortality. However, it was noted that larger and more comprehensive studies are needed.

In this study, the association between increased mortality and factors such as low albumin, total cholesterol, hemoglobin, and lymphocyte count was established. Similarly, the analysis in this study revealed an association between elevated creatinine levels, low systolic blood pressure, and elevated urea levels with increased mortality. This phenomenon can be explained by the adverse effects of factors like reduced cardiac output, renal perfusion impairment, hypertension, and diabetes on the heart and kidneys. Additionally, low serum sodium levels were noted to be associated with increased mortality in decompensated HF patients[23]. Similar observations in different studies have shown that low serum sodium levels increase short and long-term mortality risk. Nevertheless, owing to the restricted patient sample size in this study, low serum sodium levels did not reach statistical significance[24]. It is noted that anemia is also an independent risk factor in decompensated HF patients. Previous studies have indicated that anemia in chronic HF patients increases hospitalization and mortality risk[25]. In this study, 58% of the patients had anemia, but despite lower hemoglobin levels in the deceased patient group, no statistically significant difference was found. This was ascribed to the limited patient cohort included in the study.

Limitations

The constraints of this study are manifold. Primarily, the relatively small size of the patient cohort may limit the applicability of the results. Additionally, crucial factors such as dietary habits and exercise patterns were not accounted for, potentially confounding the results. The exploration of variables like malnutrition was also inadequate. The retrospective design introduces uncertainty in establishing causal relationships definitively. Furthermore, relying on data from a single center may limit the extrapolation of findings to other healthcare settings. The follow-up period of only one year might not suffice to fully understand the long-term implications of the results. Moreover, the study primarily focused

on the initial creatinine-albumin value, neglecting other potentially influential variables. These limitations underscore the need for more comprehensive investigations to enhance the robustness and applicability of the findings.

CONCLUSION

Our research revealed an association between one-year, all-cause mortality in patients with systolic decompensated HF and decreased albumin levels, elevated creatinine levels, and an increased creatinine-to-albumin ratio. These findings suggest that the creatinine-to-albumin ratio may serve as a valuable prognostic indicator for these patients, providing insights without additional costs. However, larger randomized controlled trials are needed to validate these findings conclusively.

Ethics Committee Approval: The research received approval from the Dicle University Ethics Committee (dated 27 November 2015, numbered 221) and consisted of individuals admitted to the hospital with a diagnosis of HF.

Conflict of Interest: The authors declared no conflicts of interest.

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

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Original Article / Özgün Araştırma

Investigation of the İn Vitro Effect of Vanillic Acid on Wound Healing via FN1 and COL1α1 Genes

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Abstract

Objective: Wound healing is characterized by the removal of dead/damaged tissue, the formation of new tissue, and finally, the restoration of the damaged tissue to its original function, starting from the moment of tissue injury. Vanillic acid (VA) is an important component of wheat bran and can heal wounds thanks to its antioxidant potential. This work aimed to investigate the dose-dependent effects of VA (1-2-4-8-16 and 32 µg/ml) in an in vitro way using a wound healing pattern in fibroblast cells.

Methods: The MTT test was performed to determine cell viability 48 hours after VA application to the cells in which the wound model was created (except for the control and wound groups). The cells were examined morphologically with an inverted microscope. ELISA and Real-Time PCR analyses were performed to determine changes in oxidative stress parameters and FN1 and COL1α1 gene expressions.

Results: The highest percentage closure rate of fibroblast cells in the in vitro wound pattern analysis and the highest percentage of cell viability by MTT analysis were determined in the VA-32 treated group. Morphological images showed that the evaluated gene expressions increased in fibroblast cells in a VA dose-dependent manner.

Conclusion: Our findings demonstrated for the first time that VA promotes cell migration and proliferation by regulating oxidative stress and FN1A and COL1α1 genes. The results of this work are thought to pioneer the use of VA in in vivo wound healing studies.

Keywords: 8-OHdG, Fibroblast, MTT, Vanillic acid

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Vanilik Asidin Yara İyileşmesi Üzerindeki İn Vitro Etkisinin FN1 ve COL1α1 Genleri Aracılığıyla Araştırılması

Öz

Amaç: Yara iyileşmesi, dokuda hasarın oluşmasıyla başlayan ölü/hasarlı yapının uzaklaştırılması, yeni dokunun oluşumu ve hasarlanmış dokunun fonksiyonlarını yerine getirmeleriyle karakterizedir. Vanilik asit (VA), buğday kepeğinin önemli bir bileşeni olup antioksidan potansiyeli sayesinde yarayı iyileştirebilmektedir. Bu çalışmada; fibroblast hücrelerinde yara iyileşmesi modeli oluşturarak VA'nın doz (1, 2, 4, 8, 16, and 32 µg/ml) bağlı etkilerinin in vitro metodlarla incelenmesi amaçlanmıştır.

Yöntemler: Yara modeli oluşturulan hücrelere (kontrol ve yara grubu haricinde) VA uygulaması yapıldıktan 48 saat sonra hücre canlılığını belirlemek için MTT testi yapıldı. İnvert mikroskop ile hücrelerin morfolojik olarak incelendi. Oksidatif stres parametrelerindeki değişiklikleri ve FN1 ve COL1α1 gen ekspresyonlarını belirlemek için de ELİSA ve Real-Time PCR analizi yapıldı.

Bulgular: In vitro yara modeli testinde fibroblast hücrelerinin en yüksek yüzde kapanma oranı ve MTT testi ile en yüksek hücre canlılık yüzdesi VA-32 uygulanan grupta tespit edildi. Morfolojik görüntülerinde ise, değerlendirilen gen ekspresyonlarının fibroblast hücrelerinde VA dozuna bağlı olarak arttığını gösterdi.

Sonuç: Bulgularımız ilk kez VA'nın oksidatif stresi ve FN1A ve COL1α1 genlerini düzenleyerek hücre göçünü ve çoğalmasını desteklediğini göstermiştir. Bu çalışmanın sonuçlarının, yapılacak olan in vivo yara iyileşmesi araştırmalarında VA'nın kullanımına öncülük edeceği düşünülmektedir.

Anahtar kelimeler: 8-OHdG, Fibroblast, MTT, Vanillik asit.

INTRODUCTION

A wound is defined as the disruption of normal anatomical integrity and function in the body by physical damage caused by any agent[1]. Wound healing is a duration that starts from the moment of tissue injury and includes results such as the removal of dead/damaged tissue, formation of new tissue, and finally remodeling of damaged tissue to fulfill its functions[2,3]. The wound healing process consists of four stages including hemostasis, inflammation, proliferation, and maturation[1,3]. Fibroblasts proliferate at the wound site and transform into myofibroblasts. They form a new connective tissue shape by secreting collagen (COL) and fibronectin (FN) to form the temporary extracellular matrix. COL accumulation, which starts after the formation of the cellular matrix, supports extracellular tissue[4].

FN is an extracellular matrix protein that determines cell adhesion, spreading, migration, proliferation, and apoptosis[5]. FN plays complementary roles in forming and regulating tissues during development and in the three phases of wound recovery (inflammation, proliferation, and remodeling). FN is active at all phases of wound recovery[6,7]. FN mediates a wide range of wounds and tissues: skin, periodontal tissues, bone, heart valves, corneal wound recovery, the tongue, and the growth of peripheral neurites[8,9].

Wheat dandruff is the resource of structurally diverse bioactive composites like phenolic acids, minerals, and polyphenols[10]. Vanillic acid (VA), a phenolic acid, is an important composite of wheat dandruff. It has been reported to have antioxidant, neuroprotective, and hepatoprotective activities[11,12]. VA is the oxidized form of vanillin and is found in high concentrations in vanilla beans and Angelica sinensis, a plant used in traditional Chinese medicine[13]. Free radical production and oxidative stress play a key role in the development of these illnesses, and VA can heal these illnesses thanks to its antioxidant

potential[14]. In addition, several studies have shown that VA has various pharmacological activities like antimicrobial, anti-inflammatory, antihypertensive, antioxidant, and inhibition of snake venom activity[15]. Based on former works, VA decreases free radical formation and lipid peroxidation, enhancing mitochondrial function, scavenging free radicals, and increasing antioxidant status[16,17].

It has been supported by many studies that VA exhibits anti-oxidative activity. However, its effect on wound healing has not been investigated. In this study, the effects of VA, a potential wound healing agent in vitro, on the cellular wound repair mechanism were investigated by considering changes in FN1 and COL1α1 gene levels.

METHODS

Cell culture

For our study, adult human dermal fibroblast, HDFa (PCS-201-012™) as the healthy cell, was purchased from ATCC. The cells were resuspended in fresh Dulbecco's modified Eagle's medium, 10% fetal bovine serum, and antibiotic 1% (penicillin, amphotericin B, and streptomycin) (St. Louis, MO, U.S.A). Cells were cultured in 6-, 24- and 96-well plates and stored in an incubator under optimal conditions (5% CO_2; 37°C)[18].

Drug administration

When the cells reached 85% confluence, they were seeded into 6 (for Real-Time PCR), 24 (for wound healing assay), and 96 well plates (for MTT). Experimental groups were defined as control, wound control, VA (1-2-4-8-16, and 32 µg/ml) were administered. The samples were incubated under optimal conditions until the first wound in the experimental groups was closed.

Wound assay

The wound test was used to appraise the migration ratio of VA in the fibroblast cell line. Fibroblast cells were seeded in a 24-well plate and incubated to 100% confluence. At the end of day 5, each well was aspirated with a sterile plastic pipette tip (yellow tip-100 µl). Cell debris was aspirated with phosphate buffer solution (St. Louis, MO, USA). They were then exposed to distinct doses of VA (1-2-4-8-16 and 32 µg/ml). Images from the central area of the wound were taken at 0-48 hours using an inverted microscope (Leica Microsystems, Germany) at ×20 magnification to assess cell migration. All experiments were performed in triplicate[19].

MTT assay

10 µL MTT (5 mg/ml concentration) was supplemented to every well and incubated for 4 hours (5% CO_2; 37 °C) to perform the MTT assay. To dissolve formazan crystals, the medium was lifted after 4 hours and 100 µL DMSO was supplemented. Cell viability was measured by optical density at 570 nm using a Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific, USA) and cell viability was calculated as %18.

Biochemical Analyses

Cell medium was gathered 1 day after toxicity administration and assayed according to the manufacturer's instructions.

Total oxidant status (TOS) The evaluation and the method used the TOS ELISA kit (Rel Assay Diagnostic® Company, Turkey). 500 µL of Reagent1 solution was supplemented to the wells containing the sample, and the initial absorbance value was read at 530 nm, for evaluated TOS assay. Then 30 µL of Reagent 2 solution was supplemented to the same well. After 10 minutes at room temperature, the second absorbance value was read. TOS values were calculated as H_2O_2 Equiv mmol/L-1.

Total antioxidant status (TAC) The evaluation and the method used the TAC ELISA kit (Rel Assay Diagnostic® Company, Turkey). 500 µL of Reagent1 solution was supplemented to the wells containing the sample, and the initial

absorbance value was read at 660 nm, for evaluated TAC assay. Then 75 µL of Reagent 2 solution was supplemented to the same well. 75 µL Reagent 2 solution was added and the second measurement was made at 660 nm. TAC values were calculated as Trolox Equiv/mmol L-1.

Lactate dehydrogenase (LDH) The evaluation and the method used the LDH ELISA kit (Cayman Chemicals, USA). Triton X-100 (10%) and 20 µL Assay buffer were supplemented to cells seeded in 96-well plates. Then it was incubated at room temperature for 1 hour. The cells were centrifuged at 400xg for 5 minutes. 100 µL of cell supernatant were transferred to new 96-well assay plates. LDH reaction solution was supplemented to each well and incubated on the plate with gentle shaking on an orbital shaker for 30 minutes at 37°C. LDH levels were measured with the absorbance optical density value at 490 nm.

8-hydroxy-2 ' deoxyguanosine (8-OHdG) The evaluation and the method used the 8-OHdG ELISA kit (Cell Biolabs, USA). Optical density was measured spectrophotometrically at 450 nm wavelengths. 8-OHdG activity was expressed as pg/mL.

Glutathione (GSH) The evaluation and the method used the GSH ELISA kit (E-EL-0026/GSH; Elabscience, USA). Optical density was measured spectrophotometrically at 450 nm wavelengths. The obtained results were given as % value.

Gene expression

A High Pure RNA Isolation commercial kit was used for RNA. The Transcriptor First Strand cDNA Synthesis (Roche, Germany) commercial kit was used to transform isolated RNAs into cDNA. The sequences of the gene-specific PCR primers are shown below.

β-actin

Forward: 5'- CACCATTGGCAATGAGCGGTTC-3'

Reverse: 5'- AGGTCTTTGCGGATGTCCACGT-3'

FN1

Forward:5′-ACAACACCGAGGTGACTGAGAC−3′

Reverse: 5′- GGACACAACGATGCTTCCTGAG-3′

COL1α1

Forward: 5′-GATTCCCTGGACCTAAAGGTGC-3′

Reverse: 5′- GCCTCTCCATCTTTGCCAGCA-3′

Then, 3 µL cDNA, 0.75 µL gene primer, and 3 µL master were supplemented to each tube for PCR of the cDNAs synthesized for β-actin, FN1, and COL1α1 gene measurements. 13.25 µL ultrapure water was supplemented to the mixture and the final volume was completed to 20 µL. After 5 min at 95 °C, expression levels were measured by beginning a cycle of 45 cycles (at 95 °C for 10 s and 60 °C for 30 s). Results are expressed as relative fold change compared to control. Using the ΔΔCt method (x), gene expressions were normalized to β-actin and expressed as fold change relative to control[20].

Ethical Approval

Ethical approval isn't necessary because commercially present cell lines are used in an in vitro study.

Statistical Analyses

Statistical analyses were made using one-way ANOVA and Tukey HSD method (SPSS 22 software). *p<0.05* was considered a statistically important difference in all tests. Results were offered as mean and standard deviation.

RESULTS

Effects of VA on cell viability

The results of MTT cell viability analysis performed 48 hours after 1-2-4-8-16 and 32 µg/mL VA applications to fibroblast cells were compared with the wound group (52.84%) and calculated as percentage cell viability. Accordingly, the results were found to be 69.85, 71.96, 79.90, 82.49, 88.41 and 95.59%, respectively. It was found that VA applications increased fibroblast viability and proliferation

depending on dose and duration. The results were statistically significant (*p<0.05, p<0.01*) (Figure 1).

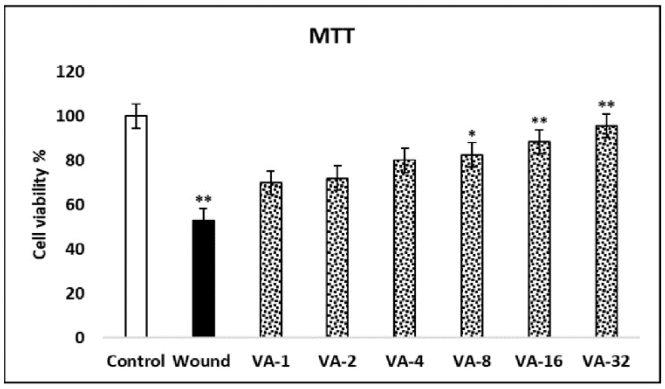

Figure 1. Dose-dependent MTT analysis graph of VA application in fibroblast cells. **p<0.05* vs. Wound group, ***p<0.01* vs. Wound group.

Effects of VA on cell morphology

When fibroblast cells were grown under appropriate conditions, it was observed that they had spindle-shaped morphology and tended to grow overlappingly. When the fibroblast cells covered the culture dish in a monolayer, the wound areas formed with the help of a sterile pipette tip were photographed at 0, 24, 36, and 48 hours and their closure was observed. It was observed that the spindle-shaped morphology of fibroblast cells did not change with different doses and durations of VA applications. According to these results, it was determined that the cells were not exposed to any stress conditions as a result of the experimental treatments. In addition, in the VA 16 and 32 µg/ml groups, it was observed that the wound line was closed at the 36th hour. In the wound group, the wound line was observed even at the 48th hour (Figure 2).

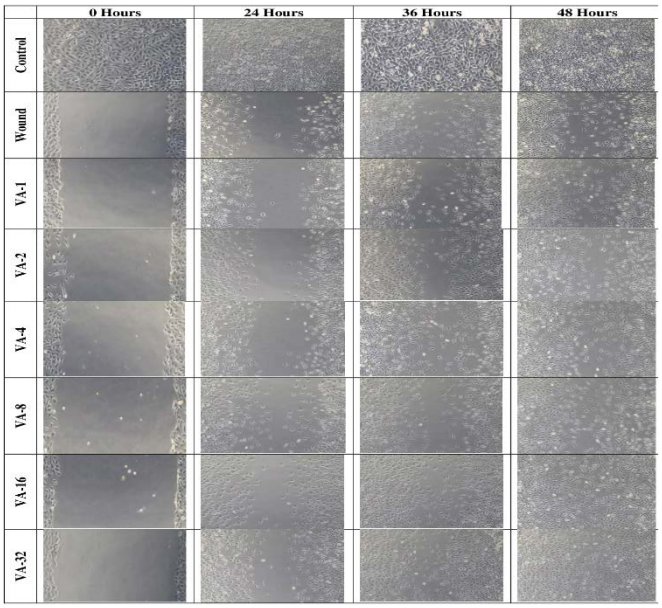

Figure 2. Inverted microscopic images of wound closure areas of fibroblast cells after VA applications.

Effects of VA on oxidative stress

In the wound group, TAC (7.59 mmol Trolox equivalent/L) and GSH (20.14%) activities were significantly decreased, while LDH (98%), TOS (63.65 H2O2 Equiv mmol/L-1), and 8-OHdG (0.594 pg/ml) levels were importantly raised compared to the control group. The results of VA groups (1-2-4-8-16, and 32 µg/ml), TAC (7.73-9.29-10.93-11.01-11.34, and 13.13 mmol Trolox equivalent/L, respectively), GSH (24.41-27.18-29.06-30.46-32.64, and 35.89%, respectively), LDH (63.53-61.49-50.81-47.67-42.24, and 30.12%, respectively), TOS (53.17-47.24-45.79-27.37-26.89, and 16.27 H2O2 Equiv mmol/L-1, respectively), and 8-OHdG levels (0.389-0.296-0.289-0.259-0.251, and 0,236 pg/ml, respectively) were given. In VA groups, TAC, GSH, LDH, TOS, and 8-OHdG activities were close to the control (Figure 3). These results are correlated with MTT. It was determined that cytotoxicity was eliminated due to VA repairing the scar tissue and contributing to cell proliferation (*p<0.05, p<0.01*).

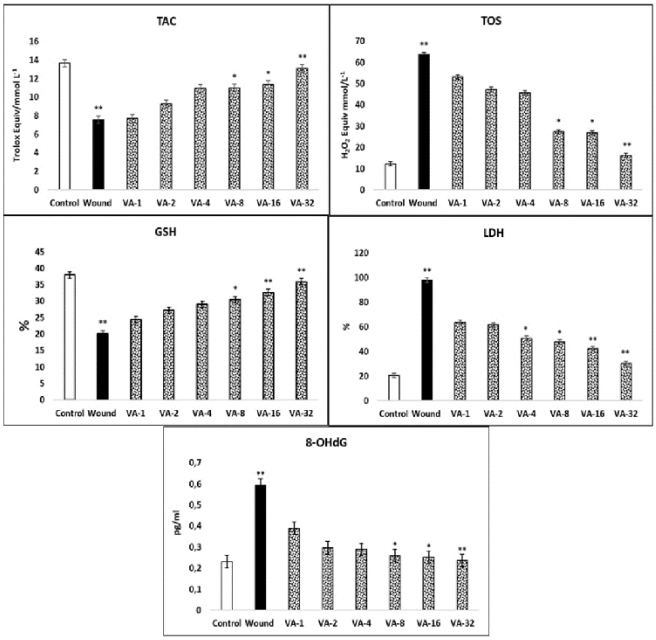

Figure 3. Oxidative Stress Results of Application Group. *$p<0.05$ vs. Wound group, **$p<0.01$ vs. Wound group.

VA regulates the expression of FN1 and COL1α1 gene

While FN1 (0.3-fold) and COL1α1 (0.4-fold) gene expressions were importantly reduced in the wound group, these gene expressions were importantly raised with the administration of VA. With the onset of healing in the wound group, the regulation of FN1 (0.4-0.4-0.5-0.6-0.7 and 0.8-fold, respectively) and COL1α1 (0.4-0.5-0.6-0.7-0.8 and 0.9-fold, respectively) gene expression increased in the VA groups (1-2-4-8-16 and 32 µg/ml). FN1 and COL1α1 are expressed together with the repair of scar tissue. According to the results we obtained, it was determined that there was a 0.9-fold increase in VA 32 µg/ml concentration. The control group was accepted as 1 and the other groups were compared with the control group. The data acquired were statistically important (*$p<0.05$, $p<0.01$*) (Figure 4). Similarly, an approximately 0.8-fold increase in FN1 gene level was determined at 32 µg/ml concentration.

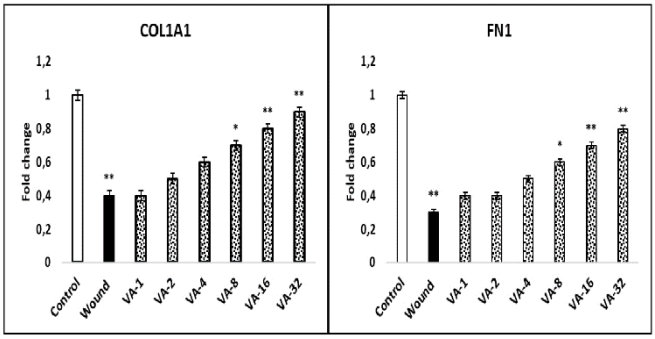

Figure 4. Gene Expression of Application Group. *$p<0.05$ vs. Wound group, **$p<0.01$ vs. Wound group.

