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CONTENTS

ORIGINAL ARTICLE

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Clinicopathological Evaluation of Renal Biopsies Among Older Adults in Turkiye

Yaşlı Türk Toplumunda Böbrek Biyopsilerinin Klinikopatolojik Değerlendirilmesi

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ABSTRACT

Aim: Kidney disease is common in older adults due to age-related structural and functional changes in the kidneys, higher rates of chronic disease, and increased drug use. As societies age, there is a rise in the prevalence of renal disease and the number of kidney biopsies being performed in older patients. This study aimed to investigate renal biopsy indications, complications, pathology results, and subsequent treatment among older adults in Turkey.

Materials and Methods: We retrospectively analyzed data from patients aged 65 and over who underwent renal biopsy in a university nephrology department between 2004 and 2023. The patients' demographic information, chronic comorbidities, biopsy indications, pre-biopsy laboratory values, post-biopsy complications, pathology results, and post-biopsy treatments were obtained by reviewing their medical records and biopsy reports.

Results: A total of 66 patients were included in the study. The median age was 73.0 years (IQR: 68.8-79.0 years) and 66.7% of the patients were men. The most common comorbidities were hypertension (83.3%), diabetes mellitus (24.3%), coronary artery disease (22.7%), and chronic kidney disease (21.2%). The most common indication for renal biopsy was nephrotic-range proteinuria (56.1%), followed by acute kidney injury (24.2%). When the pathology results were examined, primary glomerulonephritis (62.1%) was the most common result, followed by secondary glomerulonephritis (21.2%) and tubulointerstitial nephritis (12.1%). The most common histopathological diagnoses in primary glomerulonephritis were membranous glomerulonephritis (39.4%) and focal segmental glomerulosclerosis (12.1%), while those in secondary glomerulonephritis were secondary amyloidosis (9.1%) and lupus nephritis (4.5%). After biopsy, 54.5% of the patients received immunosuppressive therapy and 34.8% received renal replacement therapy. No post-biopsy complications were observed.

Conclusion: Although the most common indication for kidney biopsy in older adults is nephrotic-range proteinuria. Kidney biopsy is the gold standard method for the diagnosis of renal parenchymal diseases and is a safe procedure in older patients, with low complication rates. Kidney biopsy should not be avoided in geriatric patients if deemed clinically necessary.

Keywords: Elderly, kidney biopsy, pathology, glomerulonephritis, complication

ÖZ

Amaç: Yaşlanmaya bağlı böbreklerde meydana gelen yapısal ve fonksiyonel değişiklikler, artmış kronik komorbid hastalıklar ve ilaç kullanım sıklığında artışa bağlı olarak yaşlılarda böbrek hastalıklarının sıklığı artmaktadır. Toplumun yaşlanmasıyla birlikte böbrek hastalıklarının prevalansı ve yaşlılarda yapılan böbrek biyopsilerinin sayısı artmaktadır. Bu çalışmada yaşlı hastalarda böbrek biyopsisi endikasyonları, komplikasyonları, patolojik sonuçları ve tedavilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Üniversitemiz nefroloji bilim dalında takip edilmekte olup 2004-2023 yılları arasında böbrek biyopsisi yapılan 65 yaş ve üzeri hastaların verileri retrospektif olarak incelendi. Hastaların demografik bilgileri, kronik hastalıkları, biyopsi endikasyonları, biyopsi öncesi laboratuvar

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değerleri, biyopsi sonrası gelişen komplikasyonlar, patoloji sonuçları ve biyopsi sonrası verilen tedaviler hastaların tıbbi kayıtları ve biyopsi raporları incelenerek kaydedildi.

Bulgular: Çalışmamıza toplam 66 hasta dahil edildi. Hastaların yaş ortancası 73,0 (IQR: 68,8-79,0) yıl olup, %66,7'si erkekti. En sık görülen kronik sistemik hastalıklar hipertansiyon (%83,3), diabetes mellitus (%24,3), koroner arter hastalığı (%22,7) ve kronik böbrek yetmezliği (%21,2) idi. Nefrotik düzeyde proteinüri (%56,1) en sık böbrek biyopsi endikasyonuydu. İkinci en sık biyopsi endikasyonunun akut böbrek hasarı (%24,2) olduğu görüldü. Patoloji sonuçları incelendiğinde primer glomerülonefrit (%62,1) en sık saptanan biyopsi sonucu iken, bunu sırasıyla sekonder glomerülonefritin (%21,2) ve tübülointerstisyel nefritin (%12,1) izlediği görüldü. Primer glomerülonefritte en sık görülen histopatolojik tanılar membranöz glomerülonefrit (%39,4) ve fokal segmental glomerüloskleroz (%12,1) iken, sekonder glomerülonefritte sekonder amiloidoz (%9,1) ve lupus nefriti (%4,5) idi. Biyopsi sonrasında hastalara en sık immünosupresif tedaviler (%54,5) uygulanırken, %34,8 hastaya ise renal replasman tedavisinin uygulandığı görüldü. Yapılan biyopsiler sonrasında komplikasyon görülmedi.

Sonuç: Yaşlılarda en sık böbrek biyopsi endikasyonu nefrotik düzeyde proteinüridir. Böbrek biyopsisi renal parankimal hastalıkların tanısında altın standart yöntemdir ve yaşlılarda düşük komplikasyon oranları ile güvenilir bir işlemdir. Klinik gereklilik halinde yaşlılarda böbrek biyopsisinden kaçınılmamalıdır.

Anahtar Kelimeler: Yaşlı, böbrek biyopsisi, patoloji, glomerülonefrit, komplikasyon

INTRODUCTION

Kidney disease is a common clinical problem in the elderly and is associated with increased mortality and morbidity. The prevalence of many kidney diseases, especially chronic kidney disease (CKD), increases in the elderly. This is mainly attributed to the increasing prevalence of traditional risk factors for kidney diseases, such as diabetes mellitus (DM), hypertension (HT) and cardiovascular diseases, and changes in the genitourinary system with aging[1,2]. Various anatomical and functional changes occur in the kidney with aging. Cortical parenchyma, functional nephron number, renal blood flow and glomerular filtration rate (GFR) decrease. Due to these aging-related changes in the kidney and increased comorbid diseases, the physiological reserves of the kidney decrease and the adaptation response to stressors deteriorates; many renal diseases, especially acute kidney injury, are observed more easily and frequently[3-5].

Renal biopsy is the sampling of renal tissue by methods such as percutaneous renal biopsy or fine needle aspiration. Renal biopsy is the gold standard in the diagnosis, treatment and prognosis of renal parenchymal diseases[6]. In the elderly population, indications for renal biopsy in nephrology practice are similar to that in all age groups. Kidney biopsy indications include nephrotic syndrome and non-nephrotic proteinuria, acute kidney injury, isolated microscopic/macroscopic hematuria, coexistence of proteinuria-hematuria, systemic diseases with loss of renal function, and renal allograft dysfunction[7].

There are special considerations when making the decision to perform renal biopsy in elderly patients. As with many invasive procedures in the elderly population, renal biopsy may have a high complication rate due to factors such as physiologic changes related to aging, accompanying comorbid diseases, polypharmacy and contrast exposure[8].

The number and proportion of the elderly population in Turkey and the world is increasing rapidly. It is estimated that there were 783 million elderly people worldwide in 2022 and that this number will increase to 1.3 billion in 2040. In Turkey, the elderly population, which was 7.18 million in 2018, increased by 21.4% in the last five years and reached 8.7 million in 2023, and this figure is expected to increase to approximately 16 million in 2040[9]. In parallel with the increasing elderly population, the number of renal biopsies in the elderly is gradually increasing[10,11]. In our country, studies evaluating the results of renal biopsy in the elderly are limited in number. In this study, we aimed to investigate the indications, complications, pathologic results and treatments of renal biopsy in elderly patients.

MATERIALS AND METHODS

Our study is a retrospective descriptive cross-sectional study among patients aged 65 years and older, who underwent ultrasonography-guided renal biopsy using a 16-18 G automatic biopsy needle between January 01, 2004 and January 01, 2023 in the department of nephrology of our hospital, a tertiary care university hospital.

Inclusion criteria were determined as;

- Having kidney biopsy performed,

- Being 65 years of age or older and being followed up in the nephrology clinic of our university.

Exclusion crtiteria were as follows;

- Having transplanted kidney biopsies,

- Undergoing biopsy for malignancy,

- Having kidney biopsy procedure that was performed in our hospital, but being followed up and treated in another hospital,

- Having unmeasured proteinuria in 24-hour urine (followed up with spot urine protein/creatinine ratio),

- Having insufficiently recorded available data,

- Being younger than 65 years of age.

Demographic information, biopsy date, chronic diseases, indications for biopsy, pre-biopsy laboratory data, complications after biopsy, pathology results, post-biopsy treatments and complications after treatment were recorded from patient files and hospital information system. Indications for biopsy were grouped as nephrotic proteinuria, acute kidney injury, non-nephrotic proteinuria and micro/macrohematuria. Nephrotic proteinuria was defined as protein excretion above 3.5 g/day in 24-hour urine, acute kidney injury as serum creatinine level ≥0.3 mg/dL or ≥0.5-fold increase from baseline in the last 48 hours or ≥1.5-fold increase from baseline in the last 7 days or urine output less than 0.5 mL/kg/hour in the last 6 hours, non-nephrotic proteinuria as the presence of proteinuria below 3.5 g/day without accompanying hematuria, and micro/macrohematuria as the presence of hematuria without accompanying proteinuria. Proteinuria was considered as the presence of more than 500 mg of protein in the 24-hour urine, microscopic hematuria was defined as the presence of ≥3 erythrocytes in each large magnification field on microscopic examination of urine sediment, and macroscopic hematuria was defined as hematuria that caused discoloration in the urine that was visible to the naked eye. Serum creatinine, albumin, uric acid, estimated GFR, 24-hour urine proteinuria and hematuria were recorded before biopsy. Creatinine clearance was calculated according to the Cockcroft and Gault[12] and MDRD-4 (Modification of Diet in Renal Disease-4)[13] formulas. Pathologic classification was made into 5 groups as primary glomerulonephritis (PGN), secondary glomerulonephritis (SGN), tubulointerstitial nephritis (TIN), vascular diseases and unclassified cases.

For all patients, renal biopsy specimens examined by light microscopy and immunofluorescence microscopy were considered adequate if there were at least 10 glomeruli in the sample[14]. Immunosuppressive treatments given to the patients after biopsy were recorded. Immunosuppressive treatments were grouped as glucocorticoids, cyclophosphamide, mycophenolate mofetil.

Complications developing after biopsy were grouped as major and minor. Complications such as bleeding requiring transfusion, macroscopic hematuria, penetration to liver, spleen, pancreas, intestine and gallbladder, pneumothorax, hemothorax, development of arteriovenous fistula and death were considered major complications and complications such as pain and perirenal hematoma were considered minor complications.

The study was conducted after obtaining the necessary permissions from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (decision no: B.30.2.ATA.0.01.00/862, date: 26.10.2023).

Statistical Analysis

Data were recorded into the Statistical Package for the Social Sciences 23.0 package program and analyses were performed using the same program. Data were presented as number (n), percentage (%) and median (minimum-maximum). Descriptive statistics were given as median and minimum-maximum median for nonparametric continuous data. Categorical data were presented as frequencies with percentages in parentheses and compared using the chi-square test. The Mann-Whitney U test was used to determine the differences in the rating scores, which were considered as continuous data. A probability value less than 0.05 was considered to be statistically significant.

RESULTS

In our study, 66 geriatric patients who underwent renal biopsy were retrospectively evaluated. The median age of the patients was 73.0 (IQR; 68.8-79.0) years and 44 (66.7%) were male. When the patients were evaluated in terms of chronic systemic diseases, HT (83.3%), DM (24.3%), coronary artery disease (22.7%) and CKD (21.2%) were detected to be the most common diseases. Demographic characteristics and underlying diseases of the patients are shown in Table 1.

When the patients were evaluated in terms of indications for renal biopsy, nephrotic proteinuria (n=37, 56.1%) was found to be the most common indication for renal biopsy. The second most common indication for biopsy was acute kidney injury (n=16, 24.2%). The median pre-biopsy serum creatinine, albumin and proteinuria levels were 2.14 (0.99-4.63) mg/dL, 2.51 (2.10-2.95) g/dl, and 4770 (1156-7644) mg/day, respectively. Indications for renal biopsy and pre-biopsy laboratory data are presented in Table 2. None of the patients developed major or minor complications after biopsy.

PGN (62.1%) was the most common biopsy result, followed by SGN (21.2%) and TIN (12.1%). Membranous glomerulonephritis (MGN) was the most common primary glomerular disease

Table 1. Demographic characteristics and underlying diseases of the patients

Age, median (IQR)	73.0 (68.8-79.0)
Sex, n (%), male	44 (66.7)
Underlying diseases, n (%)	
HT	55 (83.3)
DM	16 (24.3)
CAD	15 (22.7)
CHF	6 (9.1)
Malignancy	5 (7.6)
COPD	5 (7.6)
CVD	2 (3.0)
Chronic liver disease	1 (1.5)
Number of disease, median (IQR)	3 (2-4)

IQR: Interquartile range, HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, CHF: Congestive heart failure, COPD: Chronic obstructive pulmonary disease, CVD: Cerebrovascular disease

(39.4%), while secondary amyloidosis was the most common secondary glomerular disease (9.1%). When pathology results were evaluated without differentiating between primary and secondary GN, the most common diagnoses were revealed to be MGN (39.4%), focal segmental glomerulosclerosis (FSGS) (12.1%), secondary amyloidosis (9.1%), and chronic TIN (9.1%) (Table 3).

Immunosuppressive treatment was administered to the patients most frequently (54.5%) after biopsy. Glucocorticoids (48.5%) and cyclophosphamide (22.7%) were the most common immunosuppressive treatments. The treatments given to the patients after biopsy are presented in Table 4.

Table 2. Renal biopsy indications of the patients and laboratory data before biopsy

Indication for biopsy, n (%)	
Nephrotic proteinuria	37 (56.1)
Acute kidney disease	16 (24.2)
Non-nephrotic proteinuria	9 (13.6)
Abnormal urinary system findings	3 (4.5)
Micro-macro hematuria	1 (1.5)
Laboratory, median (IQR)	
Proteinuria level (mg/day)	4770 (1156-7644)
Hematuria level (≥3 erythrocytes/hpf)	4 (2-25)
Serum uric acid (mg/dL)	6.0 (5.3-7.4)
Serum creatine (mg/dL)	2.14 (0.99-4.63)
Serum albumin (g/dL)	2.51 (2.10-2.95)
eGFR Cockcroft and Gault[12] (mL/min/1.73 m^2)	34.2 (15.2-78.5)
eGFR MDRD (mL/min/1.73 m^2)	27.3 (11.2-72.2)

IQR: Interquartile range, eGFR: Estimated glomerular filtration rate, MDRD: Modification of Diet in Renal Disease-4

Table 3. Pathology results of renal biopsy, n (%)

PGN	**41 (62.1)**
MGN	26 (39.4)
FSGS	8 (12.1)
MPGN	3 (4.5)
Crecentric glomerulonephritis	1 (1.5)
Other	3 (4.5)
SGN	**14 (21.2)**
Secondary amyloidosis	6 (9.1)
Lupus nephritis	3 (4.5)
ANCA-associated vasculitis	2 (3.0)
DM	2 (3.0)
Primary amyloidosis	1 (1.5)
TIN	**8 (12.1)**
Chronic TIN	6 (9.1)
Acute TIN	2 (3.0)

PGN: Primary glomerulonephrite, FSGS: Focal segmental glomerulosclerosis, MPGN: Membranoproliferative glomerulonephrite, SGN: Secondary glomerulonephrite, DM: Diabetes mellitus, TIN: Tubulointerstitial nephritis

Table 4. Treatments given to patients after biopsy, n (%)

Immunosuppressive therapy	36 (54.5)
Glucocorticoid	32 (48.5)
Cyclophosphamide	15 (22.7)
Mycophenolate mofetil	4 (6.1)
Immunoglobulin modulator	3 (4.5)

DISCUSSION

Renal biopsy is the gold standard for determining whether glomerular lesions are acute or chronic, reversible or treatable, regardless of age[14]. Identification of renal lesions by biopsy enables more accurate identification of renal pathologies without being dependent on diagnostic methods such as creatinine-based GFR (eGFR) calculations, which can be affected by many age-related factors. Thus, it allows the selection of the right treatment modalities. It helps to avoid inappropriate treatments, especially immunosuppression, and related complications. Early diagnosis and correct treatment may be of vital importance in the elderly, especially in the frail elderly population. In the literature, it has been shown that renal damage tends to become chronic faster in the elderly compared to young people due to low renal reserve and decreased renal mass and function[15].

In our study, which included a total of 66 geriatric patients who underwent kidney biopsy, it was observed that kidney biopsy was performed more frequently in male patients (66.7%), in line with the literature[16,17]. It is known that, starting from the fourth decade of aging, there is a decrease in kidney size due to a decrease in the renal cortical parenchyma and the number of functional nephrons. It has been shown that the decrease in kidney size is greater in the male gender[3]. It has also been shown that gender is one of the determinants of age-related decline in renal functions, that most of the damage that occurs in the kidney with age is related to androgen production, and that medical castration can slow the progression of these changes[18,19]. Kidney biopsy is performed more frequently in men, which may be due to the fact that renal functions and renal parenchyma loss are greater in men and the number of cases is lower. In our study, the most common chronic systemic diseases in patients who underwent kidney biopsy were found to be HT (83.3%), DM (24.3%), CAD (22.7%) and CKD (21.2%). Studies have reported that HT is the most common chronic disease in elderly patients who underwent biopsy, with rates ranging from 24.1% to 78%[20-24]. The rate of DM was reported to be 15.3% by Ozturk et al.[20] and 29.4% by Tuğcu et al.[23], similar to that in our study. As renal reserves decrease with aging, additional diseases that may cause kidney disease, especially DM, atherosclerotic vascular diseases and HT, facilitate the development of new kidney pathologies. Studies have shown that approximately 5-10% of the elderly have a decrease in kidney function with age, despite the absence of any accelerating factors, while no measurable decrease is detected in 30% of them[25]. eGFR can be expected

to decrease with aging. However, normal eGFR values have also been detected, especially in normotensive elderly people[5]. This shows us the contribution of chronic comorbid diseases to the progression of renal dysfunction.