DISCUSSION

During the wound-healing process, local and blood-borne fibroblasts proliferate and migrate to wound sites to form wound granulation tissue. Fibroblasts provide the formation of a new extracellular matrix under the influence of various cellular factors and some fibroblasts differentiate into myofibroblasts to help the wound contract[20]. However, various substances with antioxidant activity have also been proven to be beneficial in wound healing[22,23]. Previous in vitro and in vivo studies disclosed that VA derived from wheat bran promoted hair growth[24,25]. Kang et al. showed that VA increased the proliferation of dermal papilla cells by activating Wnt/β-catenin and PI3K/Akt paths[26]. The antioxidant effect of VA showed a healing effect on the wound. It provided the closure of the wound line in a shorter time compared to the wound group. In addition, the gradual decrease of oxidative stress, which rose in the wound group, and the VA group triggered the healing of the wound area in a shorter time. During wound healing, FN is one of the first and most abundant extracellular matrix components that accumulate in that area[27,28]. FN triggers fibril formation together with extracellular matrix formation. Accumulation of FN matrix in wounds stimulates collagen and contributes to wound contraction. In addition, FN can bind to other cells to further stabilize the extracellular matrix[7]. In this study, the effect of VA on the healing duration of fibroblast cells

was investigated. According to our findings, it was determined that increased oxidative stress and cellular cytotoxicity in the wound area were eliminated by VA and the wound line was closed faster than the normal process.

In vitro studies have shown that FN polymerization leads to the composition and stability of the extracellular matrix and cell-matrix adhesion[7,29]. FN polymerization enables the accumulation of COL types I and III in the extracellular matrix and results in the stabilization of COLI matrix fibrils. A study by Shi and Sottile showed that membrane-type matrix metalloproteinase 1 promotes extracellular matrix FN turnover by regulating the cleavage of large FN fibrils and subsequent endocytosis of α5β1 integrin[30]. They also showed that inhibition of FN polymerization accelerated myofibroblast migration.

Type I collagen is the most expressed collagen in the skin, tracked by type III and type IV, which conduce to the stability of the epidermis and are responsible for tension[31]. In this study, it was confirmed that VA significantly raised COL1α1 expression in wound cells. In contrast, the decrease in migration of fibroblasts in the wound group led to a decrease in COL1α1. Recent studies have shown that reduction in collagen expression in early granulation tissue promotes myofibroblast differentiation and increased scar deposition in cutaneous wounds[32]. In their Type 1 diabetes study, Black et al. reported that a 40% reduction in collagen deposition delayed the wound healing process[33]. Another study demonstrated that overexpression of collagen in fibrotic lungs is a key factor in tissue dysfunction[34].

CONCLUSION

Our findings show for the first time that VA increases migration and/or proliferation of fibroblasts by regulating oxidative stress and FN1 and COL1α1 genes. This activity may be related to the production of FN1 and COL1α1, which are considered important targets for modulation of the tissue repair duration and play an important role in the wound healing process. It is thought that the results of this study will lead to the use of VA in vivo wound healing research.

Ethics Committee Approval: Ethical approval isn't necessary because commercially present cell lines are used in an in vitro study.

Conflict of Interest Declaration: The author declares that he has no conflict of interest.

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Original Article / Özgün Araştırma

The prognostic value of free triiodothyronine/free thyroxine ratio in short-term outcomes after left ventricular assist device implantation

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Abstract

Background: Despite advancement in technology adverse events after left ventricular assist device(LVAD) implantation continue to be the main barrier to more widespread use of it. Therefore, it is vital to identify prognostic factors to reduce adverse cardiac events after LVAD implatation. Thyroid dysfunction is associated with a poor prognosis in individuals with heart failure. The objective of this study is to examine the correlation between thyroid hormones and adverse cardiovascular events following LVAD implantation.

Method and Results: The study included a total of 36 patients, with an average age of 60 ± 9 years, of which 77.7% were male. Although there was no significant relationship between TSH and FT3 levels and post-operative adverse events($p>0.05$ for both), there was a significant association between FT3/FT4 and all-cause and cardiovascular mortality($p<0.001$ for both). ROC analysis revealed that FT3/FT4 values lower than 1 were significantly associated with adverse events(all $p < 0.05$). There was no significant difference between the two groups in terms of age, gender, BMI, etiology of heart failure, EF, sPAP, smoking status and the presence of comorbid diseases, BNP, TSH and FT3 levels($p>0.05$ for all). All-cause mortality, cardiac mortality, arrhythmia prevelance, vasopressor need(day) and duration of ICU stay were significantly higher in FT3/FT4 < 1 group(all $p < 0.05$).

Conclusion: Our findings suggest that FT3/FT4 ratio might be useful as a biomarker of short-term adverse clinical outcomes in patients who underwent LVAD implantation.

Keywords: Left ventricular assist device; Heart failure; Thyroid disfunction; FT3/FT4 ratio; Cardiac mortality

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Sol ventrikül destek cihazı implante edilen hastalarda serbest triiyodotironin/serbest tiroksin oranının kısa vadeli olumsuz klinik sonuçlar açısından prognostik değeri

Öz

Giriş ve Amaç: Teknolojideki ilerlemelere rağmen, sol ventrikül destek cihazı(LVAD) implantasyonu sonrası gelişen olumsuz olayların sıklığı, bu teknolojinin daha geniş çapta kullanımının önünde engel olmaya devam etmektedir. Bu nedenle, LVAD implantasyonu sonrası olumsuz kardiyak olayları azaltmak için prognostik faktörleri belirlemek hayati önem taşımaktadır. Tiroiddisfonksiyonu, kalp yetmezliği hastalarında kötü prognozla ilişkilidir. Bu çalışmanın amacı, LVAD implantasyonu sonrası olumsuz kardiyovasküler olaylar ile tiroid fonksiyonu arasındaki ilişkiyi incelemektir.

Yöntem ve Bulgular: Toplam 36 hasta (ortalama yaş 60 ± 9, %77,7 erkek) çalışmaya dahil edildi. Cerrahi sonrası olumsuz olaylar ile TSH ve FT3 düzeyleri arasında anlamlı bir ilişki olmamasına rağmen (her ikisi için de $p>0.05$), FT3/FT4 oranı ile tüm nedenlere bağlı ve kardiyovaskülermortalite arasında anlamlı bir ilişki bulundu (her ikisi için de $p<0.001$). ROC analizi, FT3/FT4 değerlerinin 1'in altında olması ile kötü prognoz arasında anlamlı ilişki olduğunu ortaya koydu (tümü için $p < 0.05$). İki grup arasında yaş, cinsiyet, BMI, kalp yetmezliği etiyolojisi, EF, sPAP, sigara içme durumu, eşlik eden hastalıkların varlığı, BNP, TSH ve FT3 düzeyleri açısından anlamlı fark yoktu (tümü için $p>0.05$). Tüm nedenlere bağlı mortalite, kardiyak mortalite, aritmi prevalansı, vazopressör gereksinimi (gün) ve yoğun bakım yatış süresi FT3/FT4 < 1 grubunda anlamlı derecede yüksekti (tümü için $p < 0.05$).

Sonuç: Bulgularımız, FT3/FT4 oranının, LVAD implante edilen hastalarda kısa vadeli olumsuz klinik sonuçların bir biyobelirteci olarak kullanışlı olabileceğini göstermektedir.

Anahtar kelimeler: Sol ventrikül destek cihazı; Kalp yetmezliği; Tiroid bozukluğu; serbestT3/serbestT4 oranı; Kardiyak mortalite.

INTRODUCTION

Thyroid hormone (TH) plays a vital role in regulating cardiovascular homeostasis by influencing heart rate (HR), cardiac contractility, myocardial relaxation and systemic vascular resistance(SVR)[1]. These hormones mainly triiodothyronine (T3) and thyroxine (T4), circulate in the bloodstream, with T3 primarily deriving from the conversion of T4 through the action of deiodinase iodothyronine enzymes in peripheral tissues.While both hormones exert biological effects, T3 is considered asthe more potent of the two[2]. Research findings indicate that reduced free T3 levels and elevated levels of thyroid-stimulating hormone (TSH) are linked to a poorer prognosis in heart failure patients, even in the absence of clinically apparent thyroid disorders[3,4].

Heart failure (HF) is a significant cardiac condition that continues to pose a substantial public health challenge, persisting as a major concern despite advancements in medical therapy. Its prevalence in developed countries typically falls within the range of 1.5 to 2.0[5,6]. A substantial number of individuals with heart failure (HF) progress to an advanced HF stage, marked by persistent symptoms despite receiving the most intensive therapy available. While heart transplantation remains the gold standard therapy for end-stage HF, LVAD therapy has emerged as a valuable alternative for individuals who are not eligible for transplantation, serving as a destination treatment option.

With an aging population and a limited supply of donor organs for transplantation, it is increasingly probable that a greater number of patients will undergo LVAD treatment in the future. The ongoing advancements in LVAD technology have led to improved clinical outcomes[7]. Notwithstanding these encouraging outcomes, post-implantation complications remain the primary impediment to broader adoption of this technology in LVAD use[8].

Hence, the identification of prognostic factors is crucial in mitigating adverse cardiac events following LVAD implantation.

This study was conducted to investigate the potential correlation between thyroid hormones and the occurrence of both all-cause mortality and adverse cardiovascular events following LVAD implantation. Thus, the aim was to identify high-risk patients during the preoperative process in order to enable early intervention.

METHODS

Patient population

In this retrospective cohort study, we included 36 patients who received Heart Mate II LVAD and Heart Mate III LVAD at our institution between December 2018 and October 2022. We collected baseline demographic characteristics, intensive care unit (ICU) duration, arrhythmia occurrences (ventricular tachycardia or ventricular fibrillation), and data on all-cause and cardiac mortality from hospital medical records. Demographics, preoperative echocardiographic findings, and pre-existing comorbid conditions were manually documented. Thyroid function parameters that masured on admission were recorded. Patients who had not undergone thyroid function tests during the preoperative process were excluded.

LVAD implantataion procedure

LVAD implantation is performed under general anesthesia in a sterile operating room environment. The surgical approach involves median sternotomy or minimally invasive techniques, depending on patient characteristics and surgeon preference. Following exposure of the heart, the left ventricle is accessed, and the LVAD pump is implanted. Careful attention is paid to cannulation of the left ventricular apex and connection of inflow and outflow cannulas to the heart chambers. The driveline is tunneled subcutaneously and exits the abdomen for connection to an external power source. Intraoperative transesophageal echocardiography was used in assessing device positioning and function. Hemodynamic monitoring guides optimal pump speed adjustment to achieve adequate cardiac output while minimizing complications such as suction events and thrombosis.

Ethics

This study received approval from the institutional ethics committee of the University(E-10840098-772.02-5426, 01.09.2023). In compliance with the Declaration of Helsinki, informed consent was obtained from all patients for their participation in the study.

Clinical outcomes

The primary endpoint of this study was cardiac mortality. Cardiac mortality was defined as death resulting from myocardial infarction, ischemia, heart failure, or cardiac arrest due to other or unknown causes. Secondary endpoints encompassed the length of ICU stay, occurrences of arrhythmias (ventricular tachycardia or ventricular fibrillation), and all-cause mortality. The survival status of the patients was determined by a comprehensive review of their medical records.

Statistical Analyses

Statistical analysis was carried out using SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation or median (interquartile range, IQR), while categorical variables were expressed as percentages. A p-value < 0.05 was considered statistically significant for all comparisons. Normal distribution was assessed using the Shapiro-Wilk test. Independent samples were compared using Student's t-test for normally distributed data, and the Mann-Whitney U test was employed for non-normally distributed data. Associations between categorical variables

among groups were analyzed using the Chi-square test. The relationship between the FT3/FT4 and both cardiac and all-cause mortality was demonstrated with separate box plot graphics. Receiver Operating Characteristic (ROC) analysis was performed to determine the cutoff FT3/FT4 value for predicting mortality.

RESULTS

The study comprised a total of 36 patients. Table 1 provides an overview of the patients' baseline characteristics. The average age of the patients was 60 years (SD 9), with 77.7% of them being male. The mean left ventricular ejection fraction (LV EF) was 19.4% (SD 3.3). Among the participants, 66.6% had coronary artery disease, 61.1% had hypertension, and 69.4% had diabetes. The median TSH levels were 1.24 µIU/mL (interquartile range: 0.46–3.32), and the mean FT3 levels in the study were 3.02 ng/dL (SD 0.24).

Table I: Baseline demographic, clinical, echocardiographic and laboratory features of patients (n=36)

	OverallPopulation (n = 36)
Age (years) mean	60 ± 9
Gender (male), n (%)	28 (77.7%)
BMI, kg/m2, mean	27.6 ± 3.2
HT, n (%)	22(61.1%)
CAD, n(%)	24(66.6%)
DM, n (%)	25(69.4%)
CVA, n (%)	4(11.1%)
CRF, n (%)	13(36.1%)
Arrhythmias, n (%)	8 (22.2%)
Smoking, n (%)	22(61.1%)
Etiology; Non-İschemic, n (%)	12 (33.3%)
Heart mate II, n(%)	14 (38.8%)
NYHA 4, n(%)	18 (50%)
LVEF, %, mean	19.4 ± 3.3
sPAP, mmHg, mean	48.6 ± 9.6
BNP, pg/mL,median	11449,6 ± 9540
TSH, µIU/mL, median	1.24 (0.46–3.32)
Free T3, ng/dL, mean	3.02 ± 0.24

BMI: Body Mass Index, HT: Hypertension, CAD: Coronary Artery Disease, DM: Diabetes Mellitus, CVA: Cerebrovascular Accident, CRF: Chronic Renal Failure, NYHA: New York Heart Association, LVEF: Left Ventricular Ejection Fraction, sPAP: Systolic Pulmonary Artery Pressure, BNP: Brain Natriuretic Peptide, TSH: Thyroid-Stimulating Hormone, T3: Triiodothyronine

Association of thyroid status with postoperative adverse events investigated. Even though there was no significant link between TSH and FT3 levels and post-operative adverse events, ($p>0.05$ for both), a substantial association was identified between FT3/FT4 and cardiovascular and all-cause mortality($p<0.001$ for both)(figure 1 and 2).

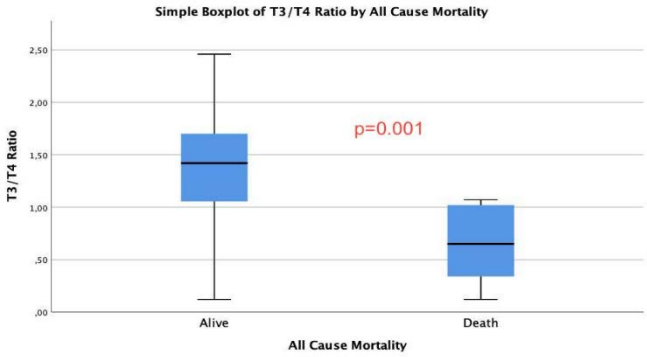

Figure 1. The box-plot graph shows association of FT3/FT4 ratio with all-cause mortality. There is a negative and significant correlation between FT3/FT4 ratio and all-cause mortality p<0.001)

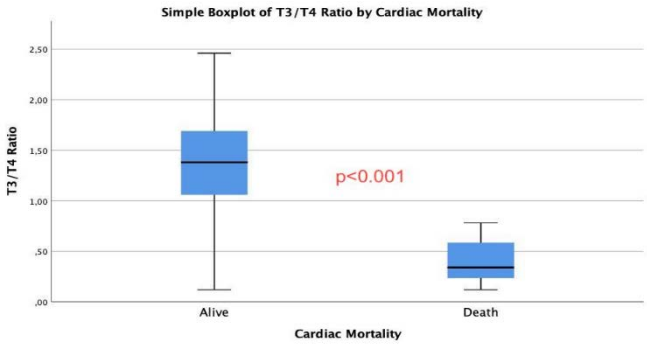

Figure 2. The box-plot graph shows association between FT3/FT4 ratio and cardiac mortality. There is a significant relationship between FT3/FT4 ratio and cardiac mortality (p<0.001)

To establish a cut-off value for FT3/FT4 to determine post-operative adverse events, we performed ROC analysis that revealed FT3/FT4 values lower than 1 were significantly associated with post-operative adverse events. According to the cut-off FT3/FT4 value, we

devided patients into 2 groups as 1) FT3/FT4 < 1 and 2) FT3/FT4 ≥ 1. Comparison of the baseline demographic, clinical and echocardiographic features between the groups defined in accordance with the specified cut-off value for FT3/FT4 are shown in Table 2. There was no substantial difference between the two groups concerning gender, age, BMI, etiology of heart failure, EF, sPAP, smoking status and the presence of comorbid diseases (coronary artery disease, diabetes mellitus, hypertension, arrhythmia, cerebrovascular disease, renal disease, peripheral artery disease), BNP, TSH and FT3 levels (all p>0.05).

Table II: Comparison of the baseline demographic, clinical, echocardiographic and laboratoryfeatures between the groups defined according to the cut-off value for FT3/FT4 ratio (n=36)

	FT3/FT4 ratio<1 (n= 12)	FT3/FT4 ratio≥1 (n= 24)	p value
Age (years) mean	57.9 ± 7.3	60.2 ± 8.2	0.230
Gender (male), n (%)	9 (75%)	19(79.1%)	0.282
BMI, kg/m2, mean	28.1 ± 3.3	27.4 ± 3.3	0.440
HT, n (%)	8 (66.6%)	14 (58.3%)	0.808
CAD, n(%)	7(58.3%)	17(70.8%)	0.343
DM, n (%)	6 (50%)	19 (79.1%)	0,157
CVA, n (%)	2 (16.7%)	2 (8.3%)	0.453
CRF, n (%)	4 (33.3%)	9 (37.5%)	0.326
Arrhythmias, n (%)	3 (25%)	5 (20.8%))	0.313
Smoking, n (%)	8 (66.6%)	14 (58.3%)	0.369
Etiology; Non-İschemic, n (%)	5 (41.6%)	7 (29.1%)	0.414
Heart mate II, n(%)	6 (50%)	8 (33.3%)	0,156
NYHA calss 4, n(%)	7 (58.3%)	11 (45.8%)	0.480
LVEF, %, mean	59.4 ± 3.9	60.1 ± 4.3	0.577
sPAP, mmHg, mean	45± 8.7	50.4 ± 9.7	0.099
BNP, pg/mL,median	6562(28871 6902)	7265(4375-9173)	0.591
TSH, µIU/mL, median	1.36 (0.68–3.71)	1.18 (0.62–3.44)	0.376
Free T3, ng/dL, mean	2.77 ± 0.38	3.09 ± 0.42	0.462

BMI: Body Mass Index, HT: Hypertension, CAD: Coronary Artery Disease, DM: Diabetes Mellitus, CVA: Cerebrovascular Accident, CRF: Chronic Renal Failure, NYHA: New York Heart Association, LVEF: Left Ventricular Ejection Fraction, sPAP: Systolic Pulmonary Artery Pressure, BNP: Brain Natriuretic Peptide, TSH: Thyroid-Stimulating Hormone, T3: Triiodothyronine

Table 3 presents a comparison of post-operative outcome variables between the groups categorized based on the FT3/FT4 cutoff value. In the FT3/FT4 < 1 group, all-cause mortality, cardiac mortality, arrhythmia prevalence, vasopressor requirement (days), and duration of ICU stay were significantly higher (all p < 0.05).

Table III: Comparison of the post-operative outcome variables between the groups defined according to the cut-off value for FT3/FT4 ratio

Outcome Variable	FT3/FT4 ratio<1 (n= 12)	FT3/FT4 ratio≥1 (n= 24)	p value
ICU Stay (days, median)	8(4-13.7)	4(3.2-6)	< 0.040
Cardiac Mortality, n (%)	7 (58.3%)	0 (0%)	< 0.001
All-cause Mortality, n (%)	6 (50%)	3 (12.5%)	0.014
Arrhythmias(VT/VF), n (%)	7 (58.3%)	2 (8.3%)	0.001
Vasopressorneed (Day, median)	5.5(3-13.5)	2.5(1-5.5)	0.019

ICU:Intensive Care Unit, VT/VF: Ventricular Tachycardia/Ventricular Fibrillation

DISCUSSION

In this study, association between thyroid function and short-term outcomes after LVAD implantation was investigated. The results indicate that FT3/FT4 was significantly associated with cardiac mortality during hospitalization after the LVAD implantation. We found a consistent negative association between FT3/FT4 and cardiac mortality, all-cause mortality, ventricular arrythmia, duration of ICU stay and need of postoperative vasopressor. On the other hand, neither TSH nor FT3 levels had shown to be associated with these end points.

In previosus studies it has been shown that cardiovascular system is an important target for TH activity. The major effects of TH on the heart are mediated by free triiodothyronine (FT3). T3 is mainly converted from T4 by deiodinase iodothyronine in peripheral tissues. It exerts influence on the vascular system as well as the heart[9]. FT3 modulates systolic contraction and

diastolic relaxation[10]. In addition to maintaining normal arteriolar remodeling, FT3 directly influences vascular smooth muscle cells, resulting in promotion of relaxation[11,12].

There are numerous cardiac conditions shown to be related to thyroid dysfunction like atherosclerosis, arrythmias, pericardial diseases, dyslipidemia and hypertension. Also there is increasing evidence suggesting that reduced thyroid function could be a contributing factor to heart failure[13,14]. Among individuals with heart failure, decreased T3 levels have been linked to myocardial scarring and abnormalities in myocardial perfusion[15]. Research has shown a connection between subclinical hypothyroidism and increased mortality rates in individuals with heart failure[16].

In research examining the link between thyroid dysfunction and heart failure, greater emphasis has been placed on evaluating FT3 and TSH levels[17,18]. However, in our study, we also prefered to examine the FT3/FT4. The FT3/FT4 represents the degree of transformation from T4 to T3, which might correlate with cardiovascular function. Therefore, there could be a significant connection between heart failure and thyroid hormone, which could be represented by the FT3/FT4 for the activity of extrathyroidal transformation of T4 to T3. Unlike prior studies, we were unable to establish a link between T3 and TSH levels and cardiovascular events. However, we demonstrated a remarkable correlation between the FT3/FT4 and prognostic outcomes following LVAD implantation. This finding might suggest that the FT3/FT4 could serve as a more effective predictor of thyroid function for end-stage heart failure patients implanted with LVAD.

Bielka at al retrospectively investigated impact of thyroid status at consecutive 147 patients who underwent LVAD implantation and they showed that there is an connection between TSH and FT3 levels and survival after LVAD implantation[19]. Nonetheless, number of studies examining the link between thyroid function and outcomes in individuals who have undergone LVAD implantation is insufficient. Our study holds significance as being the first investigation to investigate the connection between the FT3/FT4 and post-LVAD cardiovascular events.

CONCLUSION

In conclusion, our study revealed that preoperatively measured FT3/FT4 ratio has an association with unfavorable outcomes in LVAD implanted patients. Our findings suggest that FT3/FT4 ratio might be a valuable biomarker of short-term adverse clinical outcomes. Close follow-up of the patients who have lower FT3/FT4, for the risk of developing postoperative adverse events can provide a significant benefit in reducing cardiovascular events by ensuring early diagnosis and treatment.

Limitations

The primary drawback of our study was the limited sample size, thus warranting the validation of these results in a larger population. Another drawbacks are the absence of a control group and lack of assesment of postoperative thyroid function.

Ethics Committee Approval: This study received approval from the institutional ethics committee of the University(E-10840098-772.02-5426, 01.09.2023). In compliance with the Declaration of Helsinki, informed consent was obtained from all patients for their participation in the study.

Conflict of Interest: The authors declared no conflicts of interest.

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Özgün Araştırma / Original Article

Hemodiyaliz Hastalarında Okült Hepatit B ile Hepatit C Prevalansı ve Fibrometer ile Karaciğer Fibrozis Düzeyinin Değerlendirilmesi

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Öz

Amaç: Bu çalışmada, rutin hemodiyalize giren HBsAg negatif hasta serum örneklerinde HBV DNA incelemesi ile okült HBV prevalansı ve HCV prevalansının saptanması hedeflendi. Aynı zamanda non-invaziv bir metot olan fibrometer ile karaciğer fibrozis düzeyi belirlenmesi amaçlandı.

Yöntemler: Çalışmaya rutin hemodiyaliz amacıyla başvuran ve tarama testlerinde HBsAg negatif olan 100 hasta dahil edildi. Hastadan alınan örneklerde, ALT, HBsAg, Anti-HBc IgG, HBV DNA, Anti HCV, HCV RNA ve aynı zamanda serum örneklerinden fibrometer çalışıldı. HBsAg negatif ve HBV DNA pozitif hastalar okült Hepatit B olarak kabul edildi.

Bulgular: Çalışmamızda 100 hastanın 4'ünde (%4) HBV DNA pozitif saptandı. HBV DNA pozitif olan vakaların ikisinde viral yük <10 IU/ml, diğer iki vakada 17 ve 16 IU/ml olarak saptandı. Bir hastada Anti HCV pozitif tespit edilirken; hiçbir hastada HCV RNA tespit edilemedi. Okült hepatit B olan hastalarda olmayanlara göre fibrozis skoru açısından anlamlı fark saptanmışken; histolojik aktivite indeksi ve nekroz skoru açısından anlamlı fark saptanmamıştır.

Sonuç: Antijen antikor bazlı seroloji testler okült HBV tanısında yetersiz kalabildiğinden, hemodiyaliz hastaları gibi özellikli gruplarda HBV DNA düzeyinin tespiti okült HBV'yi erken tanımak, siroz ve hepatoselüler kanser gibi önemli komplikasyonları önleyebilmek açısından önem arz etmektedir. Literatürde daha önce okült Hepatit B ve fibrometer ile ilgili yapılmış bir çalışmanın bulunmamış olması, çalışmanın önemini artırmıştır.

Anahtar kelimeler: Okült Hepatit, Hemodiyaliz, Fibrometer, Fibrozis

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Prevalence of Occult Hepatitis B and Hepatitis C in Hemodialysis Patients and Evaluation of Liver Fibrosis Level with Fibrometer

Abstract

Objective: In this study, it was aimed to determine the HCV prevalence and the occult HBV prevalence by HBV DNA analysis in serum samples of HBsAg patients undergoing routine hemodialysis. It was also aimed to determine the liver fibrosis level with fibrometer, which is a non-invasive method.

Methods: The study included 100 patients who applied for routine hemodialysis and were negative for HBsAg in screening tests. In samples from the patient, ALT, HBsAg, Anti-HBc IgG, HBV DNA, Anti HCV, HCV RNA and fibrometer were studied from serum samples from the same system. HBsAg negative and HBV DNA positive occult hepatitis B were accepted.

Results: HBV DNA was found positive in 100 positive 4 (4%). Viral load was <10 IU/ml in two of the HBV DNA positive cases, and 17 and 16 IU/ml in the other two cases. While Anti HCV positive was detected in one patient; HCV RNA could not be detected in any patient. While there was a significant difference in scores compared to those who did not have occult B; The score of histological activity and necrosis were not significantly different.

Conclusion: Since antigen asntibody-based serology tests may be insufficient in the diagnosis of occult HBV, determination of HBV DNA level in special groups such as hemodialysis patients is important in terms of early recognition of occult HBV and preventing important complications such as cirrhosis and hepatocellular cancer. The absence of a previous study on occult hepatitis B and fibrometer in the literature has increased the importance of this study.

Keywords: Occult Hepatitis, Hemodialysis, Fibrometer, Fibrosis.

GİRİŞ

Okült Hepatit B virüs infeksiyonu (OBİ), serumda HBsAg saptanmazken, plazma veya karaciğerde HBV DNA'nın düşük düzeyde olsa saptanmasıdır. Anti-HBc IgM-G ve anti HBs pozitif veya negatif olabilir. OBİ prevalansı bölgeden bölgeye değişmekle birlikte genellikle %1-14 arasında değişmektedir[1,2]. Özellikle riskli olan gruplar; kan donörleri, hemodiyaliz hastaları, erkek sex işçileri, HIV ile infekte hastalar, damar içi ilaç bağımlıları, HCV pozitif hastalar ve hepatit B açısından endemik bölgede yaşayanlardır[3,4].

Okült Hepatit C infeksiyonu (OCİ), anti HCV antikorlarının serumda saptanmaması karaciğer hepatosit veya periferal mononükleer hücrelerinde moleküler yöntemlerle HCV RNA saptanmasıdır. 2004 yılında OCİ tanımı yapıldıktan sonra, bu hastalık tablosu iki şekilde kategorize edildi4. Birinci grup, anti HCV antikor negatifliği ve serum HCV RNA negatifliği olan seronegatif OCİ olarak kategorize edilirken; anti HCV antikor pozitifliği ve serum HCV RNA negatifliği olan grup seropozitif veya sekonder OCİ olarak adlandırıldı. Epidemiyoloji ile ilgili çok çalışma yokken, yapılan bir çalışmada kriptojenik karaciğer hastalarında OCİ prevalansı %8,9-10 arasında saptanmıştır[5,6].

Kronik hepatitlerde, karaciğer fibrozis ve nekroz tanımlaması için, karaciğer biyopsisi hala altın standart olarak kabul edilse de özellikle son yıllarda, non-invaziv pek çok metot geliştirilmiştir. Bunlardan en çok karşımıza çıkanlar, Hepatik Elastografi (FibroScan), ARFI (Acoustic Radiation Force Impulse), Super Sonic Imaging (ShearWave Elastografi), MR spektroskopi, MR elastografi, splenik dopler İmpedans hepatik elastografi (fibroscan), serum fibrozis direkt ve indirekt göstergeleridir[7,8].

Fibrometer, son yıllarda sık kullanılan non-invaziv yöntemlerden biridir. Serum biyokimyasal belirteçlerle, karaciğer fibrozis düzeyini indirekt olarak göstermektedir. En sık

kullanılan biyobelirteçler, alfa 2 makroglobulin, ALT, AST, GGT, trombosit, üre ve protrombonin zamanıdır. En çok kullanılan patolojiler, viral hepatitler, non-alkolik yağlı karaciğer hastalığı (NAFLD) ve alkolik karaciğer hastalığıdır. Bulguların değerlendirilmesinde Metavir skorlama sistemi kullanılmaktadır[9].

Bu çalışmada HBsAg seronegatif hemodiyaliz endikasyonu ile başvuran hastalarda hepatit C prevalansı ve OBİ prevalansı saptanması hedeflenmiştir. Aynı zamanda fibrometer ile karaciğer fibrozis düzeyinin belirlenmesi amaçlanmıştır.

YÖNTEMLER

Hastalar

Çalışmaya rutin hemodiyaliz amacıyla başvuran ve tarama testlerinde HBsAg negatif olan 100 hasta dahil edildi. Çalışmaya 1 Temmuz 2019 ile 1 Temmuz 2022 tarihleri arasında merkezimize başvuran, 18 yaş üstü, HBsAg negatif olan ve hemodiyalize giren hastalar dahil edildi. Yazılı onam vermeyen, periton diyaliz programına alınan ve 18 yaş altı hastalar çalışma dışı bırakıldı. Hastalar, hemodiyaliz süresi ve altta yatan hastalık açısından sorgulandı. Hastalardan alınan örneklerden, ALT, AST, PT, APTT, INR, HBsAg, Anti-HBc IgM, Anti-HBc IgG, HBeAg, Anti-HBeAg, Anti-HCV çalışıldı.

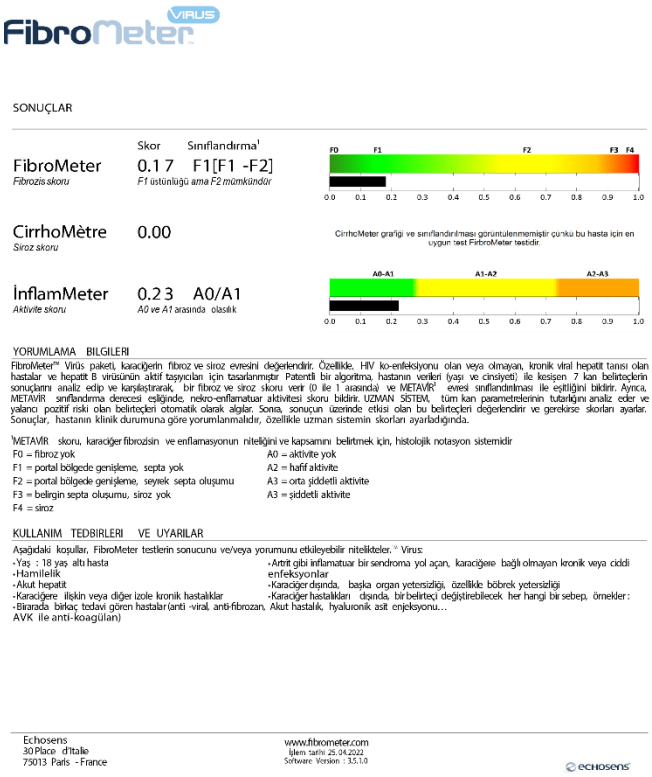
HBV DNA ve HCV RNA Analizi

Hastalardan alınan plazma örneklerinde, HBV DNA ABBOTT M2000RT HBV kiti ile, HCV RNA ise ABBOTT M2000RT HCV kiti ile kantitatif olarak çalışılmıştır. 1 IU/ml =3.41 kopya/ml olarak kabul edilmiştir.

Fibrometer

Hastalardan alınan serum örnekleri 5 dakika boyunca 5000 devir/dk santrifüj edilerek plazmasına ayrıldı; ayrılan plazmalar fibrometer testi için ayrı bir tüpe alındı. Örnekler hizmet alımı şeklinde şehir dışında bir firmaya soğuk zincir kurallarına uyularak transferi yapıldı. Aynı gün gönderilmeyen ürünler -70 °C'de özel saklama koşullarında saklandı. Fibrometer analizinde, protrombin oranı, gama glutamil transpeptidaz, trombosit, alfa2-makroglobulin, AST, ALT ve üre biyobelirteci kullanıldı. Fibrometer olarak, Echosens firmasına ait olan FibroMeter™ Virüs paketi, Software Versiyon: 3.5.1 kullanıldı.

FibroMeter™ Virüs paketi, karaciğerin fibrozis ve siroz evresini değerlendirmektedir. Özellikle, HIV ko-infeksiyonu olan veya olmayan, kronik viral hepatit tanısı olan hastalar ve hepatit B virüsünün aktif taşıyıcıları için tasarlanmıştır. FibroMeter™ Virüs paketi, hastanın verileri (yaşı ve cinsiyeti) ile 7 kan belirteçlerin sonuçlarını analiz ve karşılaştırma yaparak bir fibrozis ve siroz skoru vermektedir (0 ile 1 arasında). Sonuçlar, METAVİR evresi sınıflandırılmasına dayanmaktadır. Ayrıca METAVİR sınıflandırmasına dayanarak nekro-enflamatuar aktivite skoru da raporlandırılmaktadır. Uzman raporlandırma sistemi, tüm kan parametrelerinin tutarlığını analiz ederek yalancı pozitif riski olabilecek belirteçleri otomatik olarak algılamaktadır. Bu sistem, sonucun üzerinde etkisi olan bu belirteçleri değerlendirerek gerektiğinde düzenlemeler yapmaktadır. Sonuçlar, hastanın klinik durumuna göre yorumlanmaktadır. Örnek bir FibroMeter™ Virüs analiz sonucuna Resim 1'de yer verilmiştir. METAVİR skoru, karaciğer fibrozisin ve inflamasyonun niteliğini ve kapsamını belirtmek için, histolojik bir sistemidir. Metavir skorlamasına Tablo 1'de yer verilmiştir.


FibroMeter VIRUS

SONUÇLAR

	Skor	Sınıflandırma[1]
FibroMeter *Fibrozis skoru*	0.17	F1[F1 -F2] *F1 üstünlüğü ama F2 mümkündür*
CirrhoMètre *Siroz skoru*	0.00	
İnflamMeter *Aktivite skoru*	0.23	A0/A1 *A0 ve A1 arasında olasılık*

CirrhoMeter grafiği ve sınıflandırılması görüntülenmemiştir çünkü bu hasta için en uygun test FibroMeter testidir.

YORUMLAMA BILGILERI

FibroMeter™ Virüs paketi, karaciğerin fibroz ve siroz evresini değerlendirir. Özellikle, HIV ko-enfeksiyonu olan veya olmayan, kronik viral hepatit tanısı olan hastalar ve hepatit B virüsünün aktif taşıyıcıları için tasarlanmıştır. Patentli bir algoritma, hastanın verileri (yaşı ve cinsiyeti) ile kesişen 7 kan belirteçlerin sonuçlarını analiz edip ve karşılaştırarak, bir fibroz ve siroz skoru verir (0 ile 1 arasında) ve METAVIR[1] evresi sınıflandırılması ile eşitliğini bildirir. Ayrıca, METAVIR sınıflandırma derecesi eşliğinde, nekro-enflamatuar aktivitesi skoru bildirir. UZMAN SİSTEM, tüm kan parametrelerinin tutarlığını analiz eder ve yalancı pozitif riski olan belirteçleri otomatik olarak algılar. Sonra, sonucun üzerinde etkisi olan bu belirteçleri değerlendirir ve gerekirse skorları ayarlar. Sonuçlar, hastanın klinik durumuna göre yorumlanmalıdır, özellikle uzman sistemin skorları ayarladığında.

[1]METAVIR skoru, karaciğer fibrozisin ve enflamasyonun niteliğini ve kapsamını belirtmek için, histolojik notasyon sistemidir

F0 = fibroz yok
F1 = portal bölgede genişleme, septa yok
F2 = portal bölgede genişleme, seyrek septa oluşumu
F3 = belirgin septa oluşumu, siroz yok
F4 = siroz

A0 = aktivite yok
A2 = hafif aktivite
A3 = orta şiddetli aktivite
A3 = şiddetli aktivite

KULLANIM TEDBIRLERI VE UYARILAR

Aşağıdaki koşullar, FibroMeter testlerin sonucunu ve/veya yorumunu etkileyebilir nitelikteler. ° Virus:
·Yaş : 18 yaş altı hasta
·Hamilelik
·Akut hepatit
·Karaciğere ilişkin veya diğer izole kronik hastalıklar
·Birarada birkaç tedavi gören hastalar (anti -viral, anti-fibrozan, AVK ile anti-koagülan)
·Artrit gibi inflamatuar bir sendroma yol açan, karaciğere bağlı olmayan kronik veya ciddi enfeksiyonlar
·Karaciğer dışında, başka organ yetersizliği, özellikle böbrek yetersizliği
·Karaciğer hastalıkları dışında, bir belirteçi değiştirebilecek her hangi bir sebep, örnekler:
Akut hastalık, hyaluronik asit enjeksyonu…

Echosens
30 Place d'Italie
75013 Paris - France

www.fibrometer.com
İşlem tarihi: 25.04.2022
Software Version : 3.5.1.0

echosens

Resim 1: Örnek Bir FibroMeter™ Virüs Analiz Sonucu

Tablo I: METAVİR Skorlama Sistemi A-B

METAVİR SKORLAMA SİSTEMİ A		
Tanımlama	Fibrozis Düzeyi	Fibrozis Ciddiyeti
Fibrozis yok	F0	
Portal bölgede genişleme, septa yok	F1	Hafif-orta fibrozis
Portal bölgede genişleme, seyrek septa oluşumu,	F2	Şiddetli fibrozis
Belirgin septa oluşumu, siroz yok	F3	Ciddi/ileri fibrozis
Siroz	F4	
METAVİR SKORLAMA SİSTEMİ B		
Kronik Hepatit Ciddiyeti	Piecemal Nekrozu (PMN) + Lobular Nekrozu (LN)= Histolojik Aktivite İndeksi	
0=Yok	PMN 0 + LN 1	A0
1=Hafif	PMN 0 + LN 1 PMN 1 + LN 0-1	A1
2=Orta	PMN 0-1 + LN 2 PMN 2 + LN 0 1	A2
3=Şiddetli	PMN 2-3 + LN 2 PMN 3 + LN 0-1-2	A3

İstatiksel Analiz

Verilerin analizi SPSS 26 (IBM SPSS, Türkiye) programı ile yapılmış ve %95 güven düzeyi ile çalışılmıştır. Kategorik (nitel) değişkenler için frekans (n) ve yüzde (%), istatistikleri, sayısal (nicel) ölçümler için ortalama ($\bar{x}$), medyan (ortanca değer), standart sapma (ss), minimum ve maksimum istatistikleri verilmiştir. Homojenite açısından gruplar değerlendirilmiş olup, homojenite sağlanamadığı durumda non parametrik testler kullanılmıştır. Çoklu grup karşılaştırmalarında ANOVA testi kullanılmıştır. P-değeri <0,05 olduğu durumda, istatiksel olarak anlamlı fark kabul edilmiştir.

Etik Kurul

Çalışmanın etik kurul onayı alınmıştır (18.04.2019, 114 nolu karar). Hastalardan gönüllü olur formu yazılı olarak alınmıştır.

BULGULAR

Çalışma periyodu boyunca merkezimize başvuran 106 hemodiyaliz hastası saptandı. HBsAg pozitif saptanan 6 hasta çalışma dışı bırakıldı. Çalışmaya dahil edilen 100 hastanın yaş ortalaması 49,9 ± 17,5 yıl (min:19 maks:86) olup, 57'si erkek, 43'ü ise kadındı. Katılımcıların ortalama hemodiyaliz süresi 5,3 ± 4,6 yıl olup, en az 1 yıl en çok 21 yıl olarak saptanmıştır. Böbrek yetmezliği dışında hastaların 33'ünde hipertansiyon, 25'inde diyabetes mellitus, 5'inde kardiyovasküler hastalık, ikisinde böbrek nakli ve 5'inde eşlik eden başka hastalık mevcuttu. Diğer demografik veriler Tablo 2'de ayrıntılı olarak belirtilmiştir.

Tablo II: Katılımcılara Ait Demografik Veriler(n=100)

Yaş (yıl)	**Değer n (%)**
Ortalama	49,9± 17,5 (min:19, max:86)
Cinsiyet	
Erkek Kadın	53 (53) 47 (47)
Hemodiyaliz Süresi (yıl)	
Ortalama	5,3±4,6 (min:1, max:21)
Altta Yatan Hastalıklar	
Hipertansiyon Diyabetes Mellitus Kardiyovasküler Hastalık Böbrek Nakli Diğer Hastalıklar	33 (33) 25 (25) 5 (5) 2 (2) 5 (5)

Çalışmaya dahil edilen hastalardan, 4 (%4) kişide HBV DNA pozitif saptandı ve OBİ olarak kabul edildi. OBİ saptanan 4 hastanın 3'ünde ALT, AST, trombosit, INR, PT-APTT değerleri normalken; bir hastada ALT, AST, INR yüksekliği mevcuttu. Bu hastanın bilinen prostat kanseri ve karaciğerde kist dışında bir özellik yoktu. Bir hastada anti HCV pozitif olarak saptanmıştır. Detaylı sorgulamasında bu hastanın daha önce HCV nedeniyle tedavi almış olduğu saptanmıştır. Hastaların hiçbirinde HCV RNA pozitifliği tespit edilmedi. OBİ saptanan hastaların ayrıntılı bilgileri Tablo 3'te yer almaktadır.