Although biopsy indications vary on a national or center basis in the literature, it has been reported in many biopsy series that the most common indication in the elderly is nephrotic proteinuria[11,23,24,26,27]. In our study, nephrotic proteinuria (56.1%) was found to be the most common biopsy indication in the elderly. Studies conducted in our country have reported that the rate of kidney biopsy performed with the indication of nephrotic proteinuria in the elderly is between 41.38% and 60%[11,21-24,26,28]. The fact that nephrotic proteinuria is the most common biopsy indication may be due to the fact that the elderly see the symptoms as a part of the natural process of aging and apply to the hospital late. Similar to our study, studies have shown that acute kidney disease (AKD) is the second most common biopsy indication in the elderly and that biopsy due to AKD is performed more frequently in the elderly than in young people[21,23,24,29]. Additionally, in two studies conducted in elderly patients, AKD was reported to be the most common indication[30,31]. Studies have proven that the incidence of AKD increases with age[32]. Among the elderly, the frequency of AKD increases significantly as age increases[33]. The elderly are prone to kidney damage due to structural and functional changes in the kidney with aging, increased comorbid diseases and polypharmacy[4]. Increased biopsy rates due to AKD in the elderly may be related to the fact that the elderly are more prone to AKD and have a higher probability of prolonging the duration of AKD and becoming chronic due to their low renal reserves.

In our study, similar to studies conducted in our country[21,22,24,34], PGN (62.1%) was the most frequently detected biopsy result, followed by SGN (21.2%) and TIN (12.1%), respectively. In studies conducted abroad and involving large patient groups, it has been shown that PGN is the most common disease in the elderly, followed by SGN and TIN[35]. In the study by Harmankaya et al.[24] in 2015, in which they evaluated 98 elderly patients, the most frequently detected PGN type was stated as MGN (14.3%). This was followed by FSGS (12.2%) and crescentic GN (6.1%)[24]. In the study by Tuğcu et al.[23] in which kidney biopsies of 109 elderly patients were evaluated, the most common causes of PGN were found to be FSGS (13.8%), MGN (10.1%) and pauci-immune glomerulonephritis (PIGN) (5.5%), respectively. In the study of Hur et al.[34], in which 121 elderly patients who underwent kidney biopsy were included, it was reported that the most common PGNs were MGN (14.8%), crescentic GN (9.92%) and FSGS (9.92%). In another study conducted by Ozdemir et al.[26] on 93 elderly patients and presented in 2022, MGN (42.8%) was found to be the most common pathology among PGNs. In the study conducted by the Turkish Nephrology Association 'Glomerulonephritis Study Group', in which 47 centers participated and which included the largest number of biopsy series regarding PGNs in our country, only primary glomerular diseases were included and 3,858 patients, 262 of whom were elderly, were evaluated. In this study, MGN (40.2%), FSGS (17.4%) and crescentic GN (15.1%) were most commonly observed in the elderly. In addition, in the period covering 2017 and before, crescentic GN (23%) was the second most common type of GN and FSGS (15.2%) was the third most common type of GN. It has been stated that as of 2017, FSGS has become the second most common GN and the frequency of FSGS in the elderly is gradually increasing[11]. Studies have shown that the incidence of FSGS in the elderly is increasing worldwide[23,36]. This increase has been attributed to increased awareness of FSGS and an increase in the incidence of FSGS in the elderly secondary to diseases such as HT and age-related nephropathy[30]. In studies conducted abroad, while MGN stands out as the most frequently detected PGN in Spain[37], Czech Republic[38], Italy[39] and England[40] in Europe, the second one changes FSGS, minimal change disease and IgA nephropathy (IgAN). In studies conducted outside Europe, PGN and MGN were most frequently detected as in all other studies in Brazil[41], South Africa[42], Ireland[43], China[44], Japan[35] and the United States[30]. It has been reported that FSGS and IgAN are the second most common. The distribution of glomerulonephritis types varies from country to country and in different regions of the same country, depending on age, gender, ethnicity, geographical region, clinicians' attitudes towards indications and years. As in recent studies conducted in our country[11,24], in our study, MGN (39.4%) was found to be the most common and FSGS (12.1%) was the second most common glomerular pathology, both among PGNs and among all patients who underwent biopsy. In terms of the frequency of PGN, the results of our study are similar to those reported by recent large-scale studies conducted in our country[11,26].

In the study conducted by Tuğcu et al.[23], while the most common cause of SGN was found to be secondary amyloidosis (22.9%), diabetic nephropathy (DN) was reported to be the second most common and lupus nephritis (LN) was reported to be at the rate of 3.6%. In the study conducted by Harmankaya et al.[24], amyloidosis was found to be the most common SGN with the rate of 15.3%, followed by PIGN (8.2%) and DN (5.1%). Hur et al.[34] found that amyloidosis (19.1%) was the most common cause of SGN, followed by GNs due to vasculitis (4.96%) and LN (1.65%). In European, American and Asian countries, it has been reported that SGN due to LN is more common than secondary amyloidosis and vasculitis[30,37,39,42]. The frequent occurrence of secondary amyloidosis in our study and in our country is due to the fact that Familial Mediterranean Fever is an endemic disease in Turkey and the most common cause of secondary amyloidosis[45]. In our study, the frequency of DN is low and the rates are consistent with the literature[23,24,34]. Although DN is the most important cause of ESRD, it is rarely

observed in biopsy results. The reason for this is that the diagnosis of DN is made clinically and biopsy is not preferred unless there is additional evidence suggesting PGN[46]. In our study, although 24.3% of the patients who underwent biopsy were diagnosed with DM, the rate of patients diagnosed with DN as a result of biopsy was 3%.

Complications seen after kidney biopsy include pain, hematoma, macroscopic hematuria, major hemorrhage (bleeding requiring transfusion or radiological/surgical intervention), septicemia, and arteriovenous fistula formation[47]. Although theoretically there is no difference in the indications for biopsy between young and old patients, biopsy can be avoided in the elderly due to concomitant systemic diseases, low life expectancy, clinicians' avoiding biopsy and immunosuppressive treatment complications, and the thought that biopsy will show findings of chronic changes such as interstitial fibrosis and atrophy rather than a treatable lesion[27,30]. The frequency of complications after kidney biopsy varies due to patient selection, procedural techniques, variability in complication definitions, and differences in post-procedure monitoring time, but is on average 5-10%[47]. Serious side effects requiring surgical intervention occur at a rate of <1% and the mortality rate is <0.1%[27]. Kajawo et al.[48] performed a meta-analysis and they stated that complication rates decreased after biopsy procedures performed under ultrasound guidance and automatic needles. In the literature, it has been reported in series including large patient groups that age is not a risk factor for biopsy complications and that there is no increase in the risk of complications in the elderly[48,49]. It was observed that there were no complications after the kidney biopsies performed in our study. It is thought that the reason why no complications were observed in our study may be related to the fact that biopsies were performed with automatic biopsy needles under ultrasound guidance, bleeding control was routinely performed with ultrasound during follow-up, protective measures were taken more frequently as the risk of complications was higher in the elderly, and the number of cases was low. Inadequate diagnosis and treatment of renal parenchymal diseases is strongly associated with the risk of ESRD and increased morbidity and mortality in the elderly[50]. Pathological diagnoses made by kidney biopsy in elderly patients can be controlled with appropriate treatment. In this way, negative health consequences and unnecessary treatment burden in elderly patients can be avoided. Kidney biopsy, which is a reliable procedure with low complication rates, should not be avoided in the elderly.

Study Limitations

The strength of our study is that it is the first study examining elderly kidney biopsies in our region. The limitations of our study are that it was retrospective and conducted in a single center.

CONCLUSION

As a result, the most common indications for kidney biopsy in the elderly are nephrotic proteinuria and AKD, and it is the most common reason for biopsy in the elderly. PGN is most commonly seen in the elderly, and MGN and FSGS are observed more frequently. Among SGN, amyloidosis and LN are the most common. Kidney biopsy is the gold standard method in the diagnosis of renal parenchymal diseases and is a reliable procedure with low complication rates in the elderly. Kidney biopsy should not be avoided in the elderly if clinically necessary.

Ethics

Ethics Committee Approval: The study was conducted after obtaining the necessary permissions from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (decision no: B.30.2.ATA.0.01.00/862, date: 26.10.2023).

Informed Consent: Retrospective study.

Authorship Contributions

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

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Maladaptive Daydreaming in Psoriasis Patients

Psoriazis Hastalarında Uyumsuz Hayal Kurma Bozukluğu

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ABSTRACT

Aim: Many psychiatric disorders are associated with psoriasis. A 16-item self-report maladaptive daydreaming (MD) scale (MDS-16) is described as a screening tool for MD. We aimed to explore whether MDS-16 scores differed in psoriasis patients compared to healthy individuals, hypothesizing higher scores and higher rates of probable maladaptive daydreamers (MDers) among psoriazis patients.

Materials and Methods: We included 184 psoriazis patients and 93 age- and sex-matched people without any cutaneous disorders in this case-control, cross-sectional, and questionnaire-based study. Dermatology Quality of Life Instrument in Turkish (TQoL) and MDS-16 were applied. We considered the participants with a total MDS-16 score ≥50 as probable MDers.

Results: Total MDS-16 score was higher in the psoriasis group (p=0.038). However, the difference between the frequencies of probable MDers was not significant (p=0.234). According to the multivariable analysis, psoriasis was not found as an independent risk factor for being probable MDer. In the psoriasis group, total MDS-16 scores were positively correlated with TQoL scores (r_s=0.259, p=0.001), which were significantly higher in probable MDers (p=0.032).

Conclusion: The association between psoriasis and MD may be related to the level of the impact of psoriasis on the quality of life.

Keywords: Maladaptive daydreaming, psoriasis, quality of life

ÖZ

Amaç: Birçok psikiyatrik bozukluk psoriazis ile ilişkilidir. On altı maddeden oluşan uyumsuz hayal kurma (MD) ölçeği (MDS-16), MD bozukluğu için bir tarama aracı olarak tanımlanmıştır. Bu çalışmada, psoriazisli bireylerde daha yüksek MDS-16 skorları ve olası uyumsuz hayal kurma bozukluğu oranı görüleceği hipotezinden yola çıkılarak, psoriazisli hastalar ile sağlıklı bireylerin MDS-16 skorları arasında fark olup olmadığını araştırmayı amaçladık.

Gereç ve Yöntem: Bu kesitsel, ankete dayalı, olgu-kontrol çalışmasına 184 psoriazis hastası ve herhangi bir deri hastalığı olmayan, yaş ve cinsiyet açısından uyumlu 93 kişi dahil edildi. Türkçe Dermatolojik Yaşam Kalite Ölçeği (TDYKÖ) ve MDS-16 uygulandı. Toplam MDS-16 puanı ≥50 olan katılımcılar olası MD bozukluğuna sahip bireyler olarak değerlendirildi.

Bulgular: Toplam MDS-16 skoru psoriazis grubunda daha yüksekti (p=0,038). Ancak, olası MD bozukluğuna sahip bireylerin sıklığı arasında anlamlı fark yoktu (p=0,234). Çok değişkenli analize göre, psoriazis, MD bozukluğu açısından bağımsız bir risk faktörü olarak saptanmadı. Psoriazis grubunda; MDS-16 skorları, TDYKÖ skorları ile pozitif korelasyon gösterirken (r_s=0,259, p=0,001), olası MD bozukluğuna sahip bireylerde TDYKÖ skorları daha yüksekti (p=0,032).

Sonuç: Psoriazis ile MD bozukluğu arasındaki ilişki, psoriazisin YK üzerindeki etki düzeyi ile ilişkili olabilir.

Anahtar Kelimeler: Uyumsuz hayal kurma, psoriazis, yaşam kalitesi

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INTRODUCTION

Maladaptive daydreaming (MD) is an extensive fantasy activity that replaces human interaction and/or interferes with functioning in various areas of life and can give rise to severe distress[1]. It may arise as a coping strategy due to psychological stress and real-life problems[2,3]. In addition, patients with MD may have other symptoms related to depression, anxiety, dissociation, and obsessive-compulsive related disorders[4].

Somer et al.[4] described a 16-item self-report MD scale (MDS-16) to discriminate between individuals with and without MD. The MDS-16 is currently used as the screening tool, translated and validated in various languages[5,6].

Psoriasis is a chronic inflammatory systemic disorder that can have a negative impact on quality of life (QoL) and cause psychological and social morbidity. Coping with psoriasis can lead to chronic stress. Psychological distress may trigger or worsen psoriasis within a vicious cycle. Patients can develop new coping strategies during the disease course[7-9].

Many psychiatric disorders, such as anxiety, eating, mood (depression or bipolar disorder), sleep, somatoform disorders, sexual dysfunction, and substance abuse, are associated with psoriasis[10,11]. Evaluating the psychosocial comorbidities is essential for assessing the severity of psoriasis during the disease course. Until now, there has been no report regarding the association between psoriasis and MD in the literature.

We hypothesized that psoriasis might trigger or enhance MD as a coping strategy. We aimed to explore whether MDS-16 scores differed in psoriasis patients compared to healthy individuals, hypothesizing higher scores and higher rates of probable maladaptive daydreamers (MDers) among psoriasis patients.

MATERIALS AND METHODS

Participants

This case-control, cross-sectional, and questionnaire-based study was conducted between August 2022 and January 2023 at the dermatology outpatient clinic in our tertiary referral hospital. Participants were divided into two groups, as the psoriasis and control group. We enrolled 184 patients with psoriasis in the psoriasis group, whereas 93 age- and sex-matched people from the same geographical region and without any cutaneous disorders were in the control group. Those younger than 18 years and those who did not accept to attend the study were excluded.

We obtained ethics approval from the Tekirdağ Namık Kemal University Non-interventional Clinical Research Ethics Committee (approval number: 2022.131.06.21, date: 28.06.2022). All participants provided informed consent before participating in the study.

Measures

Sociodemographic and Clinical Data

For all participants, we recorded data on age, sex, smoking, alcohol use, body mass index (BMI), comorbidities, regularly used medicines, marital status (single, married, divorced, widowed), education levels (elementary/middle school, high school, Bachelor's/Master's degree), occupation status, income level (low, middle, high), and residence area. In addition, age at the psoriasis onset, disease duration, involvement of special sites (scalp, nail, genital, flexural, and palmoplantar regions), and therapies used for psoriasis were recorded in the psoriasis group.

Psoriasis Area Severity Index

Psoriasis area severity index (PASI) is the most commonly used scale to evaluate psoriasis severity[12]. The patients with PASI scores ≥10 or body surface area ≥10% are accepted to have moderate-severe psoriasis. However, in some patients, PASI scores may not exactly reflect the disease severity because of the impact of the disease on QoL. Despite a PASI score <10, patients with psoriatic arthritis, involvement of visible or special areas, presence of resistant plaques, itching/pain/burning sensations, or Dermatology Life Quality Index >10 are also considered as moderate-severe psoriasis[13,14]. In the psoriasis group, we defined the severity of psoriasis in light of this information.

Quality of Life Assessment

We used the Dermatology QoL instrument in Turkish (TQoL) to assess the impact of psoriasis on the participants' QoL over the last month. The TQoL is a validated, five-point Likert-type (0=never to 4=always) self-report questionnaire consisting of 11 items, each conceptualized to measure different domains, including cognitive, emotional, social, daily activity, sexual life, and symptoms. The total possible score ranges between 0 and 44. The lower the score, the better the QoL[15,16].

MDS-16 Scale

The MDS-16 was used to assess the potential MD cases. It is a 16-item self-report questionnaire on a Likert-type scale ranging from 0% (none of the time/never) to 100% (all of the time/extremely frequent) with 10% increments. The total score is the average of 16 items[4,6]. Higher scores indicate a higher potential for MD. Metin et al.[5] made a validity and reliability study of the Turkish version of the MD scale.

Somer et al.[6] reported the optimal cutoff score as 40 in screening patients with MD with a near perfect sensitivity. However, a cutoff score of 50 had 100% specificity (no false-positive case) and very accurate sensitivity in that study. Because we did not perform clinical interviews, total scores of ≥50 were considered probable MDers[17,18]. The Cronbach's α for the MDS-16 in this study was 0.913.

Statistical Analysis

The Statistical Package for the Social Sciences v.25 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. P<0.05 was considered statistically significant.

The normality of the distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables were presented as median (interquartile range) or mean±standard deviation, and categorical variables as frequency and percentage.

The Cronbach's α was measured for the internal consistency of the MDS-16 questionnaire. According to the normality test results, the independent samples t-test or the Mann-Whitney U test was used for comparisons between two groups, and the Kruskal-Wallis test was used if the number of groups was greater than two. The Dunn-Bonferroni approach was performed for multiple comparisons after the Kruskal-Wallis test. The Pearson's chi-square or Fisher-Freeman-Halton test was employed to compare categorical variables. The correlations between continuous variables were examined using the Spearman correlation test.

We performed the enter method of multivariable logistic regression analysis to assess the association between independent risk factors and MD. To verify whether the model fits the data, we employed the Hosmer and Lemeshow test. The independent variables with p<0.05 in the univariable logistic regression test were then included in multivariable logistic regression. We defined the dependent variable by dichotomizing probable MDers (total MDS-16 score ≥50) and non-probable MDers (total MDS-16 score <50). Independent variables were the study group (psoriasis or control), age, sex, smoking, alcohol use, BMI, marital status, education level, occupation status, income level, residence area, and psychiatric comorbidities. In addition, age at the psoriasis onset, psoriasis duration, involvement of special sites, TQoL score, PASI, and treatment used for psoriasis were defined as independent variables in the psoriasis group.

RESULTS

Comparison of Sociodemographic Characteristics and Total MDS-16 Scores Between Study Groups

The data of study groups were statistically similar in terms of age, sex, marital status, BMI, and income levels. The comparisons of sociodemographic and clinical data for the groups are presented in Table 1.

Table 1. Comparison of sociodemographic and clinical data between study groups

	Variables	Psoriasis group (n=184)	Control group (n=93)	p
Age	Mean±SD Median (IQR)	42.51±13.42 42 (21)	41.34±11.27 42 (17)	0.562[a]
Sex (n, %)	Female Male	91, 49.5% 93, 50.5%	50, 53.8% 43, 46.2%	0.498[b]
Marital status (n, %)	Single Married Divorced Widowed	43, 23.4% 129, 70.1% 7, 3.8% 5, 2.7%	19, 20.4% 69, 74.2% 5, 5.4% 0	0.386[c]
BMI (n, %)	<18.5 (underweight) ≥18.5 and <25 (normal) ≥25 and <30 (overweight) ≥30 and <40 (obesity) ≥40 (severe obesity)	4, 2.2% 51, 27.7% 82, 44.6% 39, 21.2% 8, 4.3%	1, 1.1% 30, 32.6% 42, 45.7% 18, 19.6% 1, 1.1%	0.630[c]
Education level (n, %)	Elementary/middle school High school Bachelor's/Master's degree	63, 34.2% 67, 36.4% 54, 29.3%	16, 17.2% 12, 12.9% 65, 69.9%	<0.001[b]
Income level (n, %)	Low (I < O) Middle (I=O) High (I > O)	67, 37.2% 92, 51.1% 21, 11.7%	27, 29.3% 47, 51.1% 18, 19.6%	0.154[b]

Table 1. Continued

	Variables	Psoriasis group (n=184)	Control group (n=93)	p
Smoking (n, %)	None Current smoker	85, 46.2% 99, 53.8%	63, 67.7% 30, 32.3%	0.001[b]
Alcohol use (n, %)	No Yes	154, 83.7% 30, 16.3%	59, 63.4% 34, 36.6%	<0.001[b]
Occupation (n, %)	No Yes	77, 44% 98, 56%	20, 23% 67, 77%	0.001[b]
Residence area (n, %)	Village District City	9, 4.9% 125, 67.9% 50, 27.2%	3, 3.2% 16, 17.2% 74, 79.6%	<0.001[b]
Psychiatric disorders (n, %)	Absent Present - Depression - Anxiety - Schizophrenia - Alcohol addiction	173, 94% 11, 6% 7 3 1 1	83, 94.3% 5, 5.7% 2 3	0.923[b]
Probable MDers (n, %)		20 (10.9%)	6 (6.5%)	0.234[b]
Total MDS-16 score	Mean±SD Median (IQR)	26.13±17.16 23.75 (25.63)	21.82±15.83 18.12 (21.56)	0.038[a]

[a]Mann-Whitney U test; [b]Pearson's chi-square test; [c]Fisher-Freeman-Halton test.
SD: Standard deviation, IQR: Interquartile range, MDS-16: Maladaptive daydreaming scale, I: Income, O: Outcome, MDers: Maladaptive daydreamers

Relationship Between Total MDS-16 Scores and Sociodemographic and Clinical Features

There were no statistically significant associations between total MDS-16 scores and sex, marital status, obesity, education level, smoking, alcohol use, occupation status, and accompanying comorbidities in both groups. The associations of total MDS-16 score with income level, residence area, and psychiatric disorders were only significant in the psoriasis group (Table 2).

There was a weak negative correlation between age and the total MDS-16 score only in the control group (r_s=-0.215, p=0.042).

In the psoriasis group, there was no significant association between total MDS-16 scores and psoriasis severity or involvement of special sites. Total MDS-16 scores were higher among patients not treated with systemic agents, yet the difference was not significant (Table 3). In addition, the total MDS-16 scores and BMI, age, PASI, disease duration, or the age of psoriasis onset were not significantly correlated. There was a weak positive correlation between TQoL and the total MDS-16 scores (r_s=0.259, p=0.001).

Sociodemographic Characteristics of Probable MDers

In both groups, sex, marital status, education levels, smoking, alcohol use, occupation status, BMI, income level, or the presence of psychiatric disorders did not significantly differ between probable MDers and the others.

Of 184 psoriasis patients, 20 (10.9%) were probable MDers. Among the probable MDers, 17 (85%) were residing in a district and 3 (15%) were in a village, whereas among non-probable MDers, 108 (65.9%) were in a district, 50 (30.5%) in a city, and 6 (3.7%) in a village (p=0.003).

In the psoriasis group, disease duration or severity, age of the onset of psoriasis, PASI, or special site involvements did not significantly differ among probable MDers compared to non-probable MDers. TQoL scores were significantly higher in probable MDers (Table 4). A much larger proportion of patients treated only with topical agents or phototherapy were probable MDers (Table 3).

Multivariable Analysis

Multivariable analyses, utilizing the enter method, were performed for all participants (Model 1), the psoriasis group (Model 2), and the control group (Model 3). According to the results of Hosmer and Lemeshow tests (p=0.064, p=0.789, and p=0.767 for Model 1, Model 2, and Model 3, respectively), the models fit the data and could be further interpreted. The explained pseudovariance (Nagelkerke, R^2) was 21.3% for Model 1, 16.3% for Model 2, and 33.7% for Model 3. The percentages of cases correctly classified were 90.8% in Model 1, 89.4% in Model 2, and 95.3 in Model 3.

Table 2. Relationship between total MDS-16 scores and sociodemographical/clinical features

Variables	Total MDS-16 score					
	Psoriasis group			Control group		
	n	Median (IQR)	p	n	Median (IQR)	p
Income level[a]						
- Low (I<O)	67	26.3 (28.1)	0.018	27	19.4 (30.63)	0.447
- Middle (I=O)	92	24.4 (25.3)		47	17.5 (18.13)	
- High (I>O)	21	11.3 (20.9)		18	16.3 (19.7)	
Residence area[b]						
- Village	9	32.5 (28.13)	0.016	3	10	0.665
- District	125	26.3 (28.75)		16	20.3 (20.8)	
- City	50	19.1 (21.56)		74	17.8 (22.8)	
Psychiatric disorders[c]						
- Absent	173	23.1 (24.7)	0.033	83	18.1 (21.3)	0.685
- Present	11	41.3 (30)		5	18.1 (47.5)	

[a]Kruskal-Wallis test. Pairwise comparisons; the differences between I<O and I>O (p=0.018) and between I=O and I>O (p=0.028) were significant.

[b]Kruskal-Wallis test. Pairwise comparisons; the differences between district and city (p=0.018), village and city (p=0.016) were significant.

[c]Mann-Whitney U test.

IQR: Interquartile range, I: Income, O: Outcome, MDS-16: Maladaptive daydreaming scale

Table 3. The relationship between MDS-16 results and treatment types in the psoriasis group

Treatment types	Probable MDers (n, %)	Total MDS-16 scores Mean±SD Median (IQR)
Topical or phototheraphy (n=34)	8 (23.5%)	32.5±19.2 32.2 (29.8)
Systemic conventional (n=58)	4 (6.9%)	23.1±15.3 21.6 (19.06)
Biologic agents (n=92)	8 (8.7%)	25.7±17.1 22.2 (27.97)
p	0.030[a]	0.058[b]

[a]Pearson's chi-square test, [b]Kruskal-Wallis test.

MDers: Maladaptive daydreamers, MDS-16: Maladaptive daydreaming scale, IQR: Interquartile range, SD: Standard deviation

The multivariable analysis revealed that residence area and psychiatric disorders for all participants, TQoL score and treatment type for psoriasis patients, and age for the control group were independent risk factors for being probable MDers. Psoriasis was not found to be an independent risk factor for MD [odds ratio (OR): 0.681; 95% confidence interval (CI): 0.169-2.75; p=0.590]. The results of multivariable logistic regression analysis are presented in Table 5.

DISCUSSION

Based on the knowledge that many psychiatric disorders are associated with psoriasis, we explored whether psoriasis patients had higher total MDS-16 scores and whether probable MDers might be more frequent among psoriasis patients. Following our hypothesis, total MDS-16 scores were significantly higher in the psoriasis group (p=0.038). However, the difference in the frequency of probable MDers between the study groups was not statistically significant (p=0.234). In addition, psoriasis was not an independent risk factor for MD according to the multivariable analyses.

Psoriasis may affect the quality of the social, personal, and sexual lives of the patients. Therefore, the patients are prone to have anxiety, depression, or other psychological disorders, yet not all patients have difficulties in adjusting to their disease[7,8]. According to previous reports, the QoL, psychological distress, or stress are not associated with the psoriasis severity and duration, or treatment modalities[7,9,19-21]. Beyond the cosmetic disfigurement of psoriasis, psychological factors and the inability to cope with stress are strong determinants of disability in psoriasis patients[9]. We observed a weak positive correlation between TQoL and the total MDS-16 scores (r_s=0.259, p=0.001), and TQoL scores were higher among probable MDs (p=0.032). In addition, according to the multivariable analysis, higher TQoL scores were associated with

a greater risk of being a probable MDer in the psoriasis group (OR: 1.055; 95% CI: 1.002-1.110). On the other hand, although total MDS-16 scores were significantly higher in the psoriasis group, the difference in the frequency of probable MDers between the study groups was not significant, and psoriasis was not among the risk factors of being a probable MDer. In addition, there was no relationship between MD and psoriasis severity, duration, or location. Among treatment modalities, a larger proportion of patients treated with skin-directed therapies (topical or phototherapy) were probable MDers. Similarly, treatment with systemic conventional (OR: 0.162; 95% CI: 0.036-0.738) or biologic agents (OR: 0.250; 95% CI: 0.073-0.854) was associated with decreased risk of being probable MDer, which may be attributed to their long-term

Table 4. Comparison of age, BMI, and TQoL scores between probable MDers and non-probable MDers

Variables	Non-probable MDers Mean±SD Median (IQR)	Probable MDers Mean±SD Median (IQR)	p
Age - Control group - Psoriasis group	42.2±11.05 44 (16) 42.53±13.27 42 (21)	29.33±6.98 26.5 (13) 42.37±15.06 42 (26)	0.006[a] 0.961[b]
BMI - Control group - Psoriasis group	26.64±4.6 25.7 (5.4) 27.46±4.98 26.95 (5.1)	27.34±2.67 28.4 (4.8) 29.63±7.78 29.35 (12.6)	0.481[b] 0.119[b]
TQoL scores - Psoriasis group	18.03±10.87 16.5 (19)	23.65±9.37 22 (15)	0.032[b]
PASI - Psoriasis group	2.7±4.6 1 (2.4)	3.1±6.5 1 (1.7)	0.806[b]

[a]Independent samples t-test, [b]Mann-Whitney U test.

MDS-16: Maladaptive daydreaming scale, IQR: Interquartile range, BMI: Body mass index, TQoL: Dermatology QoL instrument in Turkish, PASI: Psoriasis area severity index, SD: Standard deviation

Table 5. Results of multivariable logistic regression analysis

Risk factors	OR	95% CI	p
All participants			
Residence area - Village - District - City	 Reference 0.616 0.106	 0.110-3.447 0.012-0.955	 0.581 0.045
Psychiatric disorder - Absent - Present	 Reference 4.88	 1.118-21.3	 0.035
Psoriasis group			
Higher TQoL scores	1.055	1.002-1.110	0.042
Treatment types - Topical or phototheraphy - Systemic convantional - Biologic agents	 Reference 0.162 0.250	 0.036-0.738 0.073-0.854	 0.019 0.027
Control group			
Older age	0.838	0.728-0.966	0.015

OR: Odds ratio, CI: Confidence interval, TQoL: Dermatology Quality of Life Instrument in Turkish

positive effects on QoL, although used for moderate/severe psoriasis. Therefore, daydreaming or MD may be a coping strategy for psoriasis patients with impaired QoL independent from the other measures of disease severity.

Reported accompanying psychopathologies may not be compatible with the extent of psoriasis lesions[11]. The correlation between psoriasis and mental disorders includes alterations of neuroimmune, serotonergic, or dopaminergic systems[10,22,23]. Low serotonin and high dopamine levels were found to be associated with psoriasis[22]. In addition, the use of serotonin reuptake inhibitors in psoriasis patients was found to be associated with a decreased need for systemic psoriasis treatment[24]. On the other hand, accompanying psychiatric comorbidities of MD, such as anxiety disorders, depressive disorder, obsessive-compulsive related disorders, have been reported[4,25-27]. MD marked by addictive features has shared mechanisms with obsessive-compulsive disorder or dissociation[27,28]. Therefore, serotonergic and dopaminergic systems may be involved in the development of MD, like psoriasis. In this study, although total MDS-16 scores were significantly higher among participants with known psychiatric disorders in the psoriasis group, psychiatric disorders were not associated with probable MDers in both groups. Nevertheless, when we analyzed both groups together in a multivariable analysis, participants with a psychiatric disorder were at 4.88 times the risk of being a probable MDers compared to those without any psychiatric disorders (95% CI: 1.118-21.3; p=0.035).

In line with previous studies, probable MDers were younger-aged adults in the control group[28,29]. In addition, age and total MDS-16 scores were reversely correlated, and older age was associated with lower risk for MD (OR: 0.838; 95% CI: 0.728-0.966). However, there was no association between age and probable MDers in the psoriasis group.

Previous studies have reported that MD is more common in individuals with lower education levels[18,30]. Our study groups were not similar in the case of the education level. The majority of participants had higher education in the control group. However, there was no association between MD and education level in either group.

In the psoriasis group, total MDS-16 scores and the frequency of probable MDers were higher among participants living in a village/district. Similarly, living in a city was linked with a decreased risk of being probable MDers (OR: 0.106; 95% CI: 0.012-0.955); however, such an association was not observed in case all participants were analyzed together. This difference may be attributed to the disproportion of participants' residence areas between the study groups. Moreover, although total MDS-16 scores were higher among low/middle-income participants, the frequency of probable MDers was not significantly different.

Study Limitations

The first limitation of our study was its cross-sectional nature. Second, because we did not correlate the scale scores with the diagnostic structured clinical interviews, there may be potential false positives in our data. Third, some sociodemographic features (education level, occupation, and residence) of the study groups were not similar. Although we found no correlation between total MDS-16 scores and education level or occupation status, total scores were significantly higher in participants with low/middle income or residing in a village/district in the psoriasis group. In addition, it would have been better to exclude cases with psychiatric disorders from the study in the first place to better understand the relationship between psoriasis and MD, independently from the other psychiatric disorders.

CONCLUSION

Although psoriasis was not found as an independent risk factor, TQoL scores were higher among probable MDers, and the higher the TQoL score, the higher the risk for MD in the psoriasis group. Therefore, the relationship between psoriasis and MD may depend on the level of impact of psoriasis on the QoL. Clinicians should better be aware of the MD as a potential accompanying psychopathology while following up patients with psoriasis and impaired life quality. Further studies with clinical interviews are needed to confirm and extend our findings.

Ethics

Ethics Committee Approval: We obtained ethics approval from the Tekirdağ Namık Kemal University Non-interventional Clinical Research Ethics Committee (approval number: 2022.131.06.21, date: 28.06.2022).

Informed Consent: All participants provided informed consent before participating in the study.

Authorship Contributions

Concept: Ö.Z., E.B., S.A., H.A., Design: Ö.Z., E.B., S.A., H.A., Data Collection or Processing: Ö.Z., E.B., S.A., H.A., Analysis or Interpretation: Ö.Z., Literature Search: Ö.Z., E.B., Writing: Ö.Z., E.B.

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Caregiver Contribution to Self-care in Ostomy Patient Index: Turkish Validity and Reliability Study

Ostomi Hastasının Öz Bakımında Bakıcı Desteği İndeksi: Türkçe Geçerlik ve Güvenirlik Çalışması

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ABSTRACT

Aim: This study was conducted to evaluate the Turkish validity and reliability of the Caregiver Contribution to Self-care in Ostomy Patient Index, to evaluate the relatives of patients with stoma who contributed to their self-care in Turkish society, and to contribute to the literature of the measurement tool.

Materials and Methods: This methodological type of research was carried out between September 2020 and January 2021 in a state and a university hospital in Tekirdağ. The research sample consisted of 223 individuals who contributed to the self-care of individuals with colostomy, ileostomy and urostomy, who applied for outpatient control. In the analysis of the data, descriptive statistics, language and content validity, confirmatory factor analysis (CFA), item analysis, internal consistency and test-retest methods were used.

Results: The Content Validity Index was 0.99 and Cronbach's alpha 0.890. According to the CFA, fit indices were within acceptable limits and all items were statistically significant in the 3 sub-dimensions of the scale. As in the original version of the scale, item 18, which was not statistically significant, was not included in the analysis and was accepted as an addition. The correlation between test-retest and scale items was 0.983 for the whole scale and between 0.973 and 0.987 for the sub-dimensions.

Conclusion: At the end of the study, it was determined that the scale was suitable for Turkish society and was valid and reliable in Turkish.

Keywords: Caregivers, ostomy, stoma, self-care, reliability, validity

ÖZ

Amaç: Bu araştırma Ostomi Hastasının Öz Bakımında Bakıcı Desteği İndeksi'nin Türkçe geçerlik ve güvenirliğinin incelenerek Türk toplumunda stoması olan hastaların öz bakımlarına katkı sağlayan yakınlarının değerlendirilmesi ve ölçme aracının literatüre katkı sağlaması amacıyla yapıldı.

Gereç ve Yöntem: Metodolojik tipteki bu araştırma Eylül 2020-Ocak 2021 tarihleri arasında Tekirdağ'daki bir devlet ve bir üniversite hastanesinde gerçekleştirildi. Araştırmanın örneklemini polikliniklere kontrol için başvuran kolostomi, ileostomi ve ürostomili bireylerin öz bakımlarına katkıda bulunan 223 kişi oluşturdu. Verilerin analizinde betimleyici istatistikler, dil ve içerik geçerliliği, doğrulayıcı faktör analizi (DFA), madde analizi, iç tutarlılık ve test-tekrar test yöntemleri kullanıldı.

Bulgular: İçerik Geçerlilik İndeksi 0,99 ve Cronbach alfa 0,890 idi. DFA'ya göre ölçeğin 3 alt boyutunda uyum indeksleri kabul edilebilir sınırlar içerisinde olup tüm maddeler istatistiksel olarak anlamlı bulundu. Ölçeğin orijinal versiyonunda olduğu gibi istatistiksel olarak anlamlı olmayan 18. madde analize dahil edilmedi ve ek olarak kabul edildi. Test-tekrar test ile ölçek maddeleri arasındaki korelasyon ölçeğin tamamı için 0,983, alt boyutları için ise 0,973 ile 0,987 arasında bulundu.

Sonuç: Araştırma sonunda ölçeğin Türk toplumuna uygun olduğu ve Türkçe ölçek olarak da geçerli ve güvenilir olduğu belirlendi.