Tablo III: Okült Hepatit B Hastalarına Ait Ayrıntılı Bilgi

Hasta	Yaş	Cinsiyet	Eşlik Eden Hastalık	HBV DNA Düzeyi (IU/ml)	Fibrozis Skoru	Aktivite Skoru
1. Hasta	22	Erkek	Kronik Böbrek Yetmezliği	10	0,72 (F2)	0,19 (A1)
2. Hasta	72	Kadın	Kronik Böbrek Yetmezliği	9	0,40 (F1)	0,03 (A1)
3. Hasta	50	Erkek	Kronik Böbrek Yetmezliği, Hipertansiyon, Diyabetes Mellitus	17	0,32 (F1)	0,32 (A2)
4. Hasta	85	Erkek	Kronik Böbrek Yetmezliği, Hipertansiyon	16	0,44 (F2)	0,05 (A1)

F1: Portal bölgede genişleme, septa yok, F2: Portal bölgede genişleme, seyrek septa oluşumu,A1: Çok hafif aktivite, A2: Hafif aktivite

Çalışmaya dahil edilen hastalardan, OBİ olan ve olmayan olarak iki gruba ayrılıp karşılaştırma yapılmıştır. OBİ grubunda, ALT ve AST ortalaması daha yüksek olmasına rağmen, hastalardan sadece bir tanesinde ekstrem değer saptandığından ayrıntılı istatiksel analizde anlamlılık saptanmadı (p=0,413). OBİ grubunda, INR ve PT değerleri yüksek saptanmış olmasına rağmen, istatiksel olarak anlamlı fark bulunmamıştır. Yine fibrozis skoru, OBİ grubunda olmayan gruba göre istatiksel olarak anlamlı yüksek bulunmuştur. Diğer parametreler arasında istatiksel olarak anlamlı farklılık saptanmadı (p=0,044). Diğer verilere ayrıntılı olarak Tablo 4'te yer verilmiştir.

Tablo IV: Okült Hepatit B Saptanan ve Saptanmayan Hastaların Karşılaştırılması

	Okült Hepatit B Saptanmayan (*n*=96)	Okült Hepatit B Saptanan (*n*=4)	Değer Aralığı	P değeri
Yaş (yıl)	49.9 ±17.3	57.2 ± 27.5		0,424
Cinsiyet (erkek/kadın)	50/46	3/1		0,355
Hemodiyaliz Süresi	5.4±4.7	2,7 ±2,06		0,254
ALT (U/L)	11.5±5,3	65±112	Kadın: 0-35 Erkek: 0-50	0,413
AST (U/L)	15.2±6,3	31.7±30	Kadın: 0-35 Erkek: 0-50	0,352
Trombosit (103/mm3)	245.7±79.6	290±105.4	155-366	0,281
PT (sn)	12.4±2.2	16.2±8.7	10-14	0,454
APTT (sn)	29.8±11.04	30±7.1	21-28	0,973
INR	1,05±0,1	1.4±0,8	0.8-1.2	0,459
Fibrozis Skoru	1,2 ±0,49	1.7±0,5		**0,044**
Aktivite indeksi	1,07±0,7	1± 0,8		0,884
Siroz İndeksi	0.05±0.01	0.04±0.01		0,954

*ALT: Aspartat aminotransferaz; ALT: Alanin aminotransferaz, sn: Saniye *Veriler ortalama ve standart sapma olarak verilmiştir.*

TARTIŞMA

HBV infeksiyonu, riskli hasta popülasyonunda aşı ve önlemlere rağmen hala önemli bir problemdir. İmmunsüpresif durum, diyaliz makinelerin ortak kullanımı, aşıya yetersiz yanıt, kan transfüzyonları ve sık hemodiyaliz seanslarına bağlı olarak artmış girişimsel işlemlerden dolayı normal popülasyona göre hepatit B ile enfekte olma riski artmış durumdadır[10].

Okült HBV infeksiyonu, okült olmayan HBV infeksiyondaki gibi hemodiyaliz veya organ transplantasyonu ya da kesici-delici alet yaralanması ile bulaşabilmektedir. Hastaların tanısının daha geç dönemde konulması, semptomların daha silik ortaya çıkması, ileride kronik karaciğer hastalığı, siroz veya hepatosellüler kanser gelişmesi açısından önem arz etmektedir[11].

Literatürde yapılan çalışmalara göre bizim çalışmamızda OBİ prevalansı daha az olmakla birlikte bu farkın, çalışmaya dahil edilen hasta grubu ve örneklem sayısından kaynaklandığı düşünülmüştür. OBİ prevalansı ülkeden ülkeye, hatta merkezken merkeze bile değişebilmektedir. Nijerya'da Akinbami ve arkadaşlarının kan donörleri ile yaptığı bir çalışmada, çalışmaya toplam 101 katılımcı dahil edilmiştir. OBİ prevalansı %3 olarak saptanmış ve ortalama viral yük 160 IU/m olarak saptanmıştır[12].

Ülkemizde hemodiyaliz hastalarında yapılan ve 121 hastanın dahil edildiği bir çalışmada OBİ

prevalansı %8,2 (n=10) olarak saptanmıştır. OBİ saptanan 7 hastada HBV DNA düzeyi 6 IU/ml altında saptanırken geri kalan üç hastanın HBV DNA düzeyleri, 108, 157.000 ve 72.5 IU/ml olarak raporlanmıştır[13].

OBİ açısından yapılan bir meta-analiz çalışmasında, çalışmaya 305 makale dahil edilmiştir. Kan donörlerinde hepatit B açısından düşük endemisite olan bölgelerde OBİ prevalansı %0,06, orta endemisite olan bölgede %0,12 ve yüksek endemisite olan bölgede ise %0,98 olarak saptanmıştır[14].

Okült hepatit B vakalarında HBV DNA düzeyi, literatürde daha düşük olarak bildirilmiştir. Yeni kantitatif PCR yöntemleri de HBV düzeyini daha düşük saptayabildiği için; OBİ tanısında kolaylık sağlamaktadır. Literatürde OBİ hastalarıyla yapılan çalışmalarda, serum DNA seviyesi genellikle çok düşük olduğu (<200 IU/ml) belirtilmiştir[15].

Ülkemizde uygulanan hepatit B aşılama şemasına, 1998 yılının ağustos ayında başlanmış olup; yayınlanan genelge ile 0-1 yaş arası bebekler ücretsiz olarak aşılanmaya başlanmıştır. Bununla birlikte, hepatit B prevalansı düşmeye başlamıştır[16]. Buna rağmen aşının OBİ'yi önlemede daha az başarılı olduğunu destekleyen çalışmalar da mevcuttur[17].

Adar ve arkadaşlarının, 10 diyaliz merkezindeki 567 hemodiyaliz hastasında yaptıkları çalışmaya göre; 8 hastada izole anti-HBc IgG pozitifliği mevcut iken sadece bir (%0,2) hastada HBV DNA pozitifliği saptanmıştır[18]. OBİ prevalansı bu çalışmada olduğu gibi farklı merkezler arası değişkenlik göstermektedir.

Fibrometer, son yıllarda özellikle hepatit, non-alkolik yağlı karaciğer hastalığı (NAYKH) gibi karaciğeri etkileyen pek çok hastalıkta kullanılan, non-invaziv tanı ve takip yöntemlerden biri olmuştur. Duyarlılık ve sensitivitesi, hastalık, fibrozis düzeyi gibi bazı parametrelerden etkilenebilmektedir. Aykut ve arkadaşlarının, NAYKH açısından biyopsi ile tanı konulmuş 88 hastanın dahil edildiği çalışmada, fibrozisi göstermede duyarlılık, %38,6 iken; spesifitesi %86,4 olarak ölçülmüştür[19].

Dinçses ve arkadaşlarının NAYKH tanılı 52 hastada yaptığı çalışmada, F2 ve F3 fibrozisi göstermede fibrometer duyarlılığını %70, spesifitesini ise %93 olarak saptamışlardır. Bu çalışmada kullanılan transient elastografiye göre fibrometerin üstünlüğü vurgulanmıştır[20]. Literatürde OBİ ile fibrometer ait herhangi bir çalışma tespit edilemediği için karşılaştırma yapılamamıştır.

SONUÇ

OBİ, özellikle hemodiyaliz gibi riskli hastalarda göz ardı edilen önemli bir hastalıktır. Hastalara erken dönemde tanı konulması hem potansiyel bulaşların önüne geçebilmek hem de takip ve tedavi süreci açısından önem arz etmektedir. Bizim çalışmamızda OBİ sıklığı, yapılan benzer çalışmalara göre düşük çıksa da soruna dikkat çekmesi açısından önem teşkil etmektedir. Fibrometer ile hastaların irdelenmesi de literatürde herhangi bir çalışma olmadığından büyük önem teşkil etmektedir. Bu konuda yapılacak daha çok çalışma, bu konudaki bilgilerimize ışık tutacaktır.

Kısıtlılıklar

Çalışmamızda, periferik kan mononükleer hücrelerinde HCV RNA bakılamadığı için okült HCV değerlendirilememiştir. Aynı zamanda fibrometer dışı herhangi bir non-invaziv metot kullanılamadığı için bunlar arasında karşılaştırma yapılamamıştır.

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Özgün Araştırma / Original Article

Tip 1 Diyabetes Mellituslu Çocuklarda Çölyak Hastalığının Sıklığı

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Öz

Giriş: Tip 1 diyabetes mellituslu (T1DM) hastalarda otoimmün hastalık sıklığı artmıştır. Literatürde T1DM'li çocuk hastalarda çölyak hastalık (ÇH) prevalansının %1-16,6 arasında değiştiği raporlanmıştır. Bu çalışmada T1DM tanısı ile izlenen hastalarda, ÇH sıklığının belirlenmesi ve ÇH'nın büyüme ve metabolik kontrol üzerine olan etkisinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Çalışmaya 424 çocuk hasta alındı. Hastaların başvuru yaşı, cinsiyeti, ortalama HbA1C düzeyler, T1DM tanı esnasında ve son başvurudaki antropometrik ölçümleri hesaplandı. Anti-doku transglutaminaz IgA (anti-DTG IgA) düzeyi pozitif saptanan hastaların biyopsi sonuçları kaydedildi. Ayrıca anti-DTG IgA düzeyi kendiliğinden düzelen olgular da kaydedildi.

Bulgular: Olguların %52,4'i erkek, yaş ortalaması 13,99 ± 4,9 ve ortalama DM süresi 4,63 ± 3 yıl idi. Anti-DTG IgA düzeyi olguların %14,8'inde pozitif saptandı. Seroloji pozitifliği hastaların %68'inde T1DM tanı esnasında, %30'unda T1DM tanısından sonraki beş yıl içinde saptandı. Seroloji pozitifliği saptanan olguların %44'ü kendiliğinden düzeldi. Diyabetli olguların %4,1'ine biyopsi ile kanıtlanmış ÇH (BKÇH) tanısı konuldu. Çölyak negatif olan grup ile BKÇH olan grubun DM tanı esnasında ve son başvurudaki antropometrik ölçümleri arasında anlamlı fark saptanmadı BKÇH olan grubun tanıda ve son başvurudaki antropometrik ölçümleri arasında anlamlı fark yoktu.

Sonuç: T1DM'li hastalarda ÇH için antikor pozitiflik oranı %15,8 iken, %4,1'inde BKÇH tespit edilmiştir. Antikor pozitifliğinin %98'i DM tanısı esnasında ya da tanıdan sonraki beş yıl içinde saptanmıştır. Antikor pozitifliği saptanan olguların %44'ü yaklaşık iki yıl içinde glüten tüketimine rağmen kendiliğinden normale geldiği tespit edilmiştir. Bu nedenle her çölyak antikor pozitifliği saptanan hastalara acil barsak biyopsisi veya glutensiz diyet tedavisi verilmesi yerine serolojik takip yapılmasını önermekteyiz.

Anahtar kelimeler: Tip 1 DM, çölyak hastalığı, çocuklar

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Frequency of Celiac Disease in Children with Type 1 Diabetes Mellitus

Abstract

Objective: The frequency of autoimmune diseases is increased in patients with type 1 diabetes mellitus (TIDM). It has been reported that the prevalence of celiac disease (CD) in pediatric patients with TIDM varies between 1-16.6%. The aim of this study was to determine the prevalence of CD in patients with T1DM and to evaluate the effect of CD on growth and metabolic control.

Methods: 424 pediatric patients were included in the study. Age at admission, gender, mean HbA1C levels, anthropometric measurements at diagnosis, and last admission were calculated. Biopsy results of patients with positive anti-tissue transglutaminase IgA (anti-DTG IgA) levels were recorded. In addition, cases with spontaneous self-resolution of anti-DTG IgA levels were also recorded.

Results: 52.4% of the cases were male, the mean age was 13.99 ± 4.9 and the mean duration of DM was 4.63±3 years. Anti-DTG IgA level was detected positive in 14.8% of the cases. Serology positivity was detected in 68% of patients at the diagnosis of TIDM and in 10% of patients within five years after diagnosis of TIDM. 44% of the cases with serology positivity resolved spontaneously. Biopsy-proven CD (BPCD) was diagnosed in 4.1% of the cases with diabetes. There was no significant difference between the anthropometric measurements of the celiac-negative group and the group with BPCD at the time of DM diagnosis and the last admission. There was no significant difference between the anthropometric measurements of the group with BPCD at diagnosis and the last admission.

Conclusion: While the antibody positivity rate for CD was 15.8% in patients with TIDM, BPCD was detected in 4.1% of them. 98% of antibody positivity was detected at or within five years after DM diagnosis. It was found that 44% of antibody-positive cases spontaneously normalized within approximately two years despite gluten consumption. For this reason, we recommend serological follow-up instead of emergency intestinal biopsy or gluten-free diet therapy for patients with celiac antibody positivity.

Keywords: Type 1 DM, celiac disease, children.

GİRİŞ

Tip1 diabetes mellitus (T1DM) genellikle pankreas beta hücrelerinin otoimmün dekstrüksiyonu sonucu ortaya çıkan ve insülin eksikliği ile karakterize otoimmün bir hastalıktır[1]. Tip 1 DM'li çocuklarda otoimmün hastalık riski normal populasyondaki çocuklara göre daha yüksektir. Yapılan çalışmalarda T1DM'ye en sık eşlik eden otoimmün hastalığın otoimmün tiroid hastalığı olduğu ve bunu çölyak hastalığının takip ettiği rapor edilmiştir[2,3]. Genel popülasyonda ÇH prevalansı %0,3 ile %1 arasında olduğu tahmin edilirken[4], Tip 1 DM'li çocuk ve ergen hastalarda bu oranın %1 ile %16,6 arasında değiştiği rapor edilmiştir[5-8]. Tip 1 DM'li çocuklarda ÇH prevelansının yüksek olması ve olgularının çoğunun asemptomatik olması nedeniyle 'Uluslararası Çocuk ve Ergen Diyabet Derneği' (ISPAD) T1DM tanısı esnasında, serum Ig A ile birlikte anti-doku transglutaminaz IgA (anti-DTG IgA) düzeyi bakılarak ÇH için tarama yapılmasını önermektedir. Asemptomatik olgularda ise iki yıl içinde ve beş yıl sonra tekrar tarama yapılmasını, eğer semptom varsa veya birinci derece akrabalarında ÇH varsa daha sık aralıklarla tarama yapılmasını önermektedir[2]. Tip 1 DM'li hastalarda asemptomatik ÇH'si olan olguların saptanması daha iyi bir metabolik kontrol ve çölyak ile ilişkili uzun dönem komplikasyonların önlenmesine olanak sağlayacaktır.

Bu çalışmadaki amacımız hastanemizde takip edilen T1DM'li hastalarımızda biyopsiyle kanıtlanmış ÇH sıklığını belirlemek ve ÇH'nın T1DM'li olgularda büyüme ve metabolik kontrol üzerine olan etkisini değerlendirmektir.

YÖNTEMLER

Çalışmaya hastanemiz çocuk endokrinolojisi kliniğinde 01.01.2013–20.09.2021 tarihleri arasında T1DM ile takip edilen, yaşları 1-18 yaş arasında değişen, toplam 424 çocuk ve adölesan olgu alındı. Çocuk ve adölesanlarda; açlık plazma glukoz düzeyinin ≥126 mg/dl olması veya diyabetin semptomları ile birlikte rastgele bakılan plazma glukoz düzeyinin ≥200 mg/dl olması veya oral glukoz tolerans testinde 120. dakikadaki glukoz düzeyinin ≥200 mg/dl olması veya HbA1C düzeyinin ≥%6,5 olması halinde diyabet tanısı konulmaktadır[1]. Çalışmaya alınan hastaların dosyaları retrospektif olarak incelendi. Hastaların başvuru yaşı, cinsiyeti, ortalama HbA1C değerleri kaydedildi. Hastaların hem T1DM tanı esnasında hem de en son başvurudaki vücut ağırlığı (VA), VA standart deviasyon skoru (SDS), boy, boy SDS'si, beden kütle indeksi (BKİ) ve BKİ SDS skorları hesaplandı. Laboratuvarımızda kullanılan yönteme göre; anti-TG IgA düzeyi < 12 IU/mL olanlar negatif çölyak serolojisi, 12-18 IU/mL olanlar borderline çölyak serolojisi, >18 IU/mL olanlar ise pozitif çölyak serolojisi olarak kabul edildi[9]. Anti-DTG IgA düzeyi Euroimmun mikroelisa cihazında ELİSA yöntemi ile çalışıldı. Çölyak serolojisi pozitif saptanan tüm hastalarda Anti-TTG IgA'nın pozitif saptanma zamanı kaydedildi. Anti-DTG IgA düzeyi pozitif olan olgulara bağırsak biyopsisi yapılıp yapılmadığı ve yapılmış ise histopatolojik özellikleri kayıt altına alındı. Biyopsi sonuçlarına göre Marsh skoru 2 ve 3 olanlar biyopsi ile kanıtlanmış çölyak hastalığı (BKÇH), 0 ve 1 olanlar potansiyel ÇH olarak değerlendirildi[10]. Anti-DTG IgA düzeyi başlangıçta pozitif, ancak zaman içinde negatifleşen ve en az altı aydır negatif kalmaya devam eden olgular çölyak serolojisinin kendiliğinden normalizasyonu olarak değerlendirildi.

Çalışmada BKÇH'si olanlar grup 1, çölyak serolojisi negatif saptanan veya pozitif iken kendiliğinden normale dönen hastalar grup 2 olarak sınıflandırıldı. Grup 1 ile grup 2'de yer alan hastaların antropometrik ve diğer özellikleri karşılaştırıldı. Ayrıca grup 1'deki olguların ÇH tanısı esnasında ve son başvurudaki antropometrik ölçümleri karşılaştırıldı.

Çalışmaya alınan hastaların boy ölçümleri Seca markalı duvara monte 1 mm'ye kadar duyarlı boy ölçer ile, VA ise Seca markalı 100 grama kadar duyarlı dijital tartı ile ölçüldü. Ölçülen VA, boy ve BKİ SDS değerleri Türk çocukları için Çocuk Endokrinoloji ve Diyabet Derneği tarafından oluşturulan uygulama (CHILD METRICS) kullanılarak hesaplandı.

Çalışma için Dicle Üniversitesi Hastanesi "Klinik Araştırmalar Etik Kurulu'ndan" 13.10.2021 tarih ve 437 sayılı karar ile etik onay alındı. Çalışmaya etik karar onayı alındıktan sonra başlandı.

İstatistiksel Analiz

Hasta verilerinin değerlendirmesinde IBM-SPSS 20.0 istatistik programı kullanıldı. Öncelikle verilerin normal dağılıp dağılmadığını tespit etmek üzere Shapiro wilk testi kullanıldı. Normal dağılım gösteren veriler ortalama ± standart sapma (SS) ile normal dağılım göstermeyen veriler ise ortanca (25-75 persantil) şeklinde gösterildi. Kategorik değişkenler sayı ve yüzde (%) ile belirtildi. Sayısal karşılaştırmalar için, ölçülen parametrelerin normal dağılımına göre bağımsız örnek t-testi veya Mann-Whitney U-testleri kullanıldı.

Diyabet tanı yaşının beş yaş altı veya beş yaş üstünde olması ile ÇH arasında ilişki olup olmadığına ki-kare testi ile bakıldı. Biyopsi ile kanıtlanmış ÇH olan olguların tanı esnasında ve son vizitteki ağırlık, boy ve BMI SDS değerleri bağımlı örneklem T test (*paired sample t-test*) ile karşılaştırıldı. Tüm istatistiksel testlerde p <0,05 değerleri anlamlı olarak ele alındı.

BULGULAR

Çalışmaya T1DM ile izlenen 222'i (%52,4) erkek, 202'i (%47,6) kız olmak üzere toplam 424 olgunun dosyası dahil edildi. Hastaların çalışma anındaki mevcut yaş ortalaması 13,99 ± 4,9 yıl (en az-en çok: 1-17,8) idi. Hastaların ortalama T1DM tanı alma yaşı 9,33 ± 4,39 yıl iken, ortalama DM süresi ise 4,63 ± 3 yıl olarak hesaplandı (Tablo 1).