Anahtar Kelimeler: Bakım veren, ostomi, stoma, özbakım, güvenirlik, geçerlik

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INTRODUCTION

A stoma is the opening that is most often created due to cancer and connects an organ to the skin[1]. Colostomy, ileostomy, and urostomy are the most common types of stoma, in which evacuation is provided by this surgical way to the abdominal wall[2,3].

The stoma has a negative impact on the quality of life by changing the daily life activities, body image and interpersonal relationships of the individual. As a result of this effect, new social, economic, emotional, and physiological priorities may emerge for the individual[4]. As a result of studies, it has been reported that the daily activities of individuals with stoma are limited by approximately 3.46 times and that approximately 30-60% of individuals with stoma experience complications in and around the stoma after surgery. The training and counseling of nurses play an important role in helping individuals cope with these problems that reduce their quality of life, adapt to the stoma, and gain self-care skills[5]. In addition, attention is drawn to the importance of social support resources, especially family members, in adapting the individual to a challenging new lifestyle[6].

Although self-care skills are carried out by the patient, as the word means, individuals often need the contributions of their families and relatives in this regard[7]. Families take an active role in giving advice on self-care skills of individuals with a stoma, making new decisions about the individual's health status and helping them to cope with their negative feelings about this situation, helping them to fulfill their self-care skills, and performing these skills instead of the individual when necessary[6,8]. Contribution to self-care can be determined by using valid and reliable tools in the evaluation of families and relatives who play an active role in the care of individuals with a stoma, and supportive training can be planned when necessary. Thus, a positive effect can be achieved in the adaptation process and quality of life of individuals with a stoma[6,7].

There is no valid and reliable scale for Turkish society in Turkish language to evaluate the relatives who participate in the care of individuals with a stoma and contribute to their self-care. As a result of this study, it is thought that this deficiency will be eliminated. The aim of this study, which was planned in methodological type, was to evaluate the Turkish validity and reliability of the "Caregiver Contribution to Self-care in Ostomy Patient Index" and to evaluate the families and relatives of patients with a stoma who contributed to their self-care in Turkish society.

MATERIALS AND METHODS

The study was planned in methodological type for the purpose of psychometric evaluation of the instrument.

The study was carried out between September 2020 and January 2021 in the general surgery, gastroenterology surgery and urology outpatient clinics of a state and a university hospital in Tekirdağ province. In order to evaluate the validity and reliability of a scale in different languages and cultures, it is recommended that the sample size should be 5-10 times the number of scale items[9]. The scale in this study consists of 22 items. The research sample consisted of 223 individuals who contributed to the self-care of individuals with colostomy, ileostomy and urostomy, who applied for outpatient control.

Instruments

As data collection tools, an 11-item "Information Form for the Caregiver of the Individual with Ostomy" and the 22-item "Caregiver Contribution to Self-care in Ostomy Patient Index" were used.

The Information Form for the Caregiver of the Individual with Ostomy consists of questions about the sociodemographic characteristics of the family and relatives who contribute to the care of the individual with a stoma.

The "Caregiver Contribution to Self-care in Ostomy Patient Index" scale, developed in English by Villa et al. (2019)[7], consists of 22 items and 3 sub-dimensions. Scale items are evaluated with a 5-point Likert-type. The first sub-dimension is "Caregiver Contribution to Self-care Maintenance", consisting of 9 items. The second sub-dimension is "Caregiver Contribution to Self-care Monitoring", consisting of 8 items, and the third sub-dimension is "Caregiver Contribution to Self-care Management", consisting of 5 items. The 18th item of the scale, which evaluates the state of experiencing complications, has a low factor load and was accepted as an add-on. The highest 110 points are obtained from the scale, and it is stated that as the total score from the scale increases, self-care also increases[7].

Study Procedures

The study sample consisted of individuals over the age of 18 years, who supported the care of the individual with a stoma (colostomy, ileostomy, or urostomy), who spoke, read and wrote in Turkish, did not have a cognitive problem that prevented them from expressing themselves, did not have a serious psychiatric diagnosis, and gave written consent to participate in the study. The data were collected by the researcher by face-to-face interview method, giving each individual approximately 30 minutes.

Ethics

In order to adapt the scale to Turkish, permission was obtained from the authors via e-mail[7]. Study procedures were reviewed and approved by the Non-interventional Clinical Research

Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine on August 27, 2020, with the decision number of 2020.195.08.04. Moreover, study procedures were reviewed and approved by the two public hospitals. The procedures used in this study adhere to the tenets of the Declaration of Helsinki, in accordance with good clinical practice. The study was carried out on a voluntary basis. Written and verbal consent was obtained from individuals who agreed to participate in the study. This study is registered in the clinical trial database with protocol number (NCT06269276).

Statistical Analysis

Data were analyzed using lavaan Project and R Project package programs. Descriptive statistical methods (mean, standard deviation, frequency, and percentage) were used to analyze sociodemographic characteristics of the participants. The Shapiro-Wilk test was used for normality tests. According to the findings examined, the paired samples t-test from dependent 2 group comparison tests was used for the scores that were in accordance with the normal distribution, and the Pearson correlation test was used to determine the direction and degree of the relationship between the two scores. The Wilcoxon Signed-Rank test, which is one of the two dependent group comparison tests, and the Spearman correlation test were used to determine the direction and degree of the relationship between the two scores for the scores that did not conform to the normal distribution. Validity and reliability methods were used in the analysis phase. The margin of error was 5% and the significance was evaluated as $p<0.05$[10,11].

Validity Analysis

In the validity analysis of the scale, language and content analysis and confirmatory factor analysis (CFA) were used. In order to ensure language and content validity, the scale was first translated from English to Turkish and from Turkish to English by two independent linguists, and a common translation text was created[12]. The Content Validity Index (CVI) was used to examine and compare the compatibility of the translated text with the original study, and 11 expert opinions were obtained. In this method, each item is scored as "1-Not Relevant", "2-But Need Some Revision", "3-Relevant, But Needs Minor Revision" and "4-Very Relevant". The CVI is considered sufficient if at least 80% of the scale items are scored as 3 or 4 and the CVI value is above 0.80[13]. The scale, which was organized with expert opinions, was applied as a pilot study to a group of 10 people who contributed to the care of the individual with a stoma, and the scale was given its final shape. Data from this group were not included in the analyses[13-15]. In this study, CFA was applied to test the validity of a previously developed scale in different languages and samples. The Diagonal Weighted Least Squares technique was preferred since the data were Likert-type in the estimation phase of CFA[9,16]. The fit indexes obtained as a result of the analysis was at the desired level and the factor loads of the items were above 0.30 according to the path diagram. In addition, the t-values of the items are considered statistically significant at the 0.05 level if they are above 1.96 and at the 0.01 level if they are above 2.56[9,12,16-19].

Reliability Analysis

In the reliability analysis of the scale, item analysis and internal consistency and test-retest reliability were used. The Cronbach's alpha reliability coefficient and item-total score reliability, which measure the relationship between each independent item in the measurement tool and the other items and the whole scale, were used. Accordingly, the Cronbach's alpha coefficient 0.40-0.60 is reliable at a lowly level; a range of 0.60-0.80 indicates quite reliability, and a range of 0.80-1.00 indicates high reliability. In item-total score reliability, a correlation coefficient over 0.30 indicates the reliability of the item[9,20]. For test-retest reliability, the scale was repeated with 70 individuals who contributed to the care of the individual with a stoma at a 2-week interval, and the scale forms were matched after the application. The Pearson and Spearman correlation coefficients (r-value) are calculated in the test-retest method. The r-value indicating the degree of reliability takes a value between -1 and +1 and must be at least 0.70 for reliability to be accepted[9].

RESULTS

Among the individuals participating in the study, 52% (n=116) were between the ages of 45 and 59 years; 64.6% (n=144) were female; 74% (n=165) were married, 36.3% (n=81) were middle school graduates, 62.3% (n=139) were not working, and 77.6% (n=173) were living with an individual with a stoma; 65% (n=145) of stomas belonged to individual's spouse and 42.2% (n=94) supported the care of the individual with stoma for 1-5 months; and 79.8% (n=178) received ostomy care training. In addition, 53.8% (n=120) of individuals with stoma had colostomy and the reason for ostomy opening in 70.9% (n=158) was cancer (Table 1).

In the validity analysis, the CVI value of the scale was found to be 0.99 in line with the expert opinions. As a result of CFA, χ^2/sd=3.349 values were between 2 and 5 and goodness of fit index (GFI), Tucker-Lewis Index (TLI), comparison of model fit indices (CFI), adjusted goodness of fit index (AGFI) values were above 0.900 (Table 2). All items were included in 3 sub-dimensions with statistical significance and the 18th item was accepted as an add-on as it was in the original version of the scale (Figure 1, Table 3).

According to the reliability analysis statistics, the Cronbach's alpha coefficients were 0.890 for the overall scale, 0.867

for the "Caregiver Contribution to Self-care Maintenance" sub-dimension, 0.921 for the "Caregiver Contribution to Self-care Monitoring" sub-dimension, and 0.458 for the "Caregiver Contribution to Self-care Management" sub-dimension, respectively. As a result of the reliability analysis, the item correlation value of the 18th item was negative, so it was excluded from the analysis and the results in Table 4 were obtained by performing the reliability analysis again. According to the findings, all corrected item correlation values of the sub-dimensions were found to be positive. In addition, it was observed that there was no significant increase in the reliability coefficients when the item was deleted in the sub-dimensions of the Caregiver Contribution to Self-care in Ostomy Patient Index. According to the findings obtained in the final analysis stage, all questions were included in the analysis, as in the original version of the Caregiver Contribution to Self-care in Ostomy Patient Index. With the test-retest method, the Pearson and Spearman correlation coefficient (r-value) was found to be between 0.973 and 0.987 for the sub-dimensions of the scale and 0.983 for the whole scale (Table 5).

DISCUSSION

In this study, the validity and reliability of a scale that evaluates the contribution of families and relatives to the self-care of individuals with a stoma in the Turkish language and Turkish society were examined. The sub-dimensions of the scale in its original form were prepared on the basis of Riegel et al. (2012)[21] "Middle-range Theory of Self-care of Chronic Illness". In the scale that evaluates the contributions of the families and relatives of individuals with a stoma, the first part of the scale, "Self-care Maintenance" is about daily routine behaviors; the second part "Self-care Monitoring" is about recognizing the stoma and its surroundings; and the third part, "Self-care Management", deals with recognizing problems and intervening behaviors[7].

The back-translation method was used in the language adaptation of the scale and a common translation text was created. The CVI value of 0.99 in this study showed that language and content validity were appropriate[13]. Also, construct validity of the scale was tested using CFA. In this study, fit indices (χ^2/sd=3.349 value below 5 and GFI, TLI, CFI and AGFI criteria above 0.900) showed acceptable fit. The factor loads of the items were found to be above 0.30 and their t-values above 2.56, at the level of 0.01, which was statistically significant. These results found that the items were correctly included in the original scale dimensions and were collected in 3 sub-dimensions[9,12,16,19-20].

In this study, Cronbach's alpha reliability coefficient was found to be 0.890 in the overall scale and in the range of 0.458-0.921 in the sub-dimensions of the scale. The overall scale was

Table 1. Sociodemographic characteristics of families and relatives contributing to the care of individuals with stoma

Variables	n	%
Age (years)		
18-29	11	4.9
30-44	40	17.9
45-59	116	52.0
60+	56	25.1
Gender		
Female	144	64.6
Male	79	35.4
Marital status		
Single	58	26.0
Married	165	74.0
Education level		
Literate	15	6.7
Primary school	77	34.5
Middle school	81	36.3
High school	34	15.2
University	16	7.1
Profession		
Working	84	37.7
Not working	139	62.3
The state of living with an individual who has a stoma		
Yes	173	77.6
No	50	22.4
Degree of closeness with the individual with the stoma		
Spouse	145	65.0
Child	60	26.9
Other	18	8.1
Stoma type of individual with stoma		
Colostomy	120	53.8
Ileostomy	69	30.9
Urostomy	34	15.2
Cause of ostomy opening		
Cancer	158	70.9
Obstruction	43	19.3
Trauma	18	8.1
Other	4	1.8
Time to support the self-care of the individual with stoma		
1-5 months	94	42.2
6-11 months	83	37.2
1-3 years	19	8.5
3-5 years	22	9.9
5 years+	5	2.2
Status of receiving ostomy care training before discharge		
Yes	178	79.8
No	45	20.2

Table 2. Fit indices obtained from confirmatory factor analysis for the Caregiver Contribution to Self-care in Ostomy Patient Index

Indices	Perfect fit	Acceptable fit	Index
χ^2/**sd** (chi-square goodness of fit)	<3	<5	**3.349**
CFI	0.97≤**CFI**≤1	0.90≤**CFI**≤0.96	**0.921**
GFI	0.95≤**GFI**≤1	0.90≤**GFI**≤0.96	**0.934**
AGFI	0.95≤**AGFI**≤1	0.90≤**AGFI**≤0.96	**0.918**
TLI	0.95≤**TLI**≤1	0.90≤**TLI**≤0.96	**0.911**

CFI: Comparative fit index, GFI: Goodness of fit index, AGFI: Adjusted goodness of fit index, TLI: Tucker-Lewis index

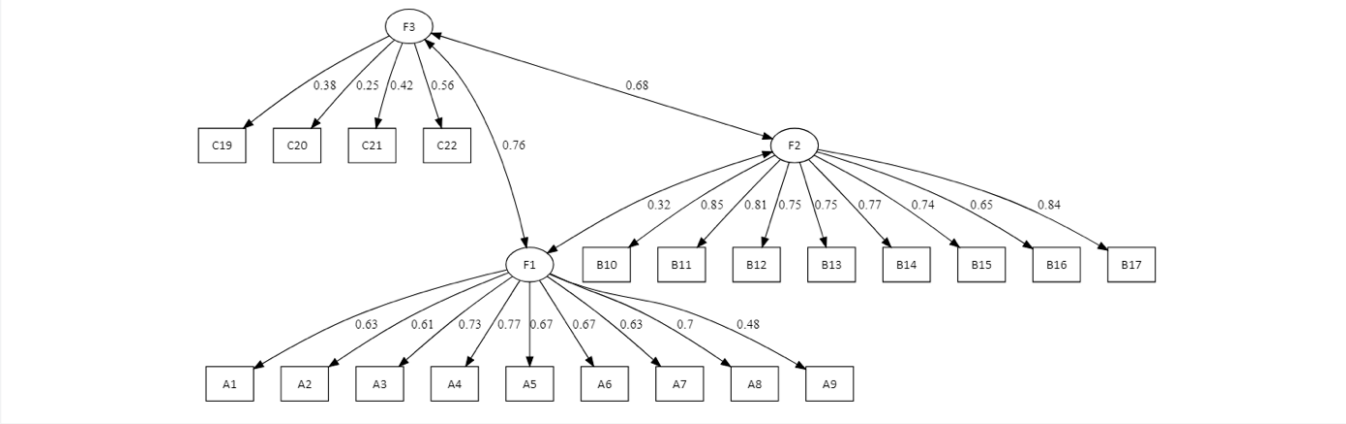

Figure 1. Caregiver Contribution to Self-care in Ostomy Patient Index confirmatory factor analysis path diagram

Table 3. Caregiver Contribution to Self-care in Ostomy Patient Index confirmatory factor analysis statistics

Sub-dimension	Item	Estimate	Standard error	t-value	p
Caregiver contribution to self-care maintenance	Item 1	1.000	-	-	-
	Item 2	0.973	0.075	13.018	<0.001
	Item 3	1.273	0.090	14.197	<0.001
	Item 4	1.247	0.087	14.315	<0.001
	Item 5	1.030	0.075	13.760	<0.001
	Item 6	1.314	0.094	13.952	<0.001
	Item 7	1.023	0.075	13.602	<0.001
	Item 8	1.120	0.079	14.188	<0.001
	Item 9	0.826	0.069	11.903	<0.001
Caregiver contribution to self-care monitoring	Item 10	1.000	-	-	-
	Item 11	0.931	0.051	18.202	<0.001
	Item 12	0.797	0.046	17.420	<0.001
	Item 13	0.825	0.047	17.586	<0.001
	Item 14	0.853	0.049	17.503	<0.001
	Item 15	0.888	0.051	17.305	<0.001
	Item 16	0.735	0.045	16.341	<0.001
	Item 17	1.026	0.057	18.049	<0.001
Caregiver contribution to self-care management	Item 19	1.000	-	-	-
	Item 20	0.622	0.091	6.805	<0.001
	Item 21	1.115	0.124	9.003	<0.001
	Item 22	1.584	0.158	10.054	<0.001

Table 4. Caregiver Contribution to Self-care in Ostomy Patient Index item analysis and internal consistency results

Sub-dimension	Item	Median	Corrected item-total correlations	Cronbach's alpha if item deleted	Cronbach's alpha
Caregiver contribution to self-care maintenance	Item 1	4.000	0.426	0.868	0.867
	Item 2	4.000	0.466	0.865	
	Item 3	4.000	0.723	0.841	
	Item 4	4.000	0.729	0.841	
	Item 5	4.000	0.648	0.849	
	Item 6	3.000	0.666	0.847	
	Item 7	4.000	0.630	0.851	
	Item 8	4.000	0.679	0.846	
	Item 9	4.000	0.454	0.867	
Caregiver contribution to self-care monitoring	Item 10	4.000	0.810	0.904	0.921
	Item 11	4.000	0.766	0.908	
	Item 12	4.000	0.724	0.911	
	Item 13	4.000	0.718	0.912	
	Item 14	4.000	0.752	0.909	
	Item 15	4.000	0.701	0.913	
	Item 16	4.000	0.618	0.920	
	Item 17	4.000	0.793	0.906	
Caregiver contribution to self-care management	Item 19	4.000	0.292	0.356	0.458
	Item 20	4.000	0.206	0.437	
	Item 21	4.000	0.345	0.302	
	Item 22	4.000	0.206	0.444	
Caregiver Contribution to Self-care in Ostomy Patient Index					0.890

Table 5. Test-retest results of Caregiver Contribution to Self-care in Ostomy Patient Index sub-dimensions

	Caregiver contribution to self-care maintenance		Caregiver contribution to self-care monitoring		Caregiver contribution to self-care management		Caregiver contribution to self-care in ostomy patient index	
	r	p	r	p	r	p	r	p
Test-retest	0.987	<0.001[P]	0.976	<0.001[S]	0.973	<0.001[S]	0.983	<0.001[S]

[P]: Pearson correlation coefficient, [S]: Spearman correlation coefficient

highly reliable. In addition, in the item-total score analysis, it was concluded that the correlation coefficient of all items was above 0.30 and there would be no increase in the Cronbach's alpha value when the item was deleted. As a result of the analysis, the Pearson and Spearman correlation coefficients of the sub-dimensions were found to be between 0.973 and 0.987. Accordingly, the sub-dimensions of the scale were highly reliable and did not change over time[9,17].