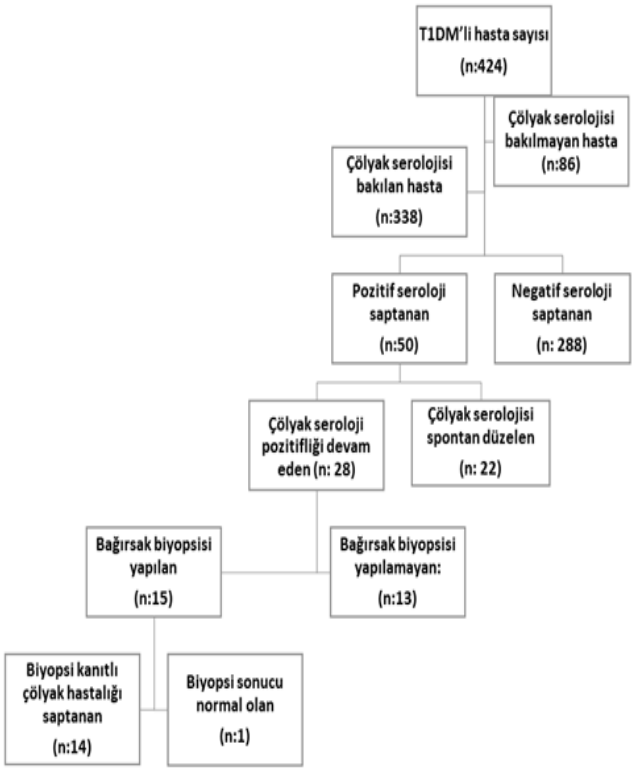
Çalışmaya alınan hastaların DM tanı anındaki ortalama boy SDS'i -0,39 ± 1,10, VA SDS'si -0,32 ±1,15 ve BKİ SDS'si -0,17 ± 1,11 iken, son başvurudaki boy, VA ve BKİ SDS'leri sırasıyla -0,41 ± 1,18, -0,33 ± 1,27 ve -0,18 ± 1,17 olarak saptandı. Olguların ortalama HbA1c düzeyleri 9,40 ± 2,42 idi (Tablo 1).

Tablo I: Hastaların genel özellikleri ve antropometrik ölçümleri

Mevcut yaşı (yıl)	13,99 ± 4,9 (2-24,42)
DM tanı yaşı (yıl)	9,33 ± 4,39(1-17,83)
DM süresi (yıl)	4,63 ± 3(0,5-16,5)
Tanıdaki boy SDS	-0,39 ± 1,10 (-3,5-(2,16))
Tanıdaki VA SDS	-0,32 ±1,15 (-3,31-(2,58))
Tanıdaki BKİ SDS	-0,17 ± 1,11 (-3,19-(3,02))
Son başvurudaki boy SDS	-0,41 ± 1,18 (-4,29-(2,11)
Son başvurudaki VA SDS	-0,33 ± 1,27 (-4,36-(2,88))
Son başvurudaki BKİ SDS	-0,18 ± 1,17 (-3,52-(2,75))
Ortalama HBA1C (%)	9,40 ± 2,42 (4,4-18,4)

DM:Diyabetes mellitus,VA:Vücut ağırlığı,SDS:Standart deviasyon skoru,BKİ:Beden kitle indeksi, HbA1C: glikozillenmiş hemoglobin

Çalışmaya dahil edilen 424 hastanın 86'sında (%20,3) ÇH için tarama yapılmamış iken, 338 hastanın 288'inde (%85,2) ÇH taraması negatif, 50'sinde (%14,8) ise anti-DTG IgA pozitif saptandı. Çölyak hastalığı için antikor pozitifliği saptanan 50 hastanın 22'sinde (%44) ortalama 22,7 (4-65) ayda spontan düzelme olduğu tespit edildi. Antikor pozitifliği devam eden 28 hastanın 15'ine (%53,6) ince barsak biyopsisi yapıldı. Biyopsi yapılan 15 olgunun birinde ÇH saptanmazken, 14'ünde ÇH ile uyumlu bulundu. Toplamda antikor bakılan 338 hastanın 14'ünde (%4,1) BKÇH tanısı konuldu (Şekil 1). BKÇH olan altı olgu (%43) March 3a, beş olgu (%36) March 3b ve üç olgu (%21) March 3c ile uyumlu idi. Antikor pozitifliği sürdüğü tespit edilen 28 hastadan 13 hastaya (%46,4) biyopsi yapılmadığı fark edildi.



Şekil 1. Tip 1 Diyabetli hastalarda çölyak hastalığı saptanma durumu

Biyopsi ile kanıtlı ÇH tanısı konulan 14 hastanın sekizi (%57,1) erkek, altısı (%42,9) kız idi. Çalışmadaki olguların tanı yaşlarına bakıldığında 424 olgudan 82'sinin (%19,3) beş yaş altı, 342'sinin (%80,7) ise beş yaş üzeri olduğu görüldü. Hem cinsiyet açısından hem de DM tanı yaşının beş yaş altı ve üstünde olması ile ÇH arasındaki ilişki Ki-kare testi ile değerlendirildi ve aradaki farkın anlamlı olmadığı görüldü (sırasıyla p değeri 0,44 ve 0,68) (Tablo 2).

Tablo II: Çölyak hastalığının Hashimoto tiroiditi, cinsiyet ve DM tanı yaşı ile olan ilişkisi

	Çölyak hastalığı (-) (n:311)	BKÇH (n:14)	P değeri
Erkek/Kız	160/151	8/6	0,44[a]
DM tanı yaşı 5 yaş altı / 5 yaş üstü	58/253	2/12	0,681[a]

[a]Ki-Kare testi, BKÇH: Biyopsi ile kanıtlanmış çölyak hastalığı, DM: Diyabetes mellitus

Çölyak hastalığı için antikor pozitifliği saptanan 50 olgunun 34'ünde (%68) antikor pozitiflik zamanının T1DM tanı esnasında olduğu tespit edildi. Geriye kalan 15 olguda (%30) tanıdan sonraki ilk beş yıl içinde, bir olguda ise (%2) diyabet tanısından 9,3 yıl sonra antikor pozitifliği tespit edildi.

Biyopsi kanıtlı çölyak hastalığı olan grubun ortanca T1DM tanı yaşı ve T1DM süresi sırasıyla 8,66 ve 4,5 yıl iken, çölyak negatif olan grubun ortanca T1DM tanı yaşı 8,83 yıl ve T1DM süresi 5,6 yıl olarak hesaplandı. Aradaki fark istatistiksel olarak anlamlı saptanmadı (sırasıyla p:0,475, p:0,516) (Tablo 3). Biyopsi kanıtlı çölyak hastalığı olan grup ile çölyak negatif olan grubun hem tanı anındaki hem de son başvurudaki ortanca boy SDS, VA SDS, BKİ SDS değerleri arasında anlamlı fark saptanmadı (p<0,05). Her iki grubun ortalama HbA1c düzeyleri arasında da fark saptanmadı (Tablo 3).

Biyopsi kanıtlı çölyak hastalığı olan hastaların tanı esnasındaki ortalama boy, VA ve BKİ SDS değerleri ile son başvurudaki değerleri arasında istatistiksel olarak anlamlı fark saptanmadı (p<0,05) (Tablo 4).

Tablo III: Çölyak negatif olan hasta grubu ile BKÇH olan hasta grubunun özelliklerinin karşılaştırılması

	Çölyak (-)	BKÇH	P değeri
Mevcut yaşı (yıl)	14,62(11,47-17,77)	12,50(10,33-16,91)	0,374
DM tanı yaşı (yıl)	8,83(6,22-12,08)	8,66(5,58-11,50)	0,475
DM süresi (yıl)	5,62(3,64-7,00)	4,50(3,33-6,08)	0,516
Tanıdaki boy SDS	-0,29(-1,05-0,36)	-0,15(-1,23-0,75)	0,827
Tanıdaki VA SDS	-0,41(-1,08-0,40)	-0,87(-1,80-0,72)	0,220
Tanıdaki BKİ SDS	-0,30(-0,92-0,61)	-0,49(-1,64-0,10)	0,165
Son başvurudaki boy SDS	-0,26(-1,12-0,40)	-0,30(-1,78-0,66)	0,946
Son başvurudaki VA SDS	-0,20(-1,06-0,50)	-0,77(-1,21-0,57)	0,490
Son başvurudaki BKİ SDS	-0,17(-0,92-0,58)	-0,54(-1,17-0,41)	0,368
Ortalama HbA1C (%)	9,62(8,32-10,78)	9,25(8,96-11,45)	0,694

BKÇH:Biyopsi ile kanıtlanmış çölyak hastalığı, DM:Diyabetes mellitus,VA:Vücut ağırlığı, SDS:Standart deviasyon skoru, BKİ:Beden kitle indeksi, HbA1C: glikozillenmiş hemoglobin

Tablo IV: Biyopsi kanıtlı çölyak hastalığı olan hastaların tanıda ve son başvurudaki antropometrik ölçümlerin karşılaştırılması

	Tanı Esnasında	Son Başvuruda	P değeri
Boy SDS	-0,40 ± 1,54	-0,55 ± 1,57	0,522
VA SDS	-0,70 ± 1,53	-0,67 ± 1,32	0,926
BKİ SDS	-0,63 ± 1,17	-0,45 ± 0,89	0,552

VA:Vücut ağırlığı, SDS:Standart deviasyon skoru, BKİ:Beden kitle indeksi

TARTIŞMA

Çalışmamızda T1DM'li hastalardan ÇH için tarama yapılan olguların %14,8'inde antikor pozitifliği saptandı. Olguların %68'inde antikor pozitifliğinin T1DM tanı esnasında gerçekleştiği görüldü. Serolojik pozitiflik saptanan olguların %44'ünün kendiliğinden normale döndüğü

gösterildi. Çalışmamızda ayrıca ÇH için serolojik pozitiflik devam eden olguların ancak %53,6'sına ince bağırsak biyopsisinin yapıldığı ve biyopsi yapılan olgular dikkate alındığında BKÇH oranının %4,1 olduğu tespit edildi.

Önceki çalışmalarda T1DM'li çocuk ve ergen hastalarda ÇH prevalansının %1 ile %16,6 arasında değiştiği bildirilmiştir[5-8]. 2017 yılında Craig ve ark. tarafından yapılan ve 52.721 çocuk ve adölesan hastanın alındığı çalışmada ÇH prevelansı %3,5 olarak tespit edilmiştir. Aynı çalışmada ÇH sıklığının ABD'de %1,9, Avustralya'da ise %7,7 olduğu rapor edilmiştir[5]. Ülkemizde T1DM'li çocuklarda yapılan çalışmalarda çölyak sıklığının %3,5-7,8 arasında değiştiği bildirilmiştir[11-14]. Şimşek ve ark.[13] tarafından 1032 T1DM'li olgunun alındığı multisentrik çalışmada ÇH için serolojik pozitiflik oranı %16,7 iken, BKÇH sıklığı %6,6 olarak saptanmıştır. Yakın zamanda ülkemizde 779 olgunun alındığı bir çalışmada olguların %15,4'ünde antikor pozitifliği saptanırken, %6,9'una BKÇH tanısı konulmuştur[14]. Bizim çalışmamızda da olguların %14,8'inde antikor pozitifliği saptanırken, %4,1'inde BKÇH teşhisi konulmuştur. Çölyak hastalığı için çalışmamızdaki antikor pozitiflik oranı ile BKÇH sıklığı hem ulusal hem de uluslararası çalışmalarla benzerlik göstermekteydi[5,9,11-14].

Son zamanlarda yapılan bazı çalışmalarda T1DM'li hastalarda saptanan çölyak seroloji pozitifliğinin %20-35 oranında glütensiz diyet verilmeksizin spontan olarak normale geldiği rapor edilmiştir[15-17]. Bu olgularda hangi mekanizma ile otoantikorların spontan olarak normale döndüğü net değildir. Bahsedilen çalışmalarda antikor pozitifliğinin spontan düzelme süresi T1DM tanısından sonraki ilk 1-2 yıl içinde gerçekleştiği rapor edilmiştir[16,17]. Yakın zamanda ülkemizde de yapılan ve 779 hastanın alındığı çalışmada olguların %23,3'ünde glutenli diyete rağmen serolojik olarak normale geldiği bildirilmiştir[14]. Çalışmamızda ÇH için antikor pozitifliği saptanan olguların %44'ünde ortalama 22,7 (4-65) ayda spontan olarak düzeldiği görülmüştür. Çalışmamızda bu oranın önceki çalışmalardan biraz daha yüksek olduğu gösterildi[9,15-17]. Çalışmamız T1DM'li olgularda geçici çölyak seroloji pozitifliğinin olabileceğini göstermektedir. Böylelikle bu olgularda hemen biyopsi yapılmasının doğru olmadığı, bunun yerine hastaların takip edilmesinin daha uygun olduğu kanısındayız.

Çölyak hastalık prevelansının normal popülasyonda kadınlarda daha yüksek olduğu bildirilmiştir[5]. Ancak T1DM'li hastalarda yapılan çalışmaların bir kısmında ÇH'nın kızlarda daha çok görüldüğü[5], bazı çalışmalarda ise erkeklerde daha çok görüldüğü bildirilmiştir[18,19]. Yakın zamanda 52.721 T1DM'li hastada yapılan, oldukça büyük örneklemli çalışmada ÇH tespit edilen hastaların %59'unun kız cinsiyette olduğu ve aradaki farkın anlamlı olduğu bildirilmiştir[5]. Çalışmamızda ÇH'nin daha çok erkeklerde görüldüğü, ancak aradaki farkın istatistiksel olarak anlamlı olmadığı gösterildi.

Literatürde önceki bazı çalışmalarda T1DM tanı yaşının küçük ve özellikle de beş yaşın altında olmasının ÇH geliştirme riskini artırdığı gösterilmiştir[5,6,20,21]. Bunun aksine T1DM tanı yaşı ile ÇH arasında ilişki olmadığını gösteren çalışmalar da mevcuttur[8,19]. Ülkemizde yapılan bir çalışmada da T1DM tanı yaşı ile ÇH arasında ilişki olmadığı rapor bildirilmiştir[9]. Bizim çalışmamızda T1DM tanı yaşı <5 olanlar ile > 5 olanlar arasında ÇH gelişimi açısından anlamlı fark saptamadık.

Tip 1 DM'li hastalarda yapılan çalışmalarda ÇH tanısı, vakaların yaklaşık yarısı T1DM tanı esnasında[15,22], geri kalan olguların çoğunun da tanıdan sonraki ilk beş yıl içinde tespit edilmiştir[20]. Dokuz kohort çalışmasını içeren bir derlemede olguların %79'unun T1DM tanısından sonraki 5 yıl içinde tanı aldığı rapor edilmiştir. Bu nedenle T1DM tanı esnasında ve sonraki beş yıl içinde ÇH taramasının önemine

vurgu yapılmıştır[7]. Ülkemizde yapılan bir çalışmada olguların %76,1'inin DM tanı esnasında ÇH tanısı aldığı raporlanmıştır[9]. Bizim çalışmamızda da olguların %68'inde T1DM tanı esnasında çölyak antikor pozitifliği saptanarak önceki çalışmalarla benzerlik göstermekteydi. Yukarıdaki tüm çalışmalar göz önünde bulundurularak T1DM tanısından sonraki ilk beş yıl içinde ÇH için taramanın ne kadar önemli olduğu rahatlıkla söylenebilir.

Tip 1 DM'li çocuklarda ÇH olan grup ile ÇH olmayan grubun antropometrik ölçümlerin karşılaştırıldığı çalışmalarda farklı sonuçlar elde edilmiştir. Bu olgularda antropometrik ölçümler açısından anlamlı fark olmadığını gösteren çalışmalar olduğu gibi[23,24], tanı esnasında boy SDS skorunun önemli ölçüde azaldığını gösteren ve glütensiz diyet ile bu durumun düzeldiğini gösteren çalışma da mevcuttur[25]. Yakın zamanda ülkemizde yapılan bir çalışmada hem tanı hem de son başvurudaki antropometrik ölçümler açısından anlamlı fark olmadığı gösterilmiştir[9]. Çalışmamızda da her iki grup arasında hem tanı esnasında hem de son başvuruda antropometrik bulgular açısından anlamlı fark saptanmadı ve bu durum önceki çalışmalarla uyumlu idi.

Önceki çalışmalarda T1DM'li olgularda ÇH varlığının metabolik kontrolü nasıl etkilediği ile ilgili çelişkili veriler mevcuttur. Bazı çalışmalarda T1DM ile birlikte ÇH varlığının glisemik kontrolleri etkilemediği[26,27], bazı çalışmalarda ise HbA1c'nin daha düşük olduğu gösterilmiştir[24]. Bizim çalışmamızda da ÇH olanlar ile olmayanlar arasında metabolik kontrol açıcından fark saptanmadı. Çalışmamızda olduğu gibi birçok çalışmada da metabolik kontrol farklı olmasa dahi ÇH'nın glisemik değişkenliğe neden olabileceği ve malabsorpsiyon nedeniyle hipoglisemi ve buna bağlı HbA1c'nin düşük seyredebileceği unutulmamalıdır.

Sonuç olarak; T1DM'li hastalarda ÇH için antikor pozitiflik oranı %15,8 tespit edilir iken, %4,1'inde BKÇH tespit edilmiştir. Antikor pozitifliğinin %98'i ya DM tanısı esnasında ya da tanıdan sonraki beş yıl içinde saptanmıştır. Antikor pozitifliği saptanan olguların %44'ü yaklaşık 2 yıl içinde glütensiz diyete rağmen kendiliğinden normale geldiği tespit edilmiştir. Bu nedenle her çölyak antikor pozitifliği saptanan hastalara acil barsak biyopsisi veya glutensiz diyet tedavisi verilmesi yerine serolojik takip yapılmasını önermekteyiz.

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Özgün Araştırma / Original Article

Mitokondri Fisyon-Füzyon Dengesinde Rol Oynayan Genlerin Nörotoksik Ortamda ifade Düzeyleri Değişimi

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Öz

Amaç: Alzheimer tipi demans, dünya çapında rastlanan demansın en yaygın görülen şekli olup, son çalışmalar, kronik hiperglisemi ve insülin direnci ile karakterize olan tip 2 diyabeti (T2D), Alzheimer hastalığı ve diğer bilişsel bozukluklar için bir risk faktörü olarak tanımlamaya başlamıştır. Alzheimer hastalığının, tip 3 diyabet (T3D) olarak önerildiği tabloda, bozulmuş insülin sinyalizasyonu, kronik hiperglisemi kaynaklı nöronal hasar, oksidatif stres, nöroinflamasyon gibi metabolik bozukluklar yer alır. Bu durum, nöronal insülin direncine yol açarak antioksidan kapasitede azalma, oksidatif hasar, mitokondriyal bozulmaya katkıda bulunarak sinirsel dejenerasyon ve bilişsel gerilemeye yol açar. Bu çalışmada, yüksek glikoz uygulayarak nörotoksisite geliştirdiğimiz nöroblastoma hücre hattında, mitokondri dinamiğinde rol oynayan DRP1, OPA1, MFN1, MFN2, FIS1 genlerinin ifade düzeylerini belirlemeyi hedefledik.

Yöntemler: İnsan nöroblastoma hücrelerine, 24 saat süre ile, 100 mM glikoz uygulayarak, nörotoksik ortam geliştirdik ve DRP1, OPA1, MFN1, MFN2, FIS1 ekspresyon seviyelerini qPCR tekniği ile belirledik.

Bulgular: Yüksek glikoz uyguladığımız grupta, kontrol grubuna oranla FIS1, DRP1 (sırasıyla 2,45-kat ve 4,61-kat) seviyelerinde artış ($p<0,05$), MFN1, MFN2, OPA1 (sırasıyla 0,69-kat, 0,58-kat ve 0,62-kat) seviyelerinde azalma ($p>0,05$) gözlemledik.

Sonuç: T2D belirtileri ile mitokondrial fragmentasyon artışı arasında korelasyon olduğu bilinmekte olup, in-vitro nörotoksik ortamda, mitokondri dinamiğinde rol oynayan moleküllerin seviyesi, artan fragmentasyonu destekler niteliktedir. T3D mekanizmasında, nörodejenerasyona katkıda bulunan mitokondriyal bozulmada yer alan moleküllerin, transkripsiyonel düzeyde değişikliklerinin aydınlatılması noktasında literatüre katkıda bulunan çalışmamız, hastalığın erken teşhisi, seyrinin yavaşlatılması ve tedavi edilmesi yönünde ilerleme kaydedilmesini sağlayacaktır.

Anahtar kelimeler: Alzheimer hastalığı, Tip 2 diyabet Mellitus, Mitokondriyal fisyon ve füzyon.

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The Expression Levels of Genes Involved in Mitochondrial Fission-Fusion Balance in A Neurotoxic Environment

Abstract

Purpose: Alzheimer's disease, the most common form of dementia worldwide, has been increasingly linked to type 2 diabetes (T2D), characterized by chronic hyperglycemia and insulin resistance, in recent studies, identifying it as a risk factor for Alzheimer's disease and other cognitive impairments. In the proposed concept of Alzheimer's disease as type 3 diabetes (T3D), metabolic disorders such as impaired insulin signaling, neuronal damage due to chronic hyperglycemia, oxidative stress, and neuroinflammation, are implicated. This condition leads to neuronal insulin resistance, contributing to decreased antioxidant capacity, oxidative damage, mitochondrial dysfunction, neuronal degeneration, and cognitive decline. In this study, we aimed to determine the expression levels of genes involved in mitochondrial dynamics, including DRP1, OPA1, MFN1, MFN2, and FIS1, in the neuroblastoma cell line subjected to high glucose-induced neurotoxicity.