The similarities of the original version of the scale with our study are that the scale items consist of 3 sub-dimensions, the CVI is 0.93 and the Cronbach's alpha is 0.972 in the internal consistency analysis.

Study Limitations

The most important limitation of our study is that it was conducted with families and relatives who contributed to the care of individuals with stoma in two public hospitals in Turkey. However, the validity and reliability of the scale in Turkish has been ensured and it is recommended to be used in other studies in Turkish society.

CONCLUSION

Individuals with stoma often need the support of their relatives in order to maintain their self-care. There is no valid and reliable scale in Turkish language to evaluate the relatives who participate in the care of individuals with a stoma and

contribute to their self-care. As a result of the research, it has been determined that the "Caregiver Contribution to Self-care in Ostomy Patient Index" is a valid and reliable scale for Turkish society in the Turkish language. This scale can be used as a guide in the evaluation of families and relatives who contribute to the self-care of individuals with a stoma in Turkish society.

Acknowledgement

We thank Villa et al., who developed the scale, for permitting to adapt the Caregiver Contribution to Self-care in Ostomy Patient Index into Turkish. The authors thank the experts who contributed with their opinions and the individuals who agreed to participate in the study.

Ethics

Ethics Committee Approval: Study procedures were reviewed and approved by the Non-interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine on August 27, 2020, with the decision number of 2020.195.08.04.

Informed Consent: Written and verbal consent was obtained from individuals who agreed to participate in the study.

Authorship Contributions

Design: T.Y., A.M., D.E., Ç.A., E.Ö., Y.D., U.C., S.Ö.G., Data Collection or Processing: T.Y., A.M., D.E., Ç.A., E.Ö., Y.D., U.C., S.Ö.G., Analysis or Interpretation: T.Y., A.M., D.E., Ç.A., E.Ö., Y.D., U.C., S.Ö.G., Literature Search: T.Y., Ç.A., Writing: T.Y., Ç.A.

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

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ORIGINAL ARTICLE

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Impact of CONUT Score at Admission on Prognosis in Older Patients with Acute Ischemic Stroke, Considering Ischemia Area: From Turkey's Second Region with the Oldest Population

Akut İskemik İnmeli Yaşlı Hastalarda İskemi Alanı Dikkate Alındığında Başvuru Sırasındaki CONUT Skorunun Prognoza Etkisi: Türkiye'nin En Yaşlı Nüfusa Sahip İkinci Bölgesinden

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ABSTRACT

Aim: The presence of malnutrition at admission in acute ischemic stroke (AIS) patients may cause poor outcomes in older patients. There are rare studies showing that Checking Nutritional Status (CONUT) score is a predictor of poor prognosis in AIS patients. We aimed to investigate the impact of CONUT score on the length of hospital stay (LOS) and intensive care need.

Materials and Methods: One hundred thirty-one of 230 patients older than 65 years old with a diagnosis of AIS were included. Patients with clinical correlation with diffusion-weighted magnetic resonance imaging examination were accepted as having ischemic stroke and classified by the Bamford classification. CONUT score was assessed within 24 hours after hospital admission.

Results: The mean age of patients was 78.15±6.9 years and 55.72% of patients were male, the mean LOS and the mean CONUT scores were 7.4±4.5 and 2.30, respectively. When patients were divided into two groups, as those requiring intensive care and with hospital stay >7 days and those with LOS <7 days, there was a significant difference between the two groups in terms of lymphocyte count, CONUT score, malnutrition level, and Bamford classification (p=0.007, p=0.002, p=0.004, p=0.030, respectively). In the crude regression model, CONUT score was determined to be possible risk factors for poor outcomes [odds ratio (OR): 1.38, p=0.002] and OR was 1.39 (p=0.003) in adjusted model for the Bamford classification.

Conclusion: Each unit increase in the CONUT score was associated with a greater risk of poor outcome in older AIS patients. Clinicians' evaluation of these patients with CONUT scoring may affect the prognosis.

Keywords: CONUT score, acute ischemic stroke, length of hospital stay, Bamford classification, older adults

ÖZ

Amaç: Akut iskemik inme (Aİİ) hastalarında başvuru sırasında malnütrisyonun varlığı yaşlı hastalarda kötü sonuçlara neden olabilir. Aİİ hastalarında Beslenme Durumunu Kontrol Etme (CONUT) skorunun kötü prognoz göstergesi olduğunu gösteren nadir çalışma vardır. Çalışmamızda CONUT skorunun hastanede yatış süresi (HYS) ve yoğun bakım ihtiyacı üzerine etkisini araştırmayı amaçladık.

Gereç ve Yöntem: Aİİ tanısı alan 65 yaş üstü 230 hastanın 131'i çalışmaya dahil edildi. Difüzyon ağırlıklı manyetik rezonans görüntüleme incelemesi ile klinik korelasyon gösteren hastalar iskemik inme olarak kabul edildi ve Bamford sınıflamasına göre sınıflandırıldı. CONUT skoru hastaneye yatıştan sonraki 24 saat içinde değerlendirildi.

Bulgular: Hastaların ortalama yaşı 78,15±6,9 yıl olup, %55,72'si erkekti. Ortalama HYS ve ortalama CONUT skoru 7,4±4,5 ve 2,30 idi. Hastalar yoğun bakım ihtiyacı olanlar ve HYS 7 günden fazla olanlar bir grup ve HYS 7 günden az olan grup olmak üzere iki gruba ayrıldığında, iki grup arasında lenfosit sayısı, CONUT skoru, malnütrisyon düzeyi ve Bamford sınıflandırması açısından anlamlı fark vardı (sırasıyla p=0,007, p=0,002, p=0,004, p=0,030). Ham regresyon modelinde CONUT skorunun kötü sonuçlar için olası risk faktörleri olduğu belirlendi [odds oran (OR): 1,38, p=0,002]. Bamford sınıflamasına göre düzeltilmiş regresyon modelinde OR: 1,39 (p=0,003) olarak tespit edildi.

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Sonuç: CONUT skorundaki her birim artış, yaşlı Aİİ hastalarında kötü sonuç riskinin artmasıyla ilişkilendirildi. Klinisyenlerin bu hastaları CONUT skorlaması ile değerlendirmesi hasta prognozunu etkileyebilir.

Anahtar Kelimeler: CONUT skoru, akut iskemik inme, hastanede kalış süresi, Bamford sınıflaması, yaşlı erişkinler

INTRODUCTION

Stroke is one of the major causes of disability and ranks as the second reason of mortality[1,2]. The relationship between stroke and mortality increases exponentially, especially after the age of 75 years[3]. Over the next 30 years, stroke is predicted to be more than quadruple due to the continuous rise in life expectancy[3].

Malnutrition has been a common problem in hospitalized older patients[4] and linked with poor outcomes, including increased mortality and morbidity in stroke patients who are most at risk for malnutrition because of dysphagia, diminished consciousness, abnormalities in perception, and cognitive deterioration[5,6]. On the other hand, compared to patients who are appropriately nourished, older adults who have had stroke and are malnourished at the time of admission are more likely to experience complications during their hospital stay, such as pneumonia, bedsores, and gastrointestinal bleeding, as well as a longer length of stay (LOS) and greater hospitalization expenditures[7]. Research indicates that malnutrition is frequently an underdiagnosed and undertreated condition[4]. Thus, the clinical nutrition guidelines advise an early evaluation of nutritional status upon admission to neurology services[5].

Different tools are used to assess nutritional status. One of the widely used practical and valid tools is the Checking Nutritional Status (CONUT) points system. The prognostic effect of the CONUT scoring system has been proven effective in cancers [8], coronary artery disease[9], heart failure[10], and atrial fibrillation[11]. Research has shown that in patients with ischemic stroke, malnutrition as determined by the CONUT score at admission is associated with mortality and length of stay[12-14]. However, there is no study on the effect of malnutrition assessed with CONUT at admission on the need for intensive care unit (ICU). Our goal was to find out how nutrition status, as measured by the CONUT score, affected longer LOS and the need for intensive care in a province with second oldest population in Turkey[15].

MATERIALS AND METHODS

Study Design and Population

The data of patients (65 years of age and older) who were hospitalized in a tertiary neurology clinic between July 2023 and December 2023 for acute ischemic stroke (AIS) treatment were enrolled retrospectively for this study. In total, 230 patients with stroke, who were older than 65 years, were registered in our study. The exclusion criteria were being at the age of <65 years, having lack of registered data, refusing treatment and leaving the hospital voluntarily, and having multiple hospital stay because of different causes. Figure 1 describes the flow chart of study population who were eligible for the study.

The Kastamonu Training and Research Hospital Research Committee granted approval and the study was conducted in accordance with the Declaration of Helsinki (no: 2023/KAEK-167, date: 20.12.2023). Written informed consent was not acquired from patients and/or relatives because of the retrospective nature of the study.

Diagnosing of Stroke

Patients who applied to the emergency department within the first 7 days after the onset of symptoms were included in the study. Computed tomography and diffusion-weighted magnetic resonance imaging (DW-MRI) examinations of all patients evaluated as having a stroke were assessed. On the other hand, those who could not undergo MRI for any reason (extreme obesity, MRI-incompatible implant, etc.) were excluded from the study. Patients with clinical correlation with DW-MRI examination, which is known to be highly sensitive in detecting the early changes and pathophysiological processes occurring in ischemic stroke, were accepted as having ischemic stroke[16]. Patients were evaluated as Total Anterior

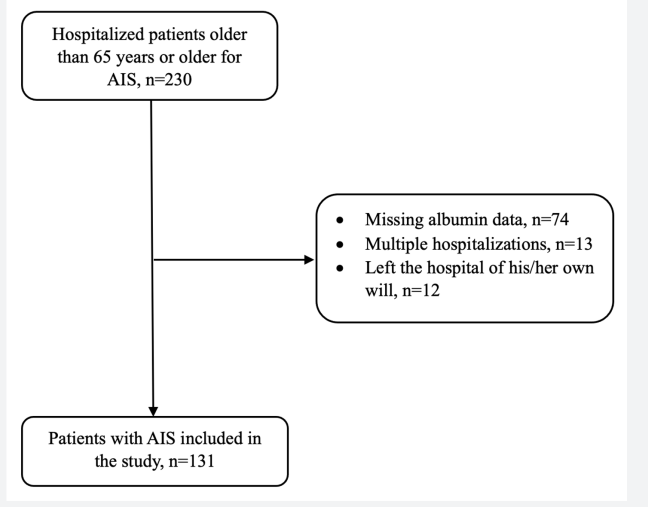

Figure 1. Flowchart of patient selection

AIS: Acute ischemic stroke, n: Number

Circulation Infarction, Partial Anterior Circulation Infarction (PACI), Posterior Circulation Infarction and Lacunar Infarction according to the Bamford criteria[17].

Assessment of Nutrition

Serum albumin, total lymphocyte count, and total cholesterol concentration were used to construct CONUT scores, which were then categorized as normal, mild, moderate or severe for scores of 0-1, 2-4, 5-8 and 9-12, respectively (Table 1)[18]. The blood samples for the CONUT score were acquired within 24 hours after hospital admission.

Demographics and Medical Data

Participants' sociodemographic information (age and gender), comorbidity assessed by the Deyo-Charlson Comorbidity Index[19] and laboratory parameters such as hemoglobin (g/dL), albumin (g/dL), lymphocyte, total cholesterol (mg/dL) and glomerular filtration rate were provided from patient charts and electronic health records at admission to hospital.

Outcomes

Longer hospitalization or in need of intensive care during hospitality in the neurology clinic is considered poor outcome.

Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences version 25.0 package program. Continuous variables were presented as mean±standard deviation, and categorical variables were presented as frequencies and percentages. The conformity of the data to the normal distribution was examined using the Kolmogorov-Smirnov test. The statistical significance of intergroup differences was assessed using χ^2 tests for categorical variables, and ANOVA test for continuous variables. Post-hoc analysis was conducted with Tukey in ANOVA test. The Mann-Whitney U test was applied for parameters that did not show normal distribution. Covariates with p≤0.05 in the univariate analysis were entered into the full logistic regression model. Results in the univariate logistic model and results in the model adjusted are presented as odds ratios (OR) and 95% confidence intervals for patients requiring intensive care or with longer hospital stay indicating poor outcome. We used "enter" method for regression models and evaluated the fit of the model with the omnibus test. Statistical significance was accepted as p<0.05.

Table 1. Assessment of Controlling Nutritional Status (CONUT) score

Parameter	Normal	Light	Moderate	Severe
Serum albumin (g/dL) Score	3.5-4.5 0	3.0-3.49 2	2.5-2.9 4	<2.5 6
Total lymphocytes (/mm^3) Score	>1600 0	1200-1599 1	800-1199 2	<800 3
Cholesterol (mg/dL) Score	>180 0	140-180 1	100-139 2	<100 3
Screening total score	0--1	2--4	5--8	9--12

RESULTS

The records of 230 hospitalized patients in neurology clinic older than 65 years because of ischemic stroke were reviewed. Seventy-four patients were excluded because of missing albumin data. Thirteen patients were excluded due to multiple hospitalization within 6 months and 12 patients were excluded because they had left the hospital voluntarily (Figure 1). One hundred thirty-one older adults with ischemic stroke were assessed for the study. The mean age of patients was 78.15±6.9 years and 55.72% of patients were men, the mean LOS and the mean CONUT scores were 7.4±4.5 and 2.30, respectively. Only 34.35% of patients had normal nutritional status on admission. PACI was the most common cause of ischemic stroke with a rate of 48.9%.

Firstly, 131 patients with ischemic stroke were divided into 3 groups, as those having hospitalization below the average, those with extended hospitalization stay and those who needed intensive care during admission (Table 2). The number of patients who stayed in the hospital for 7 days or less was 79, the number of those who stayed in the hospital for more than 7 days was 39, and the number of patients who needed intensive care during the hospitalization was 13. There was a significant age difference among three groups (p=0.013). The mean age of the group needing intensive care was significantly higher than the group LOS >7 (p=0.016) and the group LOS ≤7 (p=0.013) in post-hoc analysis results. Similarly, CONUT score differed among three groups (p<0.001). The highest mean CONUT score was 4.23 in patients requiring intensive care unit and it was significantly higher than in the group LOS >7 (p=0.013) and the group LOS ≤7 (p<0.001) in post-hoc analysis results. There was also a significant difference among the three groups when the nutritional status of the patients was staged according to the CONUT score (p=0.007). When the causes of ischemia were classified according to the Bamford, PACI was the most common in all groups, and there was a significant difference among the three groups (p=0.026).

Secondly, patients were divided into two groups: one group with those requiring intensive care and having hospital stay >7 days, and one group with those with LOS ≤7 days (Table 3). Of 131 patients, 52 patients had poor outcomes (Table 3). There was a significant difference between the two groups in terms of lymphocyte count, CONUT score,

Table 2. Demographic and clinic data of study population

	Total	LOS ≤7 n=79	LOS >7 n=39	ICU n=13	p value
Age	78.15±6.9	77.66±6.67	77.39±6.23	83.46±8.6	**0.013**
Gender (male, %)	73 (55.72%)	48 (60.76%)	21 (53.85%)	4 (30.76%)	0.126
Total cholesterol (mg/dL)	175.63±43.98	178.76±44.73	173.31±34.90	163.38±62.03	0.470
Lymphocyte (/mm^3)	1707.25±859.78	1844.43±872.99	1615.90±823.73	1147.69±644.68	0.018
Albumin (g/dL)	3.72±0.41	3.77±0.34	3.70±0.43	3.49±0.65	0.074
Hemoglobin (g/dL)	12.75±1.86	12.96±1.81	12.61±1.85	11.97±2.13	0.182
GFR	75.74±28	78.11±26.92	72.51±27.03	71.07±37.16	0.489
CONUT score	2.30±1.93	1.86±1.61	2.56±1.96	4.23±2.39	**<0.001**
Malnutrition level					**0.007**
Normal	45 (34.35%)	35 (44.30%)	10 (25.64%)	0	
Light	75 (57.25%)	41 (51.89%)	23 (58.97%)	10 (76.92%)	
Moderate	10 (7.63%)	3 (3.80%)	5 (12.82%)	2 (15.38%)	
Severe	2 (1.52%)	0	1 (1.35%)	1 (7.69%)	
CCI	6.48+1.33	6.34±1.31	6.64±1.42	6.84+1.14	0.303
Bamford classification					**0.026**
LACI	26 (19.8%)	22 (27.85%)	2 (5.13%)	2 (15.38%)	
PACI	64 (48.9%)	33 (41.77%)	21 (53.85%)	10 (76.92%)	
POCI	31 (23.7%)	19 (24.05%)	11 (28.21%)	1 (7.70%)	
TACI	10 (7.6%)	5 (6.33%)	5 (8.11%)	0	

LOS: Length of hospital stays, ICU: Intensive care unit, GFR: Glomerular filtration rate, CONUT: Controlling Nutritional Status, CCI: Charlson comorbidity index, LACI: Lacunar Infarction, PACI: Partial anterior circulation infarction, POCI: Posterior circulation infarction, TACI: Total anterior circulation infarction

Table 3. Demographic and clinic data of study population according to hospital stay and poor outcome

	Total n=131	LOS ≤7 n=79	Poor outcome n=52	p value
Age	78.15±6.9	77.66±6.67	78±7.30	0.316
Gender (male, %)	73 (55.72%)	48 (60.76%)	27 (51.92%)	0.153
Total cholesterol (mg/dL)	175.63±43.98	178.76±44.73	170.83±42.79	0.312
Lymphocyte (/mm^3)	1707.25±859.78	1844.43±872.99	1498.85±803.28	**0.007**
Albumin (g/dL)	3.72±0.41	3.77±0.34	3.65±0.49	0.109
Hemoglobin (g/dL)	12.75±1.86	12.96±1.81	12.45±1.92	0.132
GFR	75.74±28	78.11±26.92	72.15±29.49	0.235
CONUT score	2.30±1.93	1.86±1.61	2.98±2.17	**0.002**
Malnutrition level				**0.004**
Normal	45 (34.35%)	35 (44.30%)	10 (19.23%)	
Light	75 (57.25%)	41 (51.89%)	33 (63.46%)	
Moderate	10 (7.63%)	3 (3.80%)	7 (13.46%)	
Severe	2 (1.52%)	0	2 (3.85%)	
CCI	6.48±1.33	6.34±1.31	6.69±1.35	0.141
Bamford classification				**0.030**
LACI	26 (19.8%)	22 (27.85%)	4 (7.76%)	
PACI	64 (48.9%)	33 (41.77%)	31 (59.61%)	
POCI	31 (23.7%)	19 (24.05%)	12 (23.07%)	
TACI	10 (7.6%)	5 (06.33%)	5 (9.61%)	

LOS: Length of hospital stays, ICU: Intensive care unit, GFR: Glomerular filtration rate, CONUT: Controlling Nutritional Status, CCI: Charlson comorbidity index, LACI: Lacunar infarction, PACI: Partial anterior circulation infarction, POCI: Posterior circulation infarction, TACI: Total anterior circulation infarction

malnutrition level, and Bamford classification. In the crude regression model, CONUT score was determined to be possible risk factors for poor outcomes (OR: 1.38, p=0.002) (Table 4). In adjusted model for the Bamford classification, CONUT score was determined to be possible risk factors for poor outcomes (OR: 1.39, p=0.003) (Table 4). The omnibus test confirmed that the model was highly significant (-2LL=156.212, $\chi^2(2)=19.787$, p<0.001).

DISCUSSION

The fact that clinicians frequently do not have enough knowledge about nutritional support in the treatment of stroke patients causes patients to be deprived of nutritional support and accelerates the worsening of their stroke outcomes[20]. The American Heart Association and American Stroke Association advise that everyone be assessed for baseline nutritional status and that any malnutrition be treated as soon as feasible in their guidelines for the early care of patients with AIS[21]. Consistent with this recommendation, in our study, we have demonstrated that nutritional status, evaluated by the CONUT score on admission, plays an important role in the need for long-term hospital stay and intensive care in AIS patients.

Patients with ischemic stroke are known to be prone to dysgeusia and therefore malnutrition[5]. In addition, malnutrition that develops after stroke has been shown to be associated with poor outcomes[22]. However, there are rare studies showing poor outcomes in patients with AIS due to the presence of malnutrition at the time of hospital admission[14,22-24]. In a study conducted on patients with AIS over the age of 75 years, it was observed that those with CONUT score >5 had a longer hospital stay[14]. Similarly, CONUT score on hospital admission has been shown to be associated with 3-month functional deterioration in older patients with AIS[23]. In our study, we showed that only one third of the patients admitted on hospital with stroke were normal in terms of nutritional status and each unit increase in the CONUT score on admission increased the risk of poor prognosis, such as the need for intensive care and/or long-term hospitalization, by 1.4 in AIS patients over the age of 65 years. This significant effect continued even when adjusted for stroke location based on the Bamford classification.

Lower serum albumin levels in stroke patients were linked to worse outcomes, as several clinical investigations have shown[25,26]. Similarly, lower lymphocyte counts and a lower total cholesterol level were significant factors associated with the 3-month poor outcome in AIS[23]. In our study, while parameters such as lymphocyte, albumin and total cholesterol levels, which make up the CONUT score, did not influence prognosis alone, a significant effect of the CONUT score on poor outcomes was observed when these parameters were evaluated together.

Table 4. Association of CONUT score at admission with poor outcome

	Crude model		Model 1	
CONUT score	OR (95% CI)	p=0.002	OR (95% CI)	p=0.003
	1.38		1.39	

Model 1: Adjusted for the Bamford classification.
OR: Odds ratio, CI: Confidence interval, CONUT: Controlling Nutritional Status

Study Limitations

This study has several limitations. Firstly, the study was conducted at a single center with a small sample size. Secondly, due to its retrospective nature, it did not provide comprehensive nutritional data on dietary intake, weight changes, or physical examination results pertaining to fat and muscle. However, considering that CONUT is a proven prognosis nutritional assessment tool for cancers[8], coronary artery disease[9], heart failure[10], and atrial fibrillation[11], it can also be used in patients with ischemic stroke. What makes our study important from other studies is that, in addition to evaluating the need for intensive care, the Bamford classification was also considered when evaluating the CONUT effect. The fact that this study consisted of patients admitted to a tertiary hospital in the province with the 2nd oldest population in Turkey also makes our study valuable[15].

Our study results reveal that most patients presenting with AIS do not have normal nutritional status at admission to hospital and each unit increase in the CONUT score increases the risk of long-term hospitalization and/or the need for intensive care regardless of location of stroke. Considering that the incidence of malnutrition and ischemic stroke increases with age, and that malnutrition makes patients more prone to poor outcomes, evaluation of patients with the CONUT score on admission, which is an objective nutritional assessment tool that is easily obtained from blood, can help predict poor outcomes, and therefore, it will also guide clinicians and the determination of health policies. The study results need to be supported by studies with larger samples.

CONCLUSION

Patients with AIS do not have already normal nutritional status at the time of hospital admission and each unit increase in the CONUT score, was associated with a greater risk of longer LOS and/or need of ICU in AIS patients at the age of 65 years or older. Clinicians' evaluation of patients with AIS by CONUT scoring, which is a simple and valid method, may affect the prognosis of the patients.

Ethics

Ethics Committee Approval: The Kastamonu Training and Research Hospital Research Committee granted approval and

the study was conducted in accordance with the Declaration of Helsinki (no: 2023/KAEK-167, date: 20.12.2023).

Informed Consent: Written informed consent was not acquired from patients and/or relatives because of the retrospective nature of the study.

Authorship Contributions

Surgical and Medical Practices: S.K.D., O.D., Consept: S.K.D., O.D., Design: S.K.D., O.D., Data Collection or Processing: S.K.D., O.D., Analysis or Interpretation: S.K.D., Literature Search: S.K.D., O.D., Writing: S.K.D., O.D.

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Investigation of the Effects of Piceatannol on Endoplasmic Reticulum Stress on Brain in Rats with Experimental Subarachnoid Hemorrhage

Deneysel Subaraknoid Kanama Modeli Oluşturulmuş Sıçanlarda Piseatannolün Beyinde Endoplazmik Retikulum Stresi Üzerine Etkilerinin Araştırılması

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ABSTRACT

Aim: Subarachnoid hemorrhage (SAH) is a common neurologic disorder that accounts for brain injury, diminished neuron function, and neuronal death. Due to various options, SAH treatment remains lacking. Endoplasmic reticulum stress (ERS) in the brain is known as the disruption of the blood-brain barrier and triggered neuronal apoptosis, and contributes to SAH pathogenesis. This study aims to investigate the effects of piceatannol (PST) on ERS and neuronal apoptosis in an experimental SAH model in rats.

Materials and Methods: For this purpose, 24 Wistar Albino male rats (8-10 w) were randomly divided into three groups (n=8); SHAM, SAH, and PST. SAH model was induced via injection of 120 µL of autologous blood into pre-chiasmatic cisterna. 30 mg/kg PST was injected intraperitoneally after 60 minutes from SAH inducement. Garcia's neurologic examination, rotarod, and horizontal bar tests were applied for neurological evaluation. Frontal cortex specimens were harvested for histological and gene expression analysis.

Results: Our results indicated that PST treatment significantly improved Garcia scores ($p<0.01$; $p<0.05$). In addition, PST decreased pyknosis ($p<0.001$) and edema ($p<0.001$) levels, and the number of damaged cells ($p<0.01$) and apoptotic cells ($p<0.05$). GRP78 (78-kDa glucose-regulated protein; $p=0.01$), ATF4 (Activating transcription factor 4; $p=0.01$), and CHOP (C/EBP homologous protein; $p<0.05$) gene expression levels of the SAH group were increased compared to SHAM. Moreover, PST significantly decreased the expression levels of p53 ($p<0.01$).

Conclusion: Our results showed that PST indicated protective effects on ERS after SAH. It could be suggested that PST might be a supportive adjuvant agent in SAH management.

Keywords: Subarachnoid hemorrhage, early brain injury, piceatannol, endoplasmic reticulum stress, apoptosis

ÖZ

Amaç: Subaraknoid kanama (SAK) insanlarda beyin hasarına ve ölüme yol açan, günümüzde henüz kesin bir tedavisi olmayan bir hastalıktır. SAK'ın patogenezi tam olarak aydınlatılmamış olmakla birlikte erken beyin hasarı (EBH) en önemli neden olarak gösterilmektedir. EBH'nin nedenlerinden bir tanesi de endoplazmik retikulum stresidir (ERS). ERS beyin hücrelerinde apoptoza ve kan-beyin bariyerinin bozulmasına yol açmaktadır. Bu çalışmada deneysel SAK modeli oluşturulmuş sıçanlarda piseatannolün (PST) frontal korteksteki ERS ve apoptoz üzerine etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada 8-10 haftalık Wistar Albino sıçanlardan SHAM (n=8), SAK (n=8) ve PST (n=8) olmak üzere üç grup oluşturuldu. SAK ve PST gruplarında, 120 µL otolog arteriyel kan prekiazmatik sisternaya enjekte edilerek SAK modeli oluşturuldu. PST grubuna SAK sonrası 60. dakikada 30 mg/kg PST intraperiteonal uygulandı. Tüm gruplarda SAK öncesi ve sonrası Garcia nörolojik muayene skorlaması yapıldı. SAK'tan 24 saat sonra frontal korteks dokuları alınarak histopatolojik ve genetik analizler gerçekleştirildi.

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Bulgular: PST uygulamasının istatistiksel olarak anlamlı düzeyde olmak üzere nörodavranışsal test sonuçlarında iyileşmeye (p<0,01; p<0,05) ve histopatolojik düzeyde piknoz (p<0,001), ödem (p<0,001) ve TUNEL+ apoptotik hücre sayısında (p<0,05) azalmaya sebep olduğu gözlendi. SAK grubunda GRP78 (p=0,01), ATF4 (p=0,01) ve CHOP (p<0,05) gen ekspresyon seviyeleri SHAM grubuna göre yüksek bulundu. PST uygulaması SAK'ta artan tüm ERS göstergelerini azaltıcı etki gösterdi. Bu azalma GRP78 için istatistiksel olarak anlamlı bulundu (p<0,05). PST ayrıca SAK'ta artan p53 (p<0,01) gen ekspresyon değerlerini azaltıcı etki gösterdi.

Sonuç: SAK sonrası artan ERS üzerine PST'nin koruyucu etki gösterdiği anlaşıldı. Bu bulgulardan yola çıkarak, SAK tedavisinde PST'nin destekleyici bir adjuvant ajan olarak kullanım potansiyeli olduğu anlaşıldı.

Anahtar Kelimeler: Subaraknoid kanama, erken beyin hasarı, piseatannol, endoplazmik retikulum stresi, apoptoz

INTRODUCTION

Subarachnoid hemorrhage (SAH) is blood flow to the subarachnoid area between the arachnoid and pia mater filled with cerebrospinal fluid. SAH is associated with high morbidity and mortality rates and is responsible for 3-10% of all strokes[1]. SAH is commonly seen in 40-60 ages, with a mortality rate of up to 50% in the 1st month after hemorrhage[2].

The main underlying mechanism of SAH pathogenesis is early brain injury (EBI) which occurs within the first 3 days following SAH. EBI is a complex pathophysiological process that includes cerebral ischemia, blood-brain barrier (BBB) leakage, brain edema, oxidative stress, activation of inflammatory pathways signaling, and neuronal apoptosis[3]. Recent studies claimed that dysfunction of intracellular organelles such as endoplasmic reticulum (ER) and mitochondria may also contribute to the pathophysiology of SAH[4]. It is well known that ER stress (ERS) is responsible for cell death after SAH and ERS triggers the apoptosis of endothelial cells and disruption of the BBB[5]. Therefore, further studies are still required for reducing ERS in SAH.

ERS has been defined as an imbalance between the protein folding capacity and the overload of protein production in ER[6]. Synthesized polypeptides are constantly at risk of misfolding or aggregating into cytotoxic complexes. Molecular chaperones counteract this cytotoxicity by protein refolding and prevention against protein aggregation[7]. Proteins are under chaperone control under normal conditions[8]. Protein folding is facilitated by ER chaperone proteins such as BiP, or GRP78 (78-kDa glucose-regulated protein) and GRP94 (94-kDa glucose-regulated protein), and enzymes such as protein disulfide isomerase and peptidyl-prolyl isomerase[9]. Accumulation of misfolded proteins in ER lumens causes alteration in the ER homeostasis and stimulates the ERS[10]. Misfolded or unfolded proteins are degraded by the ER-associated protein degradation (ERAD) control system within the ER and maintain the protein balance[11]. However, sometimes the ERAD mechanism becomes insufficient to maintain protein balance, and in such cases, the unfolded protein response (UPR) signaling is activated in the cell[12-14]. This pathway is an important signaling mechanism required to restore homeostasis in ER function[15]. Activation of this pathway is mediated by three key sensor proteins, called (a) protein kinase RNA-like ER kinase (PERK), (b) inositol-requiring enzyme-1 (IRE1), and (c) activating transcription factor 6 (ATF6).

It has been reported in many studies that ERS and oxidative stress contribute to the pathogenesis of EBI in SAH[16,17]. Therefore, the reduction or complete elimination of ERS in SAH is considered among the treatment options. For this purpose, research on many molecules continues intensively. One of the candidate molecules considered for the reduction of ERS is piceatannol (PST).

PST is commonly found in blueberries, grapes, and passion fruit seeds, which is a hydroxyl analog of resveratrol (RES)[18]. PST has a higher bioavailability rate than RES[19-21]. This has led to the evaluation of PST as an alternative molecule to RES. The therapeutic aspect of PST is realized thanks to its anti-inflammatory, anti-oxidative, and anti-proliferative properties[22].

In addition, studies have also demonstrated that PST reduces or prevents ERS[13,23]. Although the effects of PST on ERS have been demonstrated in cells and organs such as the liver, osteoblast cells, and endothelial cells, its effect on ERS in SAH has not yet been investigated.

Therefore, our study aimed to investigate whether PST has an effect on ERS levels in the frontal cortex in SAH.

MATERIALS AND METHODS

This study was established with the approval of Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee with a number of 2021/02-08. The animal experimentation process of the study was performed at Çanakkale Onsekiz Mart University Experimental Research Application and Research Center.

Animals

In this study, 24 male Wistar rats (8-10-week-old and 200-300 g) were used. The rats were fed ad-libitum during the

experimental period at room temperature of approximately 20±2 °C on a 12-hour light-dark cycle. The rats were randomly divided into three groups (n=8) as follows;

SHAM (n=8): The sham SAH model was established for this group. The PST solvent was administered intraperitoneally (i.p.).

SAH (n=8): The SAH model was created with 120 µL of non-heparinized fresh autologous arterial blood injected into the pre-chiasmatic cisterna for 10 seconds. PST solvent was administered i.p.

PST (n=8): PST (dissolved in distilled water containing 2% ethanol) at a dose of 30 mg/kg was injected i.p. after 60 minutes of SAH inducement.

Preparation and Administration of Piceatannol

PST (Cayman Chemical; Cat. no: 10083-24-6) was first dissolved in 99% ethanol and then diluted with distilled water. The final ethanol concentration of the carrier solution was 2%. The solution was prepared just before injection and freshly administered i.p. at a dose of 30 mg/kg 60 minutes after the SAH inducement. The SHAM and SAH groups were injected with PST dissolved solution via the same route and at the same time points.

Establishment of the SAH Model

For the SAH model, first, the anterior regions of the skulls of rats were shaved under general anesthesia (60-80 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride). The tail was opened with a vertical incision and 120 µL arterial blood was collected from the tail artery. The skin and muscles were opened with a vertical incision in the frontal region and the bregma bone junction was reached. Using the stereotaxy device, the needle was tilted 30 degrees 2 mm to the right of the sagittal plane and placed 7 mm anterior to the bregma in the midline with a 1.5 mm diameter burr hole. 120 µL of non-heparinized fresh autologous arterial blood was injected into the pre-chiasmatic cisterna with a 30 G needle in 10 seconds. The incision site was sutured and closed.

In the SHAM group, all stages of the SAH model were performed, 30 G needle was kept for 10 seconds into the pre-chiasmatic cisterna, but no blood was injected into the area[24].

Neurobehavioral Assessment

All rats were subjected to neurologic tests, the day before the start of experimental procedures. The same tests were repeated 24 hours after SAH. For this purpose, Garcia's neurologic examination was performed.

Evaluation of Garcia's Neurological Assessment

Garcia's neurological assessment was used to evaluate neurologic and sensory functions. The evaluation was performed 24 hours before and after SAH by two blind observers[25].

Six tests including spontaneous activity (0-3 points), symmetry in four limb movements (0-3 points), forepaw extension (0-3 points), climbing (1-3 points), body proprioception (1-3 points) and vibration sense (1-3 points) were evaluated with a total score between 3 and 18.

Euthanasia and Tissue Harvesting

At the end of the study (24 hours after SAH), all rats were euthanized by cervical dislocation under general anesthesia, and the frontal cortex specimen was rapidly removed on an ice block. Total brain tissues from 3 of the 8 animals in each group were harvested to determine brain edema. Brain tissues of the remaining animals were used for histopathologic (kept in 4% formaldehyde) and genetic (stored at -80 °C) analysis.