Method: We induced a neurotoxic environment in human neuroblastoma cells by applying 100 mM glucose for 24 hours and determined the expression levels of DRP1, OPA1, MFN1, MFN2, and FIS1 using the qPCR technique.

Results: In the group exposed to high glucose, we observed an increase in the levels of FIS1 and DRP1 (2,45-fold and 4,61-fold, respectively) compared to the control group ($p<0,05$), while there was a decrease in the levels of MFN1, MFN2, and OPA1 (0,69-fold, 0,58-fold, and 0,62-fold, respectively), although statistically insignificant ($p>0,05$).

Conclusion: There is a known correlation between mitochondrial fragmentation and symptoms of T2D, and our findings support increased fragmentation in an in vitro neurotoxic environment by showing alterations in the levels of molecules involved in mitochondrial dynamics. Our study contributes to the literature by shedding light on transcriptional changes of molecules involved in mitochondrial dysfunction, contributing to neurodegeneration in the mechanism of T3D. This will aid in early diagnosis, slowing the progression, and treating the disease.

Keywords: Alzheimer Disease's, Type 2 Diabetes Mellitus, Mitochondrial Fission and Fusion.

GİRİŞ

T2D, multiple organ bozulması ve çoklu kronik komplikasyonla ilişkili sistemik bir hastalıktır, bunlar arasında beyin ve sinir sistemi de bulunmaktadır. Diyabet ilişkili merkezi sinir sistemi komplikasyonları ve diyabeti olan kişilerin sık sık bellek ve dikkatlerinin kötüleştiği, araştırmacılar ve klinik uzmanlar tarafından 100 yıldan fazla bir süredir bilinmektedir[1]. Literatür, T2DM'nin bilişsel fonksiyonun çeşitli alanlarında azalmış performansla ve beyinde yapısal anormalliklerle güçlü bir şekilde ilişkili olduğuna işaret etmektedir. Bu durumda bilişsel gerileme, nöropsikolojik testlerle ölçülen hafif bilişsel bozukluk (MCI), Alzheimer hastalığı (AH), vasküler demans (VD) ve diyabetik olmayan kontrol gruplarıyla karşılaştırıldığında başka demans türlerine yol açabilir. T2D, demans riskinde %50 artışla ilişkilidir[2].

Şimdiye kadar yapılan epidemiyolojik çalışmalar, metabolik sendrom ve T2D'in, vasküler demans ve Alzheimer hastalığı görülme riskini önemli ölçüde arttırdığını göstermektedir[3]. Beyin fonksiyonu, glikozun oksidatif metabolizmasına bağlıdır. Diyabette sıklıkla gelişen hiperglisemi atakları, hem merkezi hem de periferik sinir sistemlerinde glukoz metabolizması ile ilişkili fonksiyonel ve yapısal bozukluklara neden olur ve bu da nöron hasarına yol açar[4]. Kronik hiperglisemi kaynaklı nöronal hasar, çoğunlukla oksidatif stres, ileri glikasyon son ürünlerinin oluşumu, nöroinflamasyon, nörotrofik faktörlerin eksikliği gibi birçok faktör nedeniyle meydana gelir[5].

Mitokondriler sürekli olarak fisyon ve füzyon adlı iki zıt süreç sayesinde şekil ve sayı değişiklikleri geçirir. Füzyon-fisyon dengesinin ince ayarlanması, hücresel uyum açısından hücreye dış uyarılara ve çevresel streslere yanıt olarak önemlidir[6]. Bu nedenle, fisyon-füzyon dengesinin değişiklikleri oksidatif stres, mitokondriyal disfonksiyon ve metabolik değişikliklere yol açar. Mitokondriyal morfolojideki değişiklikleri düzenleyen başlıca proteinler, fission protein 1 (Fis1), dynamin-related protein 1 (Drp1), mitofusin (Mfn) ve optic atrophy 1 (Opa1) gibi dinamik moleküllerdir. Drp1 mitokondriyal fisyonu düzenlerken, Mfn1, Mfn2 ve Opa1 sırasıyla dış ve iç mitokondriyal membranların füzyonunu düzenler[7,8]. Mitokondri dinamiğinde bozulma, Alzheimer ve Parkinson gibi yaygın görülen nörodejeneratif hastalıkların yanı sıra kardiyovasküler hastalıklar, T2D ve kanserde moleküler ve hücresel patogenezde daha genel bir rol oynuyormuş gibi görünmektedir[6,8].T2D'in klinik komplikasyonları arasında yer alan dislipidemi, hiperglisemi, insülin direnci gibi komplikasyonların önemli bir nedeni, hiperglisemi tarafından artan mitokondriyal reaktif oksijen türleri (ROS) üretimidir. T2D'de mitokondriyal morfolojinin ortak bir özelliği, artan bir fragmentasyondur[8]. Hipotezimiz, kronik hipergliseminin tetiklediği oksidatif stres ve nörotoksisite durumunda, mitokondriyal morfolojideki değişiklikleri düzenleyen genlerin, diyabetin tetiklediği, Alzheimer' ın neden olduğu komplikasyonların oluşumu ve hastalığın ilerlemesinin kontrol altına alınması noktasında moleküler terapötik hedef olabileceği üzerine kurulmuştur. Patolojik ve moleküler süreçleri ile ilgili çok fazla çalışma yapılmasına rağmen, T2D ve AH' nin birlikte ilerlediği, birinin diğerini tetiklediği, multiple faktörlü durum, çok bilinmeyenli bir denklemdir. Literatürde, T3D' e mitokondriyal disfonksiyonun katkısına dair yeterli çalışma bulunmamaktadır. Yaptığımız bu çalışmada, yüksek glikoz uygulayarak hiperglisemi modeli geliştirdiğimiz nörotoksik çevrede nöroblastoma hücre hattında (SH-SY5Y), mitokondri dinamiğinde rol oynayan DRP1, OPA1, MFN1, MFN2 ve FIS1 genlerinin ifade düzeylerini belirlemeyi amaçladık.

YÖNTEMLER

Hücre Kültürü

Çalışmamızda SH-SY5Y (insan nöroblastoma) hücre hattı kullanılmıştır. SH-SY5Y hücre hattı, Prof. Dr. Gizem Dönmez Yalçın' dan (Adnan Menderes Universitesi) temin edilmiştir ve hücrelerin pasaj sayısı 100' den azdır. %10 fetal bovine serum içeren Dulbecco'nun modifiye edilmiş Eagle ortamı ve Ham's F12 ortamında kültüre alındı. Normal koşullar altında (17,5 mmol/L glukoz içeren ortam, %10 fetal bovine serum, 100 U/mL penisilin ve 100µg/ml streptomisin, %5 CO2, 37°C) 24 saat inkübasyon ve 12 saat hücre döngüsü senkronizasyonu sonrası, çalışma gruplarımız, normal glukoz (NG; 17,5 mmol/L glukoz) ve yüksek glikoz (HG;100 mmol/L glukoz) olarak belirlendi. Fetal bovine serumu Gibco (Thermo Fisher Scientific, USA), Dulbecco's Modified Eagle's Medium (DMEM, 12-741F), Dulbecco's Phosphate Buffered Saline (DPBS, 17-513), Penisilin-Streptomisin karışımı (17602) ve Tripsin-EDTA çözeltisi (17-161F) Lonza (Lonza Group, Switzerland) firmalarından temin edildi. İn vitro hiperglisemik model oluşturmak için, SH-SY5Y hücreleri üzerindeki farklı glikoz konsantrasyonları uygulaması yapıldı. D (+) glikoz, merck millipore' dan temin edilidi (108337). Hiperglisemik model grup için, nihai konsantrasyonu 100 mM olan glikoz, önceki çalışmada tarif edildiği gibi altı kuyucuklu plakalara eklenmiştir[9,10]. Literatür taraması sonucu, 24 saat ve 100 mM dozunda yaklaşık %50 hücre ölümü gözlendiği için, bu doz seçilmiştir. SH-SY5Y hücreleri (1x105 hücre/3 ml medyum) altı kuyulu petrilere ekildi. Hücreler 24 saat 37˚C sıcaklık, %95 nem ve %5 CO_2 ortamında inkübasyona bırakıldı. Hücreler

konfluent faza ulaştıktan sonra, 24 saat ve 100 mM dozda glikoz uygulanarak, hiperglisemik ortam oluşturuldu.

Mitokondriyal Genlerin mRNA Düzeylerinin Belirlenmesi

Nörotoksik ortamda SH-SY5Y hücrelerinde, mitokondri dinamiğinde rol oynayan DRP1, OPA1, MFN1, MFN2 ve FIS1 genlerinin ifade düzeylerini belirlemek amacı ile Real time PCR (RT-PCR) yöntemi uygulandı. İn-vitro deney sonrasında, hem normal glikoz düzeyi olan gruptaki hücrelerden, hem de yüksek glikoz uygulanan gruptaki hücrelerden, trizol (Invitrogen, USA) yöntemi ile RNA izole edildi ve elde edilen RNA miktarı ve saflıkları NanoDrop 8000 (Thermo Fisher, USA) cihazı ile 260/280 nm absorbans aralığında belirlendi. Total RNA saflığı 1.8-2.0 civarında olan örnekler çalışmaya dahil edildi. Elde edilen total RNA'lardan TaqMan™ Revers Transkripsiyon kiti (Applied Biosystems, 4304134) ile, kit protokolüne uygun olarak, RNA'dan komplementer DNA (cDNA) sentezi Thermal Cycler (BioRad T100) cihazında gerçekleştirilmiştir. Gene özgü olacak şekilde 20-25 baz uzunluğunda, bağlanma sıcaklığı 60-61^{o}C ve GC baz oranının %50-55 olacak şekilde dizayn edilen, DRP1, OPA1, MFN1, MFN2 ve FIS1 primerleri kullanarak RT-PCR reaksiyonu gerçekleştirildi[11]. Kullanılan primer dizileri Tablo 1' de verilmiştir. RT PCR, SYBR® (Qiagen, Almanya) kiti ve Thermo Fischer Step One Plus cihazı ile yapıldı. SYBR Green PCR Master Mix (1x) 12,5 µl, cDNA 500 ng, forward primer 0.5 µM ve reverse primer 0.5 µM içeren PCR karışımına, toplam reaksiyon hacmi 25 µl olacak şekilde nükleaz içermeyen su eklendi. Hazırlanan reaksiyon karışımı, 95 °C'de 5 dk, 95°C'de 15 sn 60 °C'de 20 sn, 72 °C'de 30 sn (40 döngü) ve 72 °C'de 10 dk sıcaklığa maruz bırakıldı. Reaksiyon sonrasında, eşik değeri belirlenerek Ct değerleri belirlendi. mRNA'lar için referans gen olarak ACTB (β-actin) kullanıldı. Her bir numune 3' lü set olarak çalışıldı. Real time PCR sonucunda elde edilen ekspresyon verilerini değerlendirmek için, $2^{-\Delta\Delta Ct}$ formülü kullanılarak glisemik ve hiperglisemik gruplar karşılaştırıldı.

Tablo I: QPCR' da kullanılan primer dizileri[11].

Gen	Forward Primer (5'-3')	Reverse Primer (5'-3')
FIS1	CTTGCTGTGTCCAAGTCCAA	GCTGAAGGACGAATCTCAGG
MFN2	ACACATGGCTGAGGTGAATG	CGTCCAGAACCTGTTCTTCTG
OPA1	GGATTGTGCCTGACATTGTG	AAGGCTTTCAACAATCTTGTCA
DRP1	CAGTGTGCCAAAGGCAGTAA	GATGAGTCTCCCGGATTTCA
MFN1	CCTGTTTCTCCACTGAAGCAC	CCTCACCAATGATGGAAAGC
ACTB	AACTGGGACGACATGGAGAA	GAAGGTCTCAAACATGATCTGG

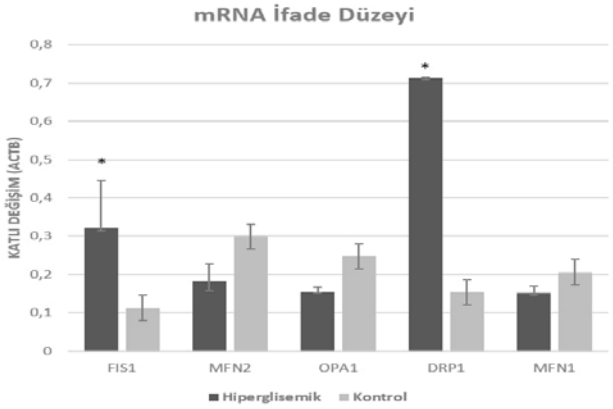
İstatistiksel Analiz

Sonuçların istatistiksel olarak karşılaştırılması "GraphPad Prism 8.0" programı ile gerçekleştirildi. Gruplar arasındaki farklılıklar tek yönlü ANOVA varyans analizi yapılarak belirlendi. Anlamlılık değeri $p<0,05$ olarak alındı.

BULGULAR

Hiperglisemik ortamda nörotoksisite geliştirdiğimiz insan nöroblastoma hücre hattında, mitokondriyal morfoloji ve fragmentasyonda görevli genlerin mRNA ekspresyonunu inceledik. 100 mM glikoz uygulayarak, nörotoksik ortam geliştirdiğimiz hücrelerde, RT-PCR tekniği ile belirlediğimiz DRP1, OPA1, MFN1, MFN2 ve FIS1 ekspresyon seviyelerine ait değişimler Şekil 1' de gösterilmiştir. Çalışma sonuçlarımıza göre, hipotezimiz ile uyumlu olarak, hiperglisemik grupta, kontrol grubuna oranla FIS1, DRP1 seviyelerinde artış, MFN1, MFN2, OPA1

seviyelerinde azalma gözlemledik. OPA1, DRP1 ve MFN1 genlerine ait değişimler istatistiksel olarak anlamlı olup (p<0,05), FIS1, MFN2 genlerine ait değişimler istatistiksel olarak anlamlı değildir (p>0,05). Yüksek glikoz uyguladığımız grupta, kontrol grubuna oranla FIS1, DRP1 (sırasıyla 2,45-kat ve 4,61-kat) seviyelerinde artış (p<0,05), MFN1, MFN2, OPA1 (sırasıyla 0,69-kat, 0,58-kat ve 0,62-kat) seviyelerinde azalma (p>0,05) gözlemledik. Sonuçlar Şekil 1' de verilmiştir.



Şekil 1. DRP1, OPA1, MFN1, MFN2, FIS1 genlerinin mRNA ekspresyon seviyeleri verileri (*p<0,05).

TARTIŞMA

T2D görülen metabolik değişiklikler ve mitokondriyal değişiklikler birebir ilişkilidir. Yapılan son çalışmalarda, mitokondrilerin sabit ve yalnız bir organel olmadığı, metabolik ihtiyaçları karşılamak için sürekli olarak morfoloji ve hücre altı dağılımda değişiklikler geçirdikleri belirlendi[6]. Mitokondri dinamiğinde meydana gelen bozukluklar, diyabet ve ilişkili nörodejeneratif hastalıkların patolojisinde, hücresel ve moleküler düzeyde önemli bir rol oynar[6,8]. Biz de yaptığımız bu çalışmada, yüksek glikoz uygulaması ile nörotoksik model geliştirdiğimiz hücre hattında, mitokondriyal düzlemde, moleküler düzeyde rol oynayan önemli genlerin ifade düzeyi farlıklıklar belirledik. Bu konu ile ilgili literatür taramalarımız sonucunda, fare ve insanda yapılan çalışmalarda, hiperglisemi ile indüklenen ROS üretimi artışı ile, artan mitokondriyal fragmentasyon ve fisyon ilişkilendirilmiştir. T2D' de ki mitokondriyal morfolojik tabloda, DRP1 düzeyinde artış, MFN2' de azalma gözlemlenmiştir[6]. Bu çalışmanın bulguları, bizim çalışmamız ile benzer bulgular içermektedir.

Yapılan başka bir çalışmada, hiperglisemi ile indüklenen ROS, hem de insülin salgısı, DRP1 tarafından indüklenen fisyonun engellenmesiyle bloke edildi[12]. Ayrıca, bozulmuş mitokondriyal füzyon, iskelet kasında insülin direnci ve karaciğere özgü MFN2 knock out farelerde, glukoz intoleransı ve artmış hepatik glukoneojenez ile ilişkilendirilmiştir[13,14]. Rat model çalışmalarında, MFN2''nin aşırı ifade edilmesinin insülin duyarlılığını artırdığı, kas ve karaciğerde lipid ara ürünlerini azalttığı belirlenmiştir[15]. Moleküler düzeyde, karaciğerdeki MFN2 ifadesi, insülin reseptörü ve glukoz taşıyıcısı GLUT2'nin artan ifadesi ve insülin yolağının aktivasyonu ile ilişkilendirilmiştir[15,16]. Bu yapılan in-vivo çalışmalar ile uyumlu olarak, bizim yaptığımız in-vitro çalışmamızda hiperglisemi durumunda DRP1 düzeyinde belirgin bir artış, MFN2 düzeyinde azalma gözlemledik.

Mitokondri biyogenezi, bir dizi transkripsiyon faktörü ile düzenlenen, mitokondri füzyon ve fisyonunun normal sağlıklı bir hücrede uyum içinde gerçekleştiği bir süreçtir[17]. T2D'de, gerçekleşen hiperglisemik tablo, farklı hücre tiplerinde normalden fazla mitokondri bölünmesine ve parçalanmasına neden olur; bu durumda aşırı ROS üretimi, azalmış mitokondriyal birleşme ve artmış mitokondriyal bölünme ile sonuçlanarak mitokondriyal işlev bozukluğuna yol açar[17].Anormal mitokondriyal biyogenez, enerji düzenlemesinde aksamalar ve nihayetinde ROS üretimini hızlandırarak diyabet patolojisine sebep olur. Bu nedenle, hiperglisemi ile tetiklenen anormal mitokondriyal dinamikler, T2D kronik komplikasyonlarının temel nedeni

olabilir[18]. Diyabetik kardiyomiyopati araştırmalarında, yağ asidi artışının kalpte, mitokondriyal yapısal yeniden şekillenmeye neden olduğu tespit edilmiştir. OPA1 ve DRP1'in post-translasyonel modifikasyonlarını düzenleyerek, mitokondriyal bölünmeyi teşvik edebileceği rapor edilmiştir[19].

T2D hastalarında vasküler endotelyal disfonksiyon ve önlenmesine yönelik yapılan bir çalışmada, mitokondriyal fragmentasyon son derece yüksek olup, FIS1 ifadesi artmıştır. FIS1 inhibisyonu ile, T2D' li hastalardan alınan damar örneklerinde, bozulmuş endotel bağımlı vazodilatasyonda iyileşme gözlemlenmiştir.Aynı çalışmada, 33 mM ve 2,5 mM olmak üzere yüksek ve düşük glukoza maruz bırakılan sağlıklı insan damarlarında nitrik oksit biyoyararlanımını korumuş ve endotel hücre tabakası bütünlüğünü iyileştirmiştir. Tam tersine, sağlıklı damarlarda FIS1' de ki ifade artışının vazodilatasyonu bozduğu ve mitokondriyal süperoksit üretimini arttırdığı tespit edilmiştir[20].

Metforminin T2D hastalarındaki, mitokondriyal fonksiyon üzerindeki etkisinin incelendiği bir çalışmada, lökositlerde yapılan analizlerde, mitokondriyal füzyon proteinleri MFN1, MFN2 ve OPA1 mRNA ve protein düzeylerinde daha düşük, FIS1 ve DRP1 protein ve gen ifade düzeylerinde daha yüksek ifadeye sahip olduğu belirlendi[21]. Tip 2 diyabette tedavi ajanı olarak kullanılan metforminin, bu etkilerin çoğunu tersine çevirerek T2D hastalarında gözlenen mitokondriyal fonksiyon ve dinamiklerini düzelttiği tespit edildi[21]. Çalışma verilerimiz, bu çalışma ile uyumludur.

Sakkaroz uygulanarak T2D modeli geliştirilen farelerin beyin mitokondriyal fonksiyonları, sağlıklı kontrol grubu farelerle karşılaştırıldığında, solunum kontrolünde, membran potansiyeli ve ATP/ADP oranlarında azalma belirlenmiştir[22]. Diyabetik Goto Kakizaki sıçanlarının beyin mitokondrilerinde yaşlanmave amiloid-β (Aβ) maruziyetinin etkileri incelenmiş ve T2D' de yaşlanma ve Aβ maruziyeti eş zamanlılığında,mitokondriyal fonksiyonun en çok bozulduğu durum olduğu rapor edilmiştir[23]. Goto Kakizaki diyabetik modeli sıçanlarla yapılan bir diğer çalışmada, Aβ1-40 uygulaması ile geliştirilen nörotoksisite durumunda, beyin mitokondrilerinde artan H_2O_2 üretimi, ardından solunum kontrol oranında ve ATP içeriğinde azalmayı indüklediği belirlenmiştir[19]. Antioksidan özelliğe sahip, CoQ10 tedavisinin bu negatif etkileri hafiflettiği gözlenmiştir[24]. T2D fenotipiyle sinir sisteminde mitokondriyal disfonksiyonu tetikleyen diğer patolojik süreçlerin özellikle belirlenmesi gerekmektedir[25].