Brain Water Content

Brain water content was evaluated to determine brain edema levels. For that purpose, the wet weight of total brains was rapidly measured and kept in an oven at +70 °C for 72 hours. After 72 hours, the tissue residues were removed from the oven and dry weights were measured. The ratio of wet and dry weight was determined as (%) described previously[26];

Brain Fluid Content=[(Wet Weight - Dry Weight) / Wet Weight] x 100%

Genetic Analysis

Total RNA Isolation

The frontal cortex samples were used for genetic analysis. 25-30 mg frontal cortex samples were weighed and homogenized. Total RNA was isolated from homogenates using PURE Link RNA MiniKit. The concentration and purity of total RNAs were determined by NanoDrop Spectrophotometer and samples with a purity ratio between 1.8-2.1 were considered usable for cDNA production. The obtained RNAs were stored at -80 °C until use[27].

cDNA Yield

cDNA yield was obtained according to the kit procedure (High Capacity cDNA Revere Transcription Kit, USA). The obtained cDNAs were run on a polymerase chain reaction (PCR) device (The Applied Biosystems®, 2720 Thermal Cycler 96-Well PCR).

Real-Time PCR (qRT-PCR) Application

The cDNA samples were amplified and then used for quantitative real-time PCR (qRT-PCR, StepOnePlus™ real-time PCR System). Gene expression levels were analyzed using TaqMan (TaqMan™ Fast Advanced Master Mix, Ampliqon, Lithuania).

Normalization was performed for GRP78, PERK, ATF4, CHOP (C/EBP homologous protein), p53 (tumor protein 53), and NF-κB (nuclear factor kappa b) genes, whose gene expression levels were analyzed in the qRT-PCR method. β-actin was used for normalization.

Histopathological Analysis

The frontal cortex tissue samples were first fixed in a 4% paraformaldehyde solution. The fixed tissue samples were placed in cassettes and washed under water for 2 hours. The tissues were passed through a series of alcohols (60%, 70%, 80%, 90%, 90%, 96%, and 100%) at increasing degrees respectively to remove water from the tissues. The tissues were then passed through xylol and embedded in paraffin. The sections were subjected to routine hematoxylin-eosin (HE) staining procedure and the stained sections were scored for cellular pyknosis and edema under a microscope (x100) according to HE staining criteria. The scoring was performed using 5 different grades Grade 0 (no findings), Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), and Grade 4 (highly severe).

TUNEL Assay

TUNEL assay was performed on 4 µm thick sections taken from paraffin blocks on slides. For this purpose, deparaffinization, rehydration, blocking of endogenous peroxidase activity, and diaminobenzidine chromogen staining procedures were performed brown stained cells were accepted as apopototic cells and apoptotic index (AI) was calculated according to below mentioned formula: AI=(apoptotic cell number/total cell number)x100.

Statistical Analysis

To determine the changes in gene expression levels, crossing point values were first obtained from qRT-PCR analysis. The results were normalized according to β-actin and gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ formula[28].

Statistical significance levels of other data were analyzed using IBM SPSS 26 software. The Kruskal-Wallis test was used for multiple group comparisons. A comparison between the two groups was performed with the Mann-Whitney U test. The value of $p<0.05$ was considered statistically significant.

RESULTS

This study was performed with 24 rats and 8 animals in each group. No death was observed in the groups during the experiment. Severe hemorrhage in the subarachnoid region was observed in rats subjected to SAH. In the PST group, it was observed that bleeding in the subarachnoid region decreased. No calculation was made for bleeding levels. Only observationally, the presence and amount of bleeding were evaluated. Brain edema data were 77.1% in the SHAM group, 77.8% in the SAH group, and 77.5% in the PST group. There was no significant difference between the groups in terms of brain edema findings.

Garcia's Score

The neurologic functions of the rats were evaluated by Garcia's neurologic examination. There was no statistically significant difference between the groups 24 hours before SAH ($p>0.05$). However, SAH and PST groups indicated significant differences 24 hours after SAH (Figure 1).

ERS Related Genes Expression Levels

In our study, GRP78, PERK, ATF4, and CHOP gene expression levels were examined. While no significant change was observed in PERK gene expression levels, statistically significant changes were observed in GRP78, ATF4, and CHOP gene expression levels ($p<0.05$ or $p<0.01$; Figure 2a-2d).

p53 and NF-κB Gene Expression Levels

The results of the study showed that p53 mRNA levels increased dramatically in the SAH group and PST strongly reduced p53 mRNA levels ($p<0.01$). However, no significant change was

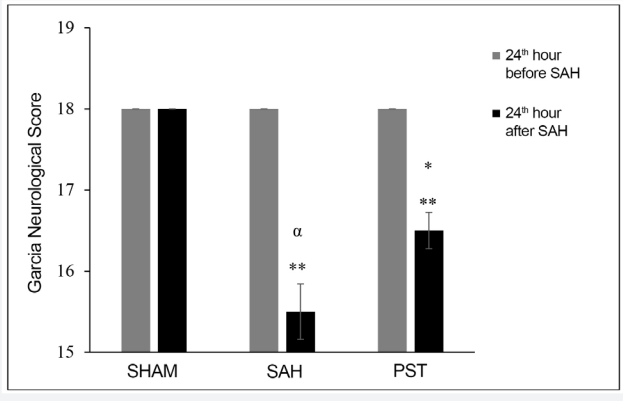

Figure 1. Comparison of Garcia's neurological scoring data
*Compared to SAH ($p<0.05$)
**Compared to SAH ($p<0.01$)
α: Comparison of before and after SAH group ($p<0.01$)

found in NF-κB gene expression levels between the groups (Figure 3a, 3b).

Hematoxylin-Eosin Staining

Frontal cortex tissue samples were evaluated for cellular pyknosis and edema by HE staining. No cellular pyknosis and edema occurred in the SHAM group. Cellular pyknosis and edema levels were significantly increased in both SAH ($p<0.001$ for both parameters) and PST (pyknosis: $p<0.01$; edema: $p<0.001$) groups compared to the SHAM group. HE staining samples of the groups are represented in Figures 4a, b, and c. The statistical significance levels of these changes are represented in Figures 5a and b.

TUNEL Results

Apoptotic cell numbers were determined by the TUNEL staining method. According to the findings, there was a statistically significant increase in the amount of TUNEL-positive cells in both SAH and PST groups compared to the SHAM group ($p<0.01$ and $p=0.05$, respectively). PST treatment significantly decreased the number of apoptotic cells after SAH (Figure 6a-6d).

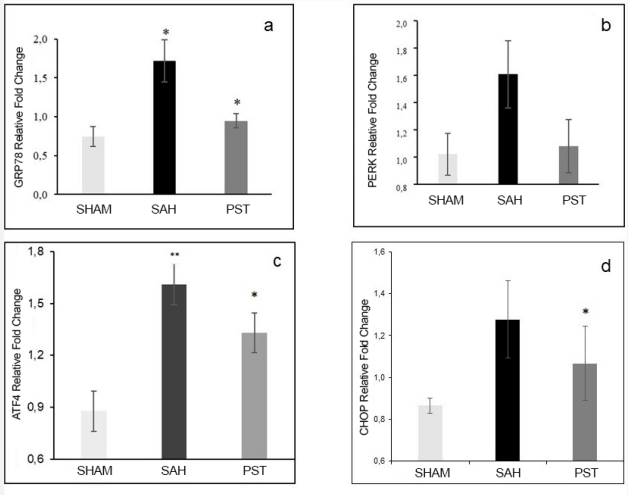

Figure 2. Representative fold changes of ERS related gene expression levels. (a) GRP78, (b) PERK, (c) ATF4, (d) CHOP.

*Compared to the SHAM group ($p<0.05$).

**Compared to the SHAM group ($p=0.01$).

DISCUSSION

In this study, the ameliorative effects of PST on ERS in the frontal cortex were demonstrated for the first time with an experimental SAH model in rats. It was determined that PST administration at 60 minutes after SAH a) improved Garcia's neurological score, b) decreased gene expression levels of ERS markers such as GRP78, PERK, ATF4, and CHOP, c) decreased p53 and NF-κB gene expression levels as inflammatory and apoptotic targets, d) altered pyknosis and edema score at the histopathological level and (e) decreased the number of TUNEL positive cells. These are the first experimental findings clarified that PST administration after SAH may improve SAH-induced ERS and reduce cell damage.

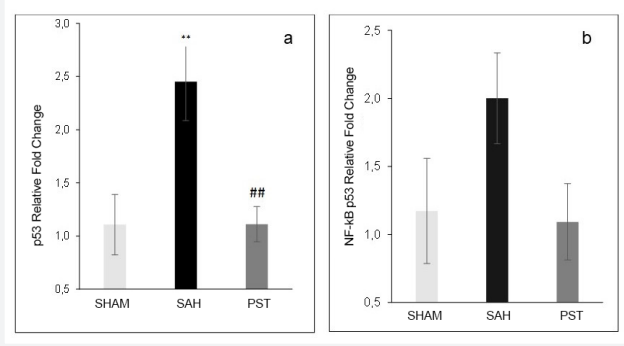

Figure 3. Representative fold changes of apoptosis and inflammatory related gene expression levels. (a) p53, (b) NF-κB. ($p>0.05$)

**Compared to the SHAM group ($p<0.01$).

##: Compared to the SAH group ($p<0.01$).

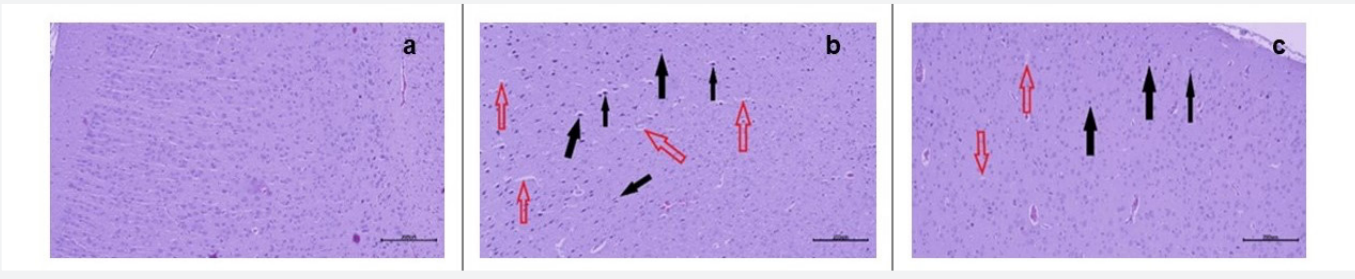

Figure 4. (a) SHAM, (b) SAH and (c) PST groups frontal corteks sections (HE, 100x). Red arrows represent edema and black arrows indicate cellular pyknosis.

In our study, the SAH model modified from Prunell et al.[24] (2003) was applied. At the end of the study, after removing the brain tissues of the animals, the subarachnoid space of all rats was checked for blood accumulation. We proved the success of our SAH model by blood clots in the subarachnoid space, neurologic score, genetic and histopathologic results.

It was observed that our results counteracted previous studies about the wet/dry brain weight ratio. Xiong et al.[29] (2020) removed the brain tissue of rats 48 hours after SAH inducement, dried at 100 °C for 48 hours, and determined the wet/dry brain ratio. The study showed that the wet/dry brain ratio increased in the SAH group and L-cysteine administration reduced brain edema. Yan et al. (2017)[30] reported that brain tissue was removed at 72 hours after SAH and divided into 4 sections. After each section was dried separately at 105 °C for 72 hours, brain edema was determined using the wet/dry brain ratio formula. Researchers claimed that this rate increases with the SAH model. In the study by Qi et al.[31] (2018), the brain tissues were removed 24 hours after SAH and dried at 105 °C for 24 hours. In this study, it was revealed that brain edema increased with SAH, and atorvastatin administration decreased brain edema. In our study, the highest wet/dry brain ratio was determined in the SAH group and PST administration decreased this ratio. However, there was no statistical difference between the groups. The reason may be the small amount of blood injection or the fact that brain edema data were recorded in only 3 rats. On the other hand, we detected edema at a histopathologic level in the brain tissue samples of the SAH group. These results suggest that our SAH model caused brain edema at the histopathologic level, but not severe enough to cause a significant increase in total brain water content.

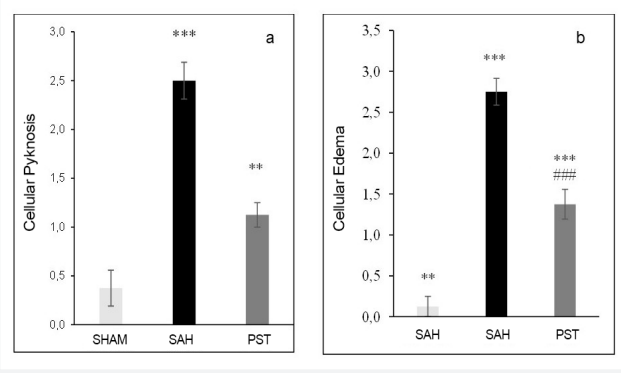

Figure 5. HE staining evaluation results of (a) cellular pyknosis and (b) edema.

**Compared to the SHAM group (p<0.01).

***Compared to the SHAM group (p<0.001).

###Compared to the SAH group (p<0.001).

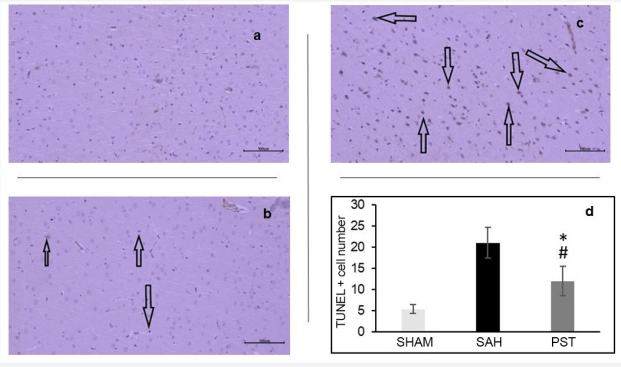

Figure 6. Apoptotic cell images of groups. (a) SHAM, (b) SAK and (c) PST (TUNEL, 200x). Black arrows represent apoptotic cells. (d) TUNEL (+) cell numbers according to AI index.

*Compared to the SAH group (p=0.05).

**Compared to the SHAM group (p<0.01).

#Compared to the SHAM group (p=0.05).

In our study, neurobehavioral test findings of all rats were recorded before the SAH model was created and compared with the results 24 hours after SAH. The neurobehavioral test was performed with Garcia's neurological assessment. Garcia's neurologic scores of all rats before SAH were 18. In the SAH group, the scores decreased to 15.5 at 24 hours. It was determined as 16.5 in the SAH group treated with PST. These results show that PST improves the Garcia neurologic score, which worsened after SAH (Figure 1). Post-SAH neurological score records are determined in almost all SAH studies. The level of deterioration in neurologic scores provides information both about the occurrence of SAH and the level of SAH. On the other hand, the effects of preventive or therapeutic agents on neurologic scores in SAH are also examined. Neurologic score data after SAH are usually recorded at 24 hours because the most significant changes occur at this time. Tian et al.[32] (2020) determined Garcia scores at the 3rd, 6th, 12th, 24th, 48th, and 72nd hours in rats in which they created a SAH model and measured the lowest score at the 24th hour. In their study, the Garcia score, which started to decrease at the 6th hour, decreased to 10 at the 24th hour and then started to increase again. They suggested that this decrease in Garcia score may be related to neuronal apoptosis at this time point[32]. Similarly, in our study, we found that the SAH group with the lowest Garcia score had the highest number of frontal cortex apoptotic cells.

The pathologic events that occur in the brain in the 24th-72nd hours after SAH are defined as EBI. Neuronal apoptosis is the most important underlying mechanism of EBI. Neuronal apoptosis is considered to be the main cause of neurologic deterioration and loss of function after SAH. Many mechanisms are known to stimulate neuronal apoptosis. These include increased ROS, excitotoxicity, synaptic dysfunction, impaired

protein degradation systems, ERS, DNA damage, mitochondrial dysfunction, and inflammation[33]. ERS, one of these factors, has recently been associated with neuronal apoptosis after SAH. Impaired ER function leads to the accumulation of unfolded proteins and consequently to ERS. ERS leads to the activation of the UPR pathway. The UPR activates ERS-responsive proteins such as PERK and ATF4. Activation of PERK stimulates the activation of ER-derived chaperones and cytokines. Moderate ERS leads to cell survival, whereas severe and sustained ERS leads to nerve cell death. A study performed by Nakka et al.[17] (2016) showed that ERS was involved in ischemia/reperfusion-induced nerve cell death. In their study, they determined that GRP78, CHOP, and ATF4 gene expression levels increased in brain tissue after ischemia/reperfusion, and high levels of neuronal damage. When they applied salubrinal, a selective eIF2α inhibitor, to reduce UPR, significant reductions in neuronal cell death. In another study, the effect of zonisamide on neuronal cell death via ERS was examined. The results revealed that zonisamide decreased ERS via CHOP and caspase-3 and prevented neuronal damage in rats with Parkinson's model[34].

In addition to neurodegenerative diseases, increased ERS after SAH has also been associated with neuronal cell death. Jiang et al.[35] (2021) showed that GRP78, CHOP, and caspase-12 gene expression levels increased after SAH in cerebral cortex tissue in rats, thus increasing neuronal cell death. Findings show that neuronal apoptosis is improved in rats given hydrogen-rich saline. Tian et al.[32] (2020) showed that the number of TUNEL-positive apoptotic cells increased at 24 hours after SAH. The researchers suggested that the increase in neuronal apoptosis may be caused by increases in GRP78, CHOP, caspase-12, and ASK1 gene expression levels. In our study, increases in GRP78, PERK, ATF4, and CHOP gene expression levels, which are indicators of ERS, were also observed after SAH and PST administration showed a therapeutic effect against these increases. We observed that both p53 and NF-κB and apoptotic nerve cell numbers were increased in the groups in which ERS indicators were also increased. These findings show that increased ERS after SAH triggers neuronal apoptosis in parallel with the reports in the literature. PST administration showed an ameliorative effect on neuronal damage by decreasing ERS.