T2D' de mitokondriyal stres faktörlerinin araştırıldığı bir çalışmada, streptozotosin (STZ) ile apoptoz ve metabolik stresin uyarıldığı fare ve insan adacık hücrelerinde, tedavi edici ajan olarak kısa zircirli yağ asitleri kullanılmış. Bu çalışmada kısa zincirli yağ asitlerinin mitokondriyal füzyon genlerinin MFN, MFN2 ve OPA1' in azalmasını önlediği, STZ maruziyeti sırasında fizyon genlerinin DRP1 ve FIS1'in artırılması engellendiği belirlenmiştir. Çalışmamızda da yüksek glikoz ile nörotoksisite geliştirilen grupta benzer sonuçlara ulaşılmıştır[26]. Arnt-benzeri protein-1'in (Bmal1)' in T2D ilişkili pankreatik B hücre yetmezliğinde araştırıldığı insülinoma (INS-1) hücre hattında, Bmal1'in azaltılmasının mitokondriyal membran potansiyelinde ve mitokondriyal mimaride olumsuz bir değişikliğe neden olduğu tespit edilmiştir. Bmal1'in silinmesi, Mfn1 ve Mfn2 mRNA ve protein ekspresyonunu azaltırken Fis1 ekspresyonunu arttırdığı belirlenmiştir[27]. Yüksek glikoz ile nörotoksisite oluşturulan, bir diyabet türü olarak kabul edilen Alzheimer'da karşılaşılan tabloya benzer bir durumun, in-vitro ortamda geliştirilmeye çalışıldığı ve mitokondri disfonksiyonunun incelendiği çalışmamıza literatürde rastlanmamıştır. Ancak

çalışmamıza ait bazı sınırlamalar mevcuttur. Mitokondri dinamiğinde rol oynayan sadece 5 gen transkripsiyonel olarak değerlendirilmiştir. Çalışma bulgularını destekler nitelikte, bu genlerin translasyonel ve biyokimyasal seviyede incelendiği, yeni çalışmalar yapılması gerekmektedir. İn-vitro hiperglisemi gibi kompleks hastalık modeli, in-vivoda ki sistemik gerçek durumu tam olarak yansıtmayabilir. İn-vivo hiperglisemik modelde, daha fazla biyobelirtecin transkripsiyonel ve translasyonel seviyede incelendiği, yeni çalışmalar planlanmaktadır.

SONUÇ

Çalışma sonuçlarımıza göre, T2D patofizyolojisinin yol açtığı durumlardan birisi olan, T3D mekanizmasında nörodejenerasyona katkıda bulunan, mitokondri dinamiğinde önemli rol oynayan moleküllerin değişimi, hastalık tablosunda artan fragmentasyonu destekler niteliktedir. Hücrenin enerji üretim fabrikası olan mitokondri biyogenezinde bozulmalar olduğunu destekleyen çalışmamız, bu yönüyle gelecekte planlanacak çalışmalara ışık tutmaktadır. Çalışma bulgularımız literatürü destekler nitelikte olup, mitokondri biyogenezinden sorumlu daha fazla molekülün incelendiği in-vitro ve in-vivo çalışmalar yapılması gerekmektedir.

Etik Kurul Onayı: Çalışma, in-vitro hücre kültürü çalışması olduğu için ve ticari olarak satılan biyolojik materyal kullanılıp, insan ve insana ait veriler kullanılmadığı için etik kurul onayı alınmamıştır.

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Özgün Araştırma / Original Article

Uzamış COVID Hastalarında Simon Görevi ile Bilişsel Etkilerin Değerlendirilmesi

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Öz

Amaç: Dünyada pandeminin sürdüğü esnada COVID-19'in uzun süreli etkileri rapor edilmeye başlandı ve 12 haftadan uzun süren olgular uzun-covid sendromu olarak ifade edildi. Uzun covid sendromunun en belirgin ifadesi sinir sistemi üzerine gösterdiği belirtilerdi. Bu etkiler uzun covid sendromuna bağlı "beyin sisi" olarak adlandırıldı. Biz de bu çalışma da COVID-19 geçirmiş bireylerde nörokognitif etkileri denetlemeyi amaçladık.

Yöntemler: Bu amaçla benzer yaş grubuna sahip, tanı aldıkları tarihten 12 hafta geçmiş COVID-19 geçiren 36 katılımcı yanı sıra COVID-19 tanısı almamış 35 birey çalışmaya dahil edildi. Bu çalışmada Simon etkisi olarak adlandırılan yönelim etkisinin test edilebilmesi için hazırlanmış işitsel bir görev katılımcılara uygulandı. Eş zamanlı olarak EEG kayıtları alındı. Görev esnasında tüm katılımcıların kayıtları üzerinde olay ilişkili potansiyel kayıtları alındı.

Bulgular: Çalışmanın davranış sonuçlarında gruplar arası test başarı oranı arasında fark yoktu. Fakat covid grubu kontrol grubuna göre görev tamamlama ve reaksiyon sürelerinde artış gözlendi. Olay ilişkili potansiyel kayıtlarında uyumlu uyaran sunumunda neredeyse tüm dalga amplitüd ve latansları iki grup için benzerdi. Bunun aksine uyumsuz uyaran sunumunda gruplar arasından N2 amplitüd ve latansları bakımından anlamlı fark gözlendi. Bununla birlikte covid grubu P3 Δt kontrol grubuna göre artmıştı. Çalışma sonuçlarımızda gözlenen covid grubundaki reaksiyon süresi artışı elektrofizyolojik ölçümlerde de doğrulandı.

Sonuç: Bulgular COVID-19'un dikkatin oluşumu ve uyarana karşın cevap hazırlığı esnasında inhibitör işlemin azaldığını. COVID-19'a bağlı yaşanan beyin sisinin dikkatin ön işlem basamaklarında meydana geldiğini gösterdi.

Anahtar kelimeler: COVID-19, Dikkat, Olay-İlişkili Potansiyeller, P3, Uzamış COVID

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Evaluation of Cognitive Effects with the Simon Task in Patients with Long COVID

Abstract

Aim: The long-term effects of COVID-19 started to be reported and it was referred to as long-covid syndrome for cases lasting longer than 12 weeks, while the pandemic was continuing in the world. The most prominent expression of long-covid syndrome was its symptoms on the nervous system. These effects were called "brain fog" due to long covid syndrome. In this study, we aimed to examine the neurocognitive effects in participants who had illness with COVID-19.

Methods: For this purpose, 36 participants of similar age group who had COVID-19 within 12 weeks of their diagnosis, as well as 35 participants who had not been diagnosed with COVID-19, were included in the study. In this study, an auditory task prepared to test the orientation effect called Simon effect was applied to the participants. EEG recordings were taken simultaneously. During the task, event-related potentials were recorded on the recordings of all participants.

Results: In the behavioral results of the study, there was no difference in the test success rate between the groups. However, the patient group showed an increase in task completion and reaction times compared to the control group. In the event-related potential recordings, almost all wave amplitude and latency were the same for the two groups in congruent stimulus presentation. On the contrary, a significant difference was observed between N2 amplitude and latencies in incongruent stimulus presentation. However, P3 Δt in the covid group increased compared to the control group. The increase in reaction time in the covid group observed in our study results was also confirmed in electrophysiologic measurements.

Conclusion: The results showed that COVID-19 decreased the inhibitory process during the formation of attention and preparation of response to stimuli. COVID-19-induced brain fog occurred in the pre-processing steps of attention.

Keywords: COVID-19, Attention, Event-Related Potentials, P3, Long COVID.

GİRİŞ

SARS-korona virüs (COVID-19) pandemisi 2020 yılından itibaren tüm dünyayı etkisi altına aldı ve 10 milyonlarca kişi bu hastalıktan etkilendi. Bu yazının hazırlandığı tarihte hastalığın tanımlanan alfa, beta, delta, omikron gibi varyantları, Eris, Pirola gibi alt varyantı bulunması sebebiyle dünya sağlık örgütü tarafından halen dikkatle takip edilmektedir[1].

Hastalığın akut fazında; başta solunum sistemi olmak üzere birçok sistemin etkilendiği bildirildi. Aşıların ortaya çıkmasıyla pandemi geriletilse de, hastalığı geçirmiş kişiler başta sinir sistemi, kardiyovasküler vb. gibi diğer sistemlerin etkilendiği ifade etmektedir[2].

COVID-19 için belirtilen nekahet döneminin 12 hafta olduğu belirtilmiş, hastalık etkilerinin 12 hafta ve üzeri görüldüğü vakalara ise uzun-covid sendromu (long-covid syndrome) adı verilmiştir[3]. Long covid veya post covid sendromun klinik gözleminde yorgunluk, nefes darlığı, koku-tat kaybı, kas ağrıları, baş ağrısı, anksiyete/depresyon ve unutkanlık konsantrasyon problemleri başta sayılan semptomlardandır[3-5]. İlerleyen nörolojik sekellere, nöropsikiyatrik ve nörokognitif bozulmalar eşlik eder. Kognitif gerileme nörolojik işlem hızında azalma ve karar mekanizmalarında gerilemeye neden olduğu için COVID-19'un kognitif etkilerine beyin sisi (brain fog) adı verilmiştir[2,4]. Bazı çalışmalar nörolojik etkileri nöroinflamasyon kaynaklı görürken[6], diğer çalışmalar virüsün doğrudan neutrofik etkilerinden kaynakladığını ifade etmektedir[7,8]. Nöroinflamasyon, sitotoksik immun reaksiyonlar veya inflamasyona bağlı kan akım değişiklikleri ile glia ve sinir hücrelerde fonksiyon bozukluğuna neden olabilir[2,6,7,9]. Çeşitli çalışmalar solunum sistemini tutan viral enfeksiyonların ilerleyici kognitif bozulmaya neden olduğunu göstermiştir[10,11]. Corona virüs etkinliğini, yüksek affinitede hücre zarında bulunan anjiotensin dönüştürücü enzim 2 (ACE-2)

reseptörlerine bağlanarak gerçekleştirirken. Solunum sistemi dışında bulunan ACE-2 reseptörleri başlıca gastrointestinal kanal epiteli ve beyin damar dokusunda bulunduğu ve virüs spike proteinlerinin kan beyin bariyerini bozduğu tespit edilmiştir[12]. Yapılan çalışmalar corona virüsün doğrudan santral sinir sistemini hedef almadığı fakat yukarıda ifade edildiği gibi artmış otoimmunite, nöroinflamasyonun ve kan beyin bariyerinde meydana gelen hasarın kognitif bozulmaya yol açtığı düşünülmektedir[12].

Nasserie ve ark. COVID-19 hastalığına yakalanmış 9751 birey üzerindeki incelemelerinde, katılımcıların %70'inin iki ay ve üzeri en az bir semptomun devam ettiğini, hastalığı hafif atlatanlar da dahil olmak üzere katılımcıların % 25'ini etkileyen bilişsel işlevlerde bozulma olduğunu ifade etmiştir[5]. Becker ve ark. 2020-2021 tarihleri arasında COVID-19 hastalığını hafif, orta, şiddetli geçirmiş hastaların nöropsikometrik incelemelerine göre; enfeksiyondan 7 ay sonra bile hastaların %10'unda dikkat, %18'inde işlem hızı düşüşü, %24'ünde hafızada gerileme, %16'sında ise yürütsel işlevlerde bozulma gözlemlemişlerdir[13]. Biz de bu çalışmada COVID-19 geçirmiş bireylerin yönelim etkisi gibi; katılımcıya çelişki yaşatan bir etki ile görev performansını, bununla birlikte dikkat süreçlerini kontrol grubu ile karşılaştırarak gözlemlemeyi amaçladık.

YÖNTEMLER

Katılımcılar ve protokol

Bu çalışmanın evreni için 80 gönüllü katılımcı ön görüldü, fakat 3 katılımcı testlere adapte olamadı, 6 katılımcının ise COVID-19 öncesi farklı bir rahatsızlığı ve/veya kronik hastalığı olduğu için toplam 9 katılımcı deneyden çıkarıldı. Çalışmanın verileri yapılan tüm testlerde herhangi bir tanı almayan 35'i kontrol, 36'sı ise nazofarenks veya orofarenksten alınan örnekleri gerçek zamanlı polimeraz zincir reaksiyon (PCR) testleri ile hastalık tanısı almış bireylerden elde edildi. Covid grubundaki katılımcılar çeşitli etkilerin hastalıktan sonra devam ettiğini beyan etti. Tüm katılımcıların sağ el tercihine sahip olduğu Oldfield el tercihi için geliştirilmiş anket ile kontrol edildi[14]. Çalışma yönteminin açıklanmasının ardından hasta ve kontrol grubundaki tüm katılımcılardan tıbbi verilerinin yayınlanabileceğine ilişkin yazılı onam belgesi alındı. Testlerden önce katılımcıların demografik verileri ve hastalığın klinik seyri sorgulandı. Teşhis alan katılımcılardan yoğun bakım öyküsünün olup olmadığı, ateş, nefes darlığı, koku/tat kaybı gastrointestinal belirtiler varsa kaydedildi.

Testlerin Uygulanması

Deney öncesi testte kullanılan sıralamadan farklı rastlantısal olarak oluşturulmuş deneme kayıtları alındı. Çalışmaya; herhangi bir duyma rahatsızlığı olmayan ses tonlarını sağlıklı biçimde işiten ve bunlar arasındaki farklılığı ayırt edebilen bireyler katılımcı olarak çalışmaya alındı.

Katılımcılara; test ile ilgili komutlar, testin amaçları bildirildi, aynı zamanda testin yapılış biçimi ekrandaki yazılı yönergeler ile aktarıldı. Katılımcıların komut tuşlarını kullanarak sol veya sağ el tercihleri ile işittikleri belirteçlere karşın yanıt oluşturması istendi. Katılımcılar yanıt esnasında mümkün olan en hızlı biçimde ve hata yapmadan testi tamamlaması gerektiği temel amaç olarak vurgulandı. Deney esnasında katılımcıların dikkatini dağıtabilecek ses veya görsel uyaranlardan kaçınıldı.

Simon görevi, EEG kaydının alınması ve OİP hesaplaması

İşitsel Simon görevi

Dikkat ve karar mekanizmalarının incelenmesine olanak sağlayan Simon görevi ses veya görsel uyaranın olduğu tarafa doğru cevap oluşturma davranışını ifade eder[15]. Literatürde Simon görevi işitsel veya görsel

görevler biçimde uygulanmaktadır. Bu çalışmada testler işitsel uyaranlar yardımı ile uygulandı. Uyaranlar 72 db ses basınç şiddetinde, kulaklık yordamı ile katılımcıya sunuldu ve katılımcıdan test esaslarına uygun cevap oluşturması beklendi. Testin esasına uygun olarak katılımcıdan en kısa sürede yanıtlarını oluşturmasını ve bu yanıtları sağ veya sol elini kullanarak gerçekleştirmesi bildirildi. Uyaranlar test katılımcılarına uyumlu (congruent); uyaran ile cevabın aynı tarafta konumlandırıldığı veya uyumsuz (incongruent); uyaran ile cevabın zıt taraflarda konumlandırıldığı bir dizi sunum gerçekleştirildi. Uyumlu ve uyumsuz uyaranlar arasında bir kalıp yoktu ve her olay rastlantısal olarak sunum programı (E-Prime, Sharpsburg, PA. ABD) tarafından belirlendi.

Bilindiği gibi canlılar uyaran tarafına doğru davranış oluşturup cevap verme eğilimindedir ve bu etki Simon etkisi olarak tanımlanmıştır[16,17]. Uyaran ile cevap arasındaki bu uyum veya uyumsuzluk katılımcının reaksiyon süresine etki eder. Esasında sinir bilimlerinde de karmaşık bu görevin çözümlenmesi; hem dikkat hem de karar oluşturma, kapasitesinin anlaşılmasına olanak tanır[16-18].

Bu deneyde verileri kaydeden sistem EOG (elektrookülografi) ve EEG (elektroensefalografi) kayıtları oluşturuldu. Uyaran sunumu ile cevap arasındaki zaman reaksiyon süresi olarak kaydedildi. Testlerin dizaynı ve uyaranların sunumu yine E-Prime yazılımı kullanılarak gerçekleştirildi. Çalışmada uyaranlar ile cevap zamanları, cevap oluşturma zamanlarının toplamları test katılımcıların verdikleri tepki süreleri, tepki sürelerinin toplamları ve testte gerçekleştirilen hata sayısı ve başarı yüzdeleri kaydedildi.

Olay İlişkili Potansiyeller

Olay ilişkili potansiyeller (OİP), EEG kayıtlarından temel bir olaya karşın istatistiksel yöntemlerle oluşturulan uyarılmış potansiyellerdendir[19]. Uyarıya bağlı oluşan dış bileşenleri ve bilişsel süreçlerin oluşturduğu iç kompleksleri bulunur. Sinir bilimlerinde olaydan ortalama 300 milisaniye sonra oluştuğu düşünülen p300 bileşeni yaygın olarak kullanılır[20].

EEG - EOG kayıtlarının alınması ve OİP dalgacıklarının hesaplaması

Elektriksel tüm kayıtlar Biopac (CA. ABD) donanımı ve bu verilerin tüm kayıt ve hesaplamaları Acqknowledge (CA. ABD) programı ile gerçekleştirildi. EEG'nin uluslararası 10-20 sistemine kullanılarak Cz bölgesine yerleştirilen aktif elektrot kullanıldı. Göz hareketlerini EEG kayıtlarından temizleyebilmek için EOG kayıtları alındı. Uyaran sunumu ve cevapların kaydı aynı anda E-Prime programı ile gerçekleştirildi. Tüm davranış kayıt ve analizleri aynı program vasıtası ile hesaplandı. Sonuç potansiyel olan OİP elde edildikten sonra uyumlu, uyumsuz uyaranlar ayrı ayrı ve bütünleşik olarak incelendi. Gruplara göre grand averaj (büyük ortalamalar) alınarak sonuç potansiyeller üzerinden bileşenler ayrıştırıldı. OİP potansiyellerinde latans, amplitüd ve tepe/tepe ölçümleri yapıldı.

İstatistiksel Değerlendirme

İstatistiksel analizlerde ortalama (m) ± standart sapma (sd) kullanıldı, anlamlılık düzeyi 0.05 belirlendi. Örneklem büyüklüğünü belirlemek için güç analizi G*Power (Heinrich-Heine Universitat, Dusseldorf, Almanya) 3.1.94 sürümü program kullanıldı ve alfa değeri 0.05, güç (1-β err prob) %80 olarak hesaplandığında her grupta 36 olmak üzere en az 72 katılımcı öngörüldü. Katılımcıların yaşanabilecek testlere uyumsuzluğu göz önüne alındığında her grupta 40 olmak üzere 80 katılımcının testlere alınması planlandı. Levene testi ile verilerin homojenliği test edildi. Shapiro-Wilk testi ile verilerin normal dağılıma uygunluğu denetlendi. Covid

ve kontrol grubu karşılaştırmalarında bağımsız örneklem t testi ile gerçekleştirildi. Grafiklerin oluşturulması ve istatistiksel analizler için IBM SPSS versiyon 25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) ve Graphpad prism v9 (Graphpad software CA, ABD) kullanıldı.

Etik Kurul Onayı

Bu çalışma uluslararası Helsinki Deklarasyonu esaslarına uygun olarak yürütülmüş, Avrasya Üniversitesi girişimsel olmayan klinik araştırmalar etik kurulu 24.06.2022 tarih 12 sayılı toplantıda; E-69268593-050-13227 sayı no'lu onayı sonrası gerçekleştirilmiştir.

BULGULAR

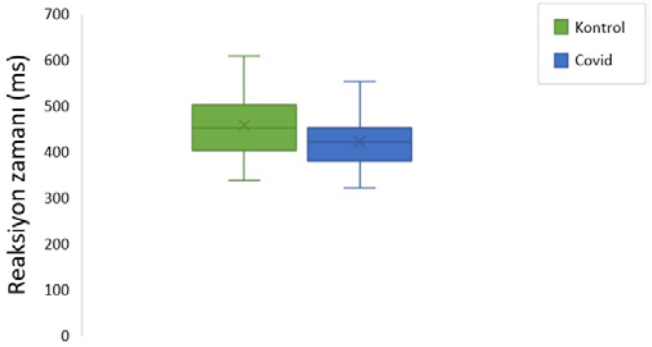
Çalışmaya katılan katılımcıların yaş ortalaması 21.6±1.82 olarak hesaplandı. Katılımcıların 40'ı kadın (%56,3), 31'i erkek (%56,3) bireylerden oluştu. Covid grubundaki katılımcıların dahil edilme kriteri olarak; NHS tarafından uzun covid süresi olarak ifade edilen 12 haftalık süreyi geçirmiş bireyler çalışmaya dahil edildi[3].

Hasta grubunun COVID-19 teşhisi aldıktan teste alınmaları için geçen süre 24.8±13.5 hafta idi. Covid grubundaki katılımcıların %38,9'u tedavi almış, %61,1'i ise herhangi bir tedavi almamıştır. Covid grubundaki katılımcıların hastalık seyri ve beyanları Tablo 1'de gösterilmiştir.

Tablo II: Covid grubunda yer alan bireylerin klinik gözlemi

	Varyans	Yüzde (%)
Hastalığın seyri	Asemptomatik	8.3
	Hafif	50.0
	Orta şiddette	41.7
Nefes darlığı	Var	30.6
	Yok	69.4
Öksürük	Var	66.7
	Yok	33.3
Halsizlik	Var	83.3
	Yok	16.7
Koku/tat kaybı	Var	55.6
	Yok	44.4
Kas ağrısı	Var	83.3
	Yok	16.7
İshal	Var	22.2
	Yok	77.8

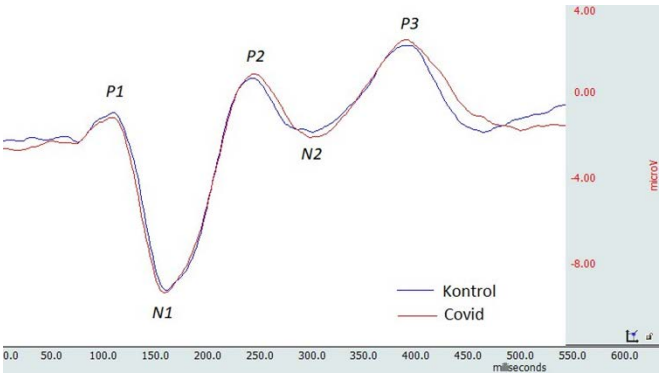
Katılımcıların test başarı oranları ve reaksiyon zamanları kaydedildi. Yapılan testlerden elde edilen sonuçlara göre covid (92.80±5.46) ve kontrol (92.14±6.45) grubu arasında başarı oranı farkı yokken (p=0.14), kontrol grubu reaksiyon zamanı covid grubuna göre düşüktü (p=0.032), (Şekil 1).



Şekil 1. Simon yönelim etkisi testinde uyaran/cevap ikilisinin gruplara ait reaksiyon zamanları

** Kontrol grubu ortalaması covid grubu ortalamasına göre düşüktü [F(1,69):0.97, p=0.032]*

Bu sonuçlar kontrol ve covid grubunun benzer başarı oranı ile testi tamamladığını, fakat yönelim etkisini çözümlemek için hasta grubunun kontrol grubuna göre daha uzun bir zaman gerektirdiğini gösterdi.

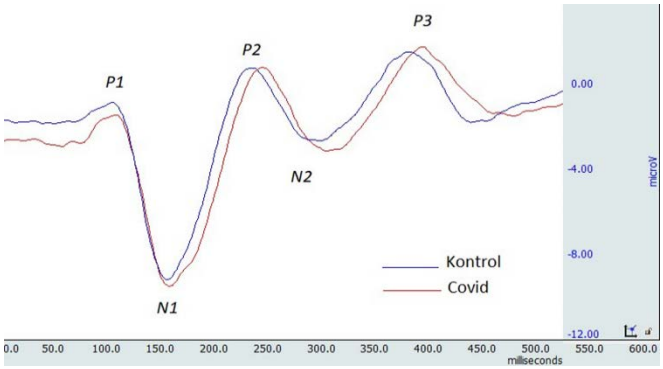


Şekil 2. Simon yönelim etkisi uygulanan katılımcılara uyumlu uyaran esnasında kaydedilen Olay ilişkili potansiyel kayıtları

Cz kanalı OİP kayıtlarında P1, N1, P2, N2, P3 gösterilmiştir. Yatay eksen milisaniye düzey eksen mikrovolt olarak gösterilmiştir. Görüntüler Acqnowledge grand (büyük) averaj kayıtlarıdır.

Olay ilişkili potansiyel incelemeleri iki yönlü olarak her iki grup üzerinde gerçekleştirildi. Uyumlu uyaran sunulduğunda olay ilişkili

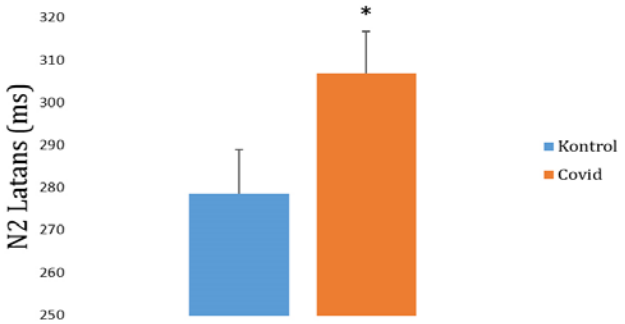
potansiyeller bakımından dalgaların oluşum biçimleri, latans ve amplitüdleri incelendiğinde (Şekil-2); P1 dalgası kontrol grubu için aktivasyon 0.74±0.03 ms, covid grubu için aktivasyon 0.76±0.002 olarak kaydedildi (p=0.35). P1 amplitüdü kontrol grubu için 1.37±0.5 µV, covid grubu için 1.28±0.43 µV olarak kaydedilmiştir P1 kompleksi için gruplar arası fark yoktur (p=0.22). P1-N1 Δ amplitüdü (tepe-tepe) covid grubu için 8.27±3.04, covid grubu için 8.17±3.04 olarak kaydedilmiştir. Her iki grup karşılaştırmalarında yine istatistiksel fark yoktur (p=0.08). P3 aktivasyonları değerlendirildiğinde; kontrol grubu 302 ms, covid grubunda 304 ms olarak kaydedildi, P3 amplitüd karşılaştırıldığında; kontrol grubu; 4.53±1.46 µV, covid grubu; 4.52±1.48 µV olarak kaydedildi, P3 amplitüdleri neredeyse her iki grupta aynıydı ve istatistiksel olarak fark yoktu (p=0.53). P3 Δt karşılaştırıldığında; kontrol grubu 164±33 ms, dalganın altında kalan alan 0.301 µV/sec, covid grubu için ise;202±23ms, dalganın altında kalan alan ise 0.370 µV/sec olarak hesaplandı. Hasta grubu benzer aktivasyon zamanlarına sahip olsa da dalga daha geniş ve alanı daha büyüktü, fakat bu fark istatistiksel olarak gösterilemedi (p=0.061). Bu sonuçlara göre uyumlu uyaran sunumunda kontrol ve covid grupları arasında bir farkın olmadığı görülmektedir.



Şekil 3. Simon yönelim etkisi uygulanan katılımcılara uyumsuz uyaran esnasında kaydedilen olay ilişkili potansiyel kayıtlarında grup ortalamaları

Cz kanalı OİP kayıtlarında P1, N1, P2, N2, P3 gösterilmiştir. Yatay eksen milisaniye düzey eksen mikrovolt olarak gösterilmiştir. Görüntüler Acqnowledge grand (büyük) averaj kayıtlarıdır.

Uyumsuz uyaran sunumunda ise olay ilişkili potansiyel kayıtlarında kompleksler arasında ayrılmalar görülmektedir (Şekil 3). P1 amplitüd ölçümleri incelendiğinde kontrol grubu 0.90±0.55 µV, covid grubu ise 1.25±0.32 µV olarak ölçülmüştür. Covid grubunda gözlenen P1 dalga yüksekliğindeki artış istatistiksel olarak gösterilememiştir (p>0.05). Benzer şekilde P1 Δt genişlikleri değerlendirildiğinde kontrol grubu 90±22 ms, covid grubu ise 125±30ms'dir ve gruplar arasında fark yoktur (p=0.073). Bununla birlikte N1 tepe değeri kontrol grubu için 6.78±1.35 µV, covid grubu için 7.15±1.12 idi ve iki grup arasında istatistiksel fark yoktu (p=0.46). N1 Δt bakımından kıyaslandığında her iki grubun da 96 ms dalga genişliğine sahip olduğu görülmüştür. P2 dalga piki incelendiğinde kontrol grubu ortalaması 3.54±0.57 µV, covid grubu ortalaması ise 3.50±0.33 µV olarak ölçüldü ve gruplar arasında fark yoktu (p=0.11). Bununla birlikte P2 dalga Δt genliği her iki grup arasında değerlendirildiğinde gruplar arasında fark bulunmadı (p=0.096). N2 amplitüdleri değerlendirildiğinde her iki grup arasında anlamlı fark görüldü [F(69)=5.64, p<0.001]. N2 latansları değerlendirildiğinde (Şekil-4) covid grubunun (306.44±10.5), kontrol grubundan (278.34±9.9) daha geç tepe değere ulaştığı görüldü [F(69)=(11.5, p<0.001)]. N2 Δt latansları bakımından dalgaların oluşum süreci benzerdi; sırasıyla kontrol grubunda 70±3 ms, covid grubunda ise 72±4 ms olarak ölçüldü ve aralarında fark bulunamadı (p=0.098).



Şekil 4. Olay ilişkili potansiyel kayıtlarına göre uyumsuz uyaran sunumunda gözlenen gruplara ait N2 latansları

*Cz kanalı N2 kompleksine ait latans (ms), * Covid grubunun N2 latansı uyumsuz uyaran sunumunda kontrol grubundan yüksek olduğu görüldü (p<0.001)*

P3 amplitüdü kontrol grubu için 3.23± 0.35 μV, covid grubu için 3.40±0.43 μV olarak ölçüldü ve gruplara arasında anlamlı bir fark yoktu (p=0.086). P3 latansı karşılaştırıldığında covid grubu (399.86±14.18) latansı, kontrol grubundan (383.22±12.8) büyük olsa da istatistiksel olarak fark gösterilemedi (p=0.061). P3 Δt covid grubunun (133±22 ms), kontrol grubu dalgacık genişliğinden (95±12 ms) daha fazla olduğu, P3'ün daha uzun bir biçimde tamamlandığı gözlendi [F(69)=8.71, p<0.001].

TARTIŞMA

Bu çalışmada COVID-19 enfeksiyonuna bağlı gelişen uzun covid sendromunun kognitif etkilerinin denetlenmesi amaçlandı. Ülkemizde COVID-19 sonrası bilişsel fonksiyonlar bakımından yapılan araştırma ve değerlendirmeler sınırlıdır. Köse A. ve ark. gerçekleştirdikleri bir anket çalışmasında covid sonrasında dikkati sürdürme yeteneğinde bozulmayı ifade etmiştir[21]. Girişte belirtildiği gibi uzun covid sendromunun mental yorgunluk, hafızanın zayıflaması ve beyin sisi olarak özetlenen etkileri[2,4,7,22], literatürde yönelim etkisi olarak ifade edilen Simon görevi ile denetlendi (21). Simon etkisi flanker veya stroop testine benzer biçimde uyaran-cevap ilişkisinin incelendiği, uyaranın yarattığı çelişki biçimine göre cevabın oluşturulduğu bir testtir[17,18,23,24].

Simon görevi bilişsel sistemde karmaşa yaratan, bu çelişki çözümlenirken merkezi sinir sisteminin incelenmesine olanak sağlayan bir görevdir[18]. Bizde bu görevinden yararlanarak hasta ve kontrol grubundaki bireylerin bilişsel işlem çözümleme performansını karşılaştırdık. Sonuçlarda da ifade edildiği gibi uyaran sunumu ve beklenen cevabın aynı tarafta olduğu; uyumlu uyaran sunumunda hasta ve kontrol grupları arasında OİP dalgaları ile ilgili bir fark bulunmadığı görüldü (Şekil 1). Ancak asıl çelişkinin yaratıldığı daha çok inhibitör sistemle denetlenen uyumsuz uyaran sunumunda ise hasta ve kontrol grubu dalgacıkları arasında farklılar gözlendi (Şekil 2). Bazı sonuçlar istatistiksel olarak gösterilemese de uyumsuz uyaran sunumunda covid grubuna ait dalgaların ortalaması ile kontrol grubuna ait dalgalarda özellikle P3'te genişleme görüldü. Aynı zamanda N2-P3 kayması gruplar arasında belirgindi (Şekil 2). N2'nin uyumsuz uyaran sunumunda uyumlu uyaran sunumuna göre genlik artışı uyumsuz uyaranın yarattığı çelişki ile açıklanır. Zhang ve arkadaşları N2 komponentinin bilişsel kontrolün ön işlemi olduğunu ifade etmiştir[25]. Folstein ve Van Petten N2 komponentini; motor işlem öncesi strateji belirleme süreci olarak ifade etmiştir. Yazar aynı zamanda "novel" yani olağanın dışında gelişen uyaranın çözümlenme sürecinde yaşanan bir mismatch negatifliği olarak tanımlamıştır[26]. Diğer bir çalışmada N2 komponentinin uyumsuz uyaran sunumunda belirginleştiği, bunun da cevap seçiminden önce bilişsel bir çelişkinin ortadan kaldırılma için gerekli inhibitör bir düzenlemenin anterior singulat korteks tarafından gerçekleştirildiğini ifade edilmiştir[27]. Davranış sonuçlarımızda gözlediğimiz covid grubunda gözlenen reaksiyon zamanındaki uzama öncelikle uyarana motor cevabın seçimi ile ilgili bir gecikme olduğu, bu gecikmenin de covid grubunda kontrol grubuna göre daha uzun olduğu anlaşılmıştır. Yine çalışmamızda gözlenen bulgulardan diğeri ise covid grubunda P3 dalgasının aktivasyonundaki gecikmedir (Şekil 2). P3 dalgası OİP çalışmalarında en fazla başvurulan dalga tipidir. Bu dalga uyaranların seçimi ile ilgili karar aşamasının özelliklerini yansıtması bakımından önemlidir.

İşitsel uyaranların sunulduğu çalışmamızda enformasyonun spaysal olarak tesbiti ve çözümlenmesinin gerçekleştirildiği alan temporal lobdan kalkarak dikkatin oluşumunda

ön işlem basamağı olan anteriyor singulat korteks alanıdır[28]. Bu bölgenin işlek bellek ve dikkatin oluşumunda görev aldığı bilinmektedir[27,28]. Çalışmamızdan elde ettiğimiz bulgulara göre; uyaranın değerlendirme sürecini ve karar aşamasını yansıtan hem N2 hem de N2-P3'ün covid grubundaki latans artışının ve aktivasyon gecikmesinin; bu komplekslerin oluşumundan sorumlu anterior singulat korteksin etkilendiğini göstermiştir. Çalışmamızı doğrular biçimde Hugon ve ark. COVID-19'a bağlı beyin sisi olarak adlandırılan dikkat, konsantrasyon eksikliği ve hafıza problem yaşadığını beyan eden iki vakada; poziron emisyon tomografi (PET) yöntemi ile flurodeoksiglukoz (FDG) tutulumunu incelemiş; singulat kortekste belirgin olarak hipometabolizmayı göstermiştir[4]. Bunun yanında Diaz ve arkadaşları orbitofrontal, frontal ve singulat bölgelerin COVID-19'dan etkilendiğini göstermiştir[9]. Bizim çalışmamızda da uyumsuz uyaranın yarattığı çelişkiye bağlı olarak covid grubunda gözlenen OİP dalgalarındaki aktivasyon kayması görevin için "telafi süresi"'ne ihtiyaç duyduğunu, uyarana karşın cevabın oluşturulması esnasında çelişkinin değerlendirilme aşamasını etkilediğini, bu işlemlerinin gerçekleştiği anterior singulat korteksin etkilendiğini göstermektedir.

Bu çalışma demografik özellikler tablosunda gösterildiği gibi, hastalığı asemptomatik ve hafif etkilerle geçirmiş bireylerin 12 hafta sonra kognitif etkileniminin varlığını, dar bir yaş aralığında sorguladı. Bunun sebebi yaşlanma ve diğer kronik hastalık risklerinin bilişsel performansı değiştirerek çalışmanın homojenitesine etki etmesinden sakınmak içindir. Popülasyonun büyük bir kesiti; hastalığı hafif ve asemptomatik olarak atlattığı için, bu çalışmanın sonuçları popülasyonun genelinde gözlenebilecek etkilerin anlaşılmasına da katkı sağlayacaktır. Benzer çalışmalar; COVID-19 sebebiyle yoğun bakımda tedavi görmüş hasta gruplarının da incelendiği daha büyük bir kesitte yapıldığında COVID-19'un kognitif sisteme etkilerinin boyutu daha net görülebilir. Bu manada bu çalışma kesitinin darlığı çalışmanın kısıtlılığı olarak nitelendirilebilir.

SONUÇ

Çalışma bulgularımız literatürde COVID-19'a bağlı gelişen singulat korteksin etkilenimini gösteren vaka örneklerini doğrulayan ve açıklayan sonuçlar ortaya çıkarmıştır. Bu sonuçlara göre reaksiyon süresinin covid grubunda uzadığını, uzun covid sebebiyle rapor edilen beyin sisinin; uyaranın çözümlenmesi aşamasındaki, dikkatin ön işlem aşamasını etkileyerek meydana geldiğini göstermiştir.

Etik Kurul Onayı: Bu çalışma uluslararası Helsinki Deklarasyonu esaslarına uygun olarak yürütülmüş, Avrasya Üniversitesi girişimsel olmayan klinik araştırmalar etik kurulu 24.06.2022 tarih 12 sayılı toplantıda; E-69268593-050-13227 sayı no'lu onayı sonrası gerçekleştirilmiştir.

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Editöre Mektup / Letter to Edıtor

İntragastrik Balon Sonrası Gelişen Nadir Komplikasyon: Akut Pankreatit

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Öz

Obezite tedavisinde intragastrik balon (İGB) kısa süreli kilo vermek için bariatrik cerrahi öncesi tercih edilen minimal invaziv yöntemdir (1). Literatürde endoskopik intragastrik balonla ilişkili akut pankreatit çok az vakada saptanmıştır

Anahtar kelimeler: Obezite, İntragastrik balon, Pankreatit

Rare Complication After Intragastric Balloon: Acute Pancreatitis

Abstract

Intragastric balloon (IGB) is the preferred minimally invasive method before bariatric surgery for short-term weight loss in obesity treatment (1). Acute pancreatitis associated with endoscopic intragastric balloon has been detected in very few cases in the literature. It is a rare, interesting case and we aimed to present it to raise awareness among physicians.

Key words: Obesity, Intragastric balloon, Pancreatitis.

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Sayın Editör;

Obezite sıklığı gittikçe artan önemli mortalite ve morbiditeyle ilişkili bir hastalıktır. Dünya genelinde yaklaşık 1.4 milyar erişkin obez veya aşırı kiloludur. Dünyada fazla kilolu olanların görülme oranı %39 ve obezite görülme oranı ise %13'tür. Obezite hipertansiyon, koroner arter hastalığı, tip 2 diyabetes mellitus ve malignite gelişiminde önemli bir risk faktörüdür[1,2]. Obezite tedavisinde yaşam tarzı düzenlenmesi, intragastrik balon veya bariatrik cerrahi gibi yöntemler kullanılır. İntragastrik balon (İGB) kısa süreli kilo vermek için bariatrik cerrahi öncesi tercih edilen minimal invaziv yöntemdir. Mekanik olarak gastrik distansiyon yaparak tokluk hissi oluşturur. İntra gastrik balon 6 ay boyunca bekletilir. Yaklaşık %15-20 kadar kilo kaybı olması beklenir[1,3]. Endoskopik intragastik balonla ilişkili akut pankreatit gelişimi nadir bir komplikasyon olup literatürde çok az vakada saptanmıştır.

35 yaş, erkek hasta, epigastrik bölgede ani başlayan sırta vuran karın ağrısı, kusma şikayetiyle başvurdu. Fizik muayenesi epigastrik bölgede hafif hassasiyet dışında olağandı. Vital bulguları stabildi. Ek hastalık, operasyon veya travma öyküsü yoktu. Alkol veya başka ilaç alımı yoktu. Yaklaşık iki hafta önce dış merkezde %0,9 NACL içerisine metilen mavisi konularak yaklaşık 450 cc şişirilmiş endoskopik İGB yerleştirilme öyküsü mevcuttu. Laboratuar tetkikleri; lökosit: 8700 UL (3900-10900), kreatinin: 0.8 mg/dl (0.7-1.2), AST: 23 U/L (0-40), ALT: 41 U/L (0-41), Total bilurubin: 0.5 mg/dl (0-1.4), GGT:18 U/L (10-71), trigliserid:113 (<200),amilaz: 1163 U/L, lipaz: 4795 U/L (13-60), CRP: 6 mg/dl (0-5). Batın tomografisinde mide lümeni içerisinde mide balonu ile uyumlu olabilecek terapötik materyal izlenmiştir. Pankreas kuyruk kesimi komşuluğunda sol anterıor pararenal yağ planlarında heterojen kirlenme ve effüzyon izlenmiştir. Pankreatit ile uyumlu saptandı. Diğer yapılar olağan idi. MRCP'de intra ve ekstrahepatik safra yolları olağan idi. Pankreatit ile uyumlu idi. Akut ödematöz pankreatit tanısıyla servise yatırıldı. Diğer pankreatit nedenleri ekarte edildi. Mevcut klinik bulguların intragastrik balonla ilişkili olması nedeniyle yatırıldıktan sonra ertesi gün endoskopik olarak balon çıkarıldı. İşlem sonrası takiplerinde klinik ve labortuar bulguların normale geldiği görüldü.

Endoskopik intragastrik balon (İGB) işlemi iyi etkinlik, düşük maliyet, düşük mortalite ve morbiditeye sahiptir. İGB yerleştirilmesi sonrası bulantı, karın ağrısı ve reflü gibi hafif komplikasyonlar gelişebilir. Ayrıca gastrik ülser, perforasyon ve barsak tıkanıklığı gibi ciddi komplikasyonlar da gelişebilir[1]. Akut pankreatit ise çok nadir gelişen bir komplikasyondur. Klinik, radyolojik ve laboratuar bulgular ile tanı desteklenmelidir. Fundus yerleşimi sonucu balonun mekanik basısı veya katater basısına bağlı görülebilir. Mutlaka balonun boyutu, şekli, hacmi ve yerleştirme konumuna dikkat edilmelidir. Kesin tedavisi balonun çıkarılmasıdır. Genellikle hafif pankreatit kliniği olup balonun çıkarılması sonrası iyileşme gözlenir[4,5].

Sonuç olarak intragastrik balonla ilişkili akut pankreatit nadir gelişebilir. Bundan dolayı balon yerleşimi, balonun boyutu ve balonun hacmi ile ilgili değişikliklerin etkisini araştıran çalışmalara ihtiyaç vardır. Son zamanlarda intragastrik balon obezite tedavisinde rutin pratikte çok sık kullanıldığı için komplikasyonlarıyla daha sık karşılaşılmaktadır. Nadir ve ciddi görülebilen komplikasyonların yönetiminde daha dikkatli olunmalıdır.

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