In our study, NF-κB gene expression levels were analyzed as a parameter that may indicate inflammatory processes. The fact that NF-κB gene expression levels, which increased after SAH, decreased by approximately 50% in the PST group suggested that PST inhibited inflammatory processes. The inhibitory effect of PST on NF-κB was demonstrated in a study[36]. According to the results of the study, it was suggested that the antioxidant and anti-inflammatory effects of PST, which occur in brain tissue and are thought to be protective against brain damage, were associated with NF-κB inhibition. In the study, the effect of PST on brain cells was examined in lipopolysaccharide-induced inflammation. In an *in vitro* study, it was reported that PST might protect the brain against damage by reducing the disruption in the BBB. In another study, the protective effects of PST in a brain ischemia/reperfusion model were examined[37]. PST is known as a SIRT1 activator. SIRT1 activation protects cells against damage by stimulating the synthesis of antioxidant and antiapoptotic factors[38,39]. Based on the study results, the antioxidant and anti-inflammatory effects of PST in a brain ischemia/reperfusion model were associated with SIRT1 activation[37]. Although the above-mentioned studies show the protective effects of PST on brain injury, only one study examining the effects of PST in SAH was found. However, PST is known as a RES analog and there are studies on the role of RES in both brain injury and SAH. In a study in which RES was administered at both very high and low doses in an SAH model, it was found that its antiapoptotic effect only appeared at high doses. In the study, RES showed this effect by inhibiting the Akt pathway[40]. In another study, RES also showed a decreasing effect on ERS with increased oxidative stress after SAH[41]. The effect of RES was examined in the prefrontal cortex similar to our study. RES significantly decreased the increased CHOP and GRP78 levels after SAH. Increased ROS accumulation in SAH triggers processes leading to ERS and apoptosis. RES, an antioxidant, shows a decreasing effect on ERS thanks to this property. In our study, similar to RES, PST decreased CHOP, GRP78, ATF4, and PERK gene expression levels, which are among the increased ERS indicators after SAH. PST also decreased the number of TUNEL-positive cells. These data suggest that PST also decreased apoptosis after SAH.

Study Limitations

In our study, the protein levels of the markers were not evaluated. In addition, only 3 rats were used for brain edema because of ethical issues.

CONCLUSION

Our results showed that PST improved the deteriorated neurologic scores after SAH, decreased apoptosis, and produced a protective effect against the ERS. These results are the first findings that PST has a protective effect on ERS after SAH. Based on these findings, PST has the potential to be used as an adjuvant agent in the treatment of SAH. However, further studies are required to elucidate other underlying mechanisms.

Ethics

Ethics Committee Approval: This study was approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee with a number of 2021/02-08.

Informed Consent: Animal experiment.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: B.Ö., M.U.

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

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ORIGINAL ARTICLE

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One Year Retrospective Review of Forensic Reports Reported in the Emergency Department

Acil Serviste Bildirimi Yapılan Adli Raporların Geriye Dönük Bir Yıllık İncelemesi

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ABSTRACT

Aim: It was aimed to investigate the characteristics of forensic cases admitted to the emergency department of Tekirdağ Namık Kemal University Hospital (TNKUH), the qualities of forensic reports and the prognosis of the cases.

Materials and Methods: All forensic cases for which a forensic report was written in the TNKUH emergency department between 01.01.2021 and 31.12.2021 were included in the study. Case records, hospital information management system and clinical patient forms and judicial notification files in the emergency department archive were examined retrospectively one by one.

Results: Analyzes were made on 1136 cases for which forensic reports were prepared in the TNKUH emergency department. While 71.8% of the patients were male, the overall average age was 33.4±15.7 years. It was observed that the highest number of patients was in the 21-30 age group with a rate of 31.3%. Among the reasons for application, traffic accidents ranked first (33.3%), followed by assault-force cases (24.1%) and work accidents (21.5%). It was determined that 89.1% of the assault-algebra cases occurred with blunt traumatic-impact action. In the conclusions of the forensic reports, no life-threatening situation was mentioned in 175 cases (15.4%), it was stated that there was a life-threatening situation in 84 patients (7.4%), and that there was no life-threatening situation in 877 (77.2%) patients. It was observed that 957 patients (84.2%) were discharged from the emergency department after their examination and treatment, 112 patients were admitted to the ward (7.5%) and intensive care units (2.4%), and 8 (0.7%) patients died in the emergency department during their examination and treatment. Most of the patients admitted to clinical wards were admitted to surgical branch wards.

Conclusion: A significant portion of forensic cases are caused by trauma. Having complete and orderly records in forensic cases is important in terms of physician safety and preventing patient victimization in the legal process that may occur afterwards.

Keywords: Emergency department, forensic case, forensic report

ÖZ

Amaç: Tekirdağ Namık Kemal Üniversitesi Hastanesi (TNKÜH) acil servisine başvuran adli olguların özellikleri ile adli raporların nitelikleri ve olayların prognozlarının araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 01.01.2021-31.12.2021 tarihleri arasında TNKÜH acil serviste adli rapor yazılmış tüm adli olgular dahil edilmiştir. Olguların kayıtları, hastane bilgi yönetim sistemi ve acil servis arşivinde bulunan klinik hasta formları ve adli bildirim dosyaları tek tek geriye dönük olarak incelenmiştir.

Bulgular: TNKÜH acil servisinde adli rapor düzenlenen 1136 vaka üzerinden analizler yapıldı. Hastaların %71,8'i erkek iken genel yaş ortalaması 33,4±15,7 yıl idi. En fazla hasta sayısının %31,3 oran ile 21-30 yaş grubunda olduğu görüldü. Başvuru nedenleri arasında trafik kazaları (%33,3) ilk sırada yer alırken, bunu sırasıyla darp-cebir olguları (%24,1) ve iş kazaları (%21,5) izlemekteydi. Darp-cebir olgularının %89,1'inin künt travmatik-etkili eylem ile gerçekleştiği saptandı. Adli raporların sonuç kısımlarında, 175 olguda (%15,4) hayati tehlike ile ilgili herhangi bir durumdan bahsedilmemişti, 84 hastada (%7,4) hayati tehlikenin bulunduğu, 877 (%77,2) hastada ise hayati tehlikeye sokan bir durum olmadığı belirtilmişti.

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957 hastanın (%84,2) tetkik ve tedavileri sonrasında acil servisten taburcu edildiği, 112 hastanın servis (%7,5) ve yoğun bakım birimlerine yatırıldığı (%2,4), 8 (%0,7) hastanın da tetkik ve tedavileri esnasında acil serviste vefat ettiği görüldü. Klinik servislere yatırılan hastaların büyük kısmı cerrahi branş servislerine yatırılmışlardı.

Sonuç: Adli olguların önemli bir kısmını travmaya bağlı sebepler oluşturmaktadır. Adli olgularda kayıtların eksiksiz ve düzenli olması, sonrasında oluşabilecek hukuksal süreçte hekim güvenliği ve hasta mağduriyetinin önlenmesi açısından önemlidir.

Anahtar Kelimeler: Acil servis, adli vaka, adli rapor

INTRODUCTION

Many emergency cases are admitted to emergency departments every day and some of these cases are forensic cases. A forensic case can be defined as any event that causes a person's physical or mental health to deteriorate, injury or death as a result of the intent, negligence, recklessness or carelessness of oneself or another person. On the basis of this definition, it is a situation where the intent, negligence, recklessness or carelessness of another person or persons is a factor in a situation where people may be physically or mentally harmed[1-6]. Forensic cases include not only applications to health service providers due to criminal incidents, but also those who frequently apply to the departments of forensic medicine in cases that will be subject to a trial, such as examinations of detainees and prisoners, entrance and exit examinations of detention, age determination, detection of illegal substance use, safe driving examinations depending on alcohol intake level, forgery of writing-signatures-documents, as well as those who frequently apply to the departments of emergency services, and those who generally apply to the Departments of Psychiatry and Neurology for reasons such as legal capacity, criminal capacity, guardianship, annulment of marriage[7]. The procedures carried out by healthcare professionals in order to determine the incident, to find evidence and traces, and to organize the treatment and prophylaxis stage in the necessary processes in order to document it in a report are called "forensic examination"[8].

Traffic accidents, firearm and explosive injuries, sharp-piercing-crushing tool injuries, battery and force (blunt traumatic-effective action) cases, occupational accidents, physical, emotional, sexual abuse and neglect of vulnerable risk groups (such as women-elderly-children-disabled and those with sexual orientation differences), poisoning, suicide attempts, falls, illegal substance use, entry and exit from detention, allegations of torture, animal scratches and bites, mechanical or chemical asphyxia, burns, exposure to electric current, suspected domestic accidents, allegations of medical malpractice and all kinds of suspicious deaths are considered forensic cases[3,4,6,9,10].

Legal Basis

Within the scope of the duties and responsibilities imposed on all physicians by national and international health legislation, emergency physicians have the responsibility to examine patients and perform the necessary medical intervention, as well as the duties of forensic medicine and official referee[11-13].

Physicians have an obligation to report forensic cases to the judicial authorities in accordance with article 280 of the Turkish Criminal Code (TCC) no. 5237[14]. In addition, Article 86 of the Regulation on the Operation of Inpatient Treatment Institutions, which includes the articles amended in 2015, states that "In cases examined and treated in inpatient treatment institutions, if there is an indication that a crime has been committed, it is obligatory to inform the public prosecutor's office or judicial police without delay in accordance with the relevant article of the Turkish Criminal Code. The items that have the quality of evidence removed from the wounded and the corpse must be delivered to the judicial authorities in the same manner and without delay"[15].

Within the framework of Law No. 1219 on the Practice of Medicine and Medical Sciences, all physicians who have the right to practice medicine in our country are responsible for taking part in forensic incidents, making forensic notifications and issuing forensic reports, as well as fulfilling the official referee duties imposed on them by law[16].

According to the Turkish Criminal Code No. 5237, which entered into force in June 2005, emergency room physicians are expected to clarify two main issues in forensic cases in which they examine and issue a report: whether the damage has caused a situation that endangers the life of the person and whether it is mild enough to be eliminated by a simple medical intervention. In this context, a standard guideline for "Evaluation of Injury Crimes Defined in the Turkish Criminal Code from the Perspective of Forensic Medicine", which was first created in September 2005 and published in June 2019 with the joint work of the Presidency of the Council of Forensic Medicine (ATK), the Association of Forensic Medicine Specialists (ATUD) and the Forensic Medicine Association, has been prepared for nationwide use[4]. Following the enactment of the Criminal Procedure Code No. 5271, the 2019 guideline was added to the circular dated 22.09.2005 and numbered 13292, regulating the "Principles to be followed in the Execution of Forensic Medicine Services" published by the Ministry of Health General Directorate of Primary Health Care Services. It was also sent to governorships by the Ministry of Health and announced to health units by provincial health directorates[17-22]. Feedback received along with community and human-based

needs is being evaluated and updates are currently underway for the 2019 guidelines.

In this cross-sectional study, we aimed to analyze the epidemiological and demographic characteristics of forensic cases, which have an important place among the admissions to the emergency department of Tekirdağ Namık Kemal University Hospital (TNKÜH), the qualities of forensic reports, their relations with other clinics, their prognosis and other related factors, and to make a comparison with basic sources of information and literature studies. In addition, according to the results, it is aimed to determine the needs for in-service trainings between clinics and to review our status of good medicine on the basis of law.

MATERIALS AND METHODS

This study was planned as a retrospective, cross-sectional, one-year archival data review, which was initiated with the approval of Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (protocol number: 2023.75.04.11, date: 25.04.2023).

Study Population and Data Collection

All forensic cases for which a forensic report was written in the emergency department of TNKÜH between 01.01.2021 and 31.12.2021 were included in the study. The case list was obtained from the hospital information management system. The records of the cases were retrospectively reviewed one by one in the hospital information management system and the clinical patient forms and forensic report files in the emergency department archive. Cases with missing data and cases that could not be reached were not included in the study. The files were analyzed in terms of age, gender, nature of the forensic event, mechanism of trauma, presence and level of ethanol, outcome of the forensic report, presence of life threat, outcome of the case in the emergency department and if the case was hospitalized in a clinic, its nature.

Statistical Analysis

Statistical Package for the Social Sciences 16.0 package program was used to analyze all data obtained from the study. The findings were analyzed statistically in terms of frequency distribution. Descriptive numerical variables were expressed as mean±standard deviation, categorical variables were expressed as number (n), and proportions were given as percentage (%).

RESULTS

Between 01.01.2021 and 31.12.2021, it was determined that a general forensic examination form was filled out and a forensic report was prepared for 1186 cases in the emergency department of TNKÜH. Statistical analyses were performed on 1136 files, since 50 cases with incomplete or inaccessible file information were excluded from the study.

The gender distribution was male with a rate of 71.8% (n=816) and female with a rate of 28.2% (n=320). The mean age was 33.4±15.7 years, the oldest case was 92 years old and the youngest case was in the zero age group. The median age of the cases was 33.4±15.7 years (minimum: 0 - maximum: 92). The highest number of cases was in the age range of 21-30 years with the rate of 31.3% (n=355). While 6.6% (n=75) of the oldest group were 61 years old and older, 3.3% (n=38) of the youngest group were in the age group of 0-10 years (Table 1).

When we analyzed the cases according to event types, traffic accidents ranked first with the rate of 33.3% (n=378), followed by assault and battery cases with 24.1% (n=274) and occupational accidents with 21.5% (n=244). In terms of the trauma mechanisms of the incidents, we see that 89.1% (n=244) of the cases of assault and battery (n=274) were caused by blunt traumatic-effective action, most of the cases of occupational accidents (n=244) were caused by blunt-crushing trauma (43.9%, n=107) and sharps injuries (42.2%, n=103), most of the suicide cases (n=65) were caused by oral drug intake (73.9%, n=48), followed by sharps injuries (21.5%, n=14), poisoning cases (n=60) were mostly caused by food intake (65%, n=39), 20% (n=12) by smoke-gas inhalation and 6.7% (n=4) by alcohol use (Table 2).

Blood alcohol level was requested from 26.1% (n=297) of the 1136 cases included in the study, and blood alcohol level was below the detection value (<10 mg/dL) in 223 of the cases. Most of the alcohol level requests were made for traffic accidents with 208 (70%) cases, 178 cases had blood alcohol levels below the detection value and 30 cases were found to be alcoholic. The highest blood ethanol level was not due to a traffic accident, but belonged to a patient admitted for suicide attempt with a blood level of 582 mg/dL (Table 2).

When we analyzed the conclusions of the forensic reports, it was observed that in 175 cases (15.4%), there was no mention of any life-threatening situation; 18 of these were reports that reported only lesions and complaints for the purpose of detention-entry-exit examination; 84 cases (7.4%) were found to be life-threatening; and in 877 cases (77.2%), it was written

Table 1. Demographic characteristics of forensic cases

Demographic data	n	%
Sex		
Male	816	71.8
Female	320	28.2
Age groups		
0-10 years	38	3.3
11-20 years	195	17.2
21-30 years	355	31.3
31-40 years	206	18.1
41-50 years	176	15.5
51-60 years	91	8
61 years and above	75	6.6
Mean age	33.4±15.7 (minimum: 0 - maximum: 92)	

Table 2. Epidemiological characteristics of forensic cases					
	n	%		n	%
Nature of the forensic case			**Trauma mechanism**		
Traffic accident	378	33.3	**Occupational accident**		
Assault and battery	274	24.1	Blunt trauma	107	43.9
Occupational accident	244	21.5	Sharp object injury	103	42.2
Falling from a height	65	5.7	Burn	8	3.3
Suicide	65	5.7			
Poisoning	60	5.3	**Suicide**		
Firearm injury	10	0.9	Taking medication orally	48	73.9
Biting and stinging	8	0.7	Sharp object	14	21.5
Illegal substance use	5	0.4			
Sharps injury	3	0.3	**Poisoning**		
Burn	3	0.3	Food intake	39	65
Electric shock	1	0.1	Smoke-gas inhalation	12	20
Sexual abuse	1	0.1	Alcohol use	4	6.7
Drowning in water	1	0.1			
Other (entry-exit examinations)	18	1.6			
Request for alcohol in blood			**Blood alcohol results**		
No	839	73.9	Below the detection value	223	75.1
Yes	297	26.1	Ethanol positive	74	24.9

that there was no life-threatening situation. Of the 84 life-threatening cases, 31 were traffic accidents and 13 were high-energy traumas such as falling from a height (Tables 2 and 3).

Again, when the final decisions of forensic reports were analyzed, it was found that 4.6% (n=52) were finalized as "definitive physician's report", 59.6% (n=677) as "physician's report expressing opinion", and 27.2% (n=309) as "provisional physician's report" (Table 3).

When the outcomes of forensic cases in the physical environment of the emergency department were analyzed, it was detected that 957 cases (84.2%) were discharged from the emergency department after examination and treatment, 112 cases (n=85 + n=27) were hospitalized in the ward and intensive care units (7.5% + 2.4%), and 8 cases (0.7%) died in the emergency department during their examination and treatment. When the forensic events of the 8 patients who died were analyzed, it was determined that two cases were traffic accidents, two cases were suicide, one case was a fall from a height, one case was firearm injury, one case was drowning in water and one case was suspected arrest.

It was observed that most of the patients (n=85) hospitalized in clinical service beds were hospitalized in surgical unit beds (35 cases in orthopedics and traumatology clinic, 20 cases in neurosurgery clinic, 8 cases in ophthalmology unit and 7 cases in thoracic surgery unit). Of the 27 cases hospitalized in the intensive care unit, 25 were hospitalized in the intensive care unit of the anesthesiology and reanimation clinic and the other two cases were hospitalized in the intensive care units of the internal medicine clinic and pediatrics clinic (Table 3).

Table 3. Conclusions of forensic reports and cases		
	n	%
Presence of a life-threatening situation		
None	877	77.2
Present	84	7.4
Not specified	175	15.4
Termination of forensic report		
Definitive physician's report	52	4.6
Provisional physician's report	309	27.2
Physician's report expressing opinion	677	59.6
Not specified	98	8.6
Ending emergency service		
Discharge	957	84.2
Hospitalization in clinic	85	7.5
Intensive care hospitalization	27	2.4
Leaving the clinic voluntarily	58	5.1
Death	8	0.7
Referral/transmission to another health institution	1	0.1
Clinic of hospitalization		
Orthopedics and Traumatology	35	3.1
Anesthesiology and Reanimation	25	2.2
Brain and Nerve Surgery	20	1.8
Ophthalmology	8	0.7
Thoracic Surgery	7	0.6
General Surgery	4	0.4
Other*	13	1.3

*Mental Health and Diseases, Ear, Nose and Throat Diseases, Urology, Internal Medicine Intensive Care, Pediatrics Intensive Care, Neurology, Pediatric Surgery, Cardiovascular Surgery

DISCUSSION

Forensic incidents constitute a considerable portion of emergency department visits. Between the dates of the study, the number of patients admitted to the emergency department for all reasons was 60,403 and the number of forensic cases was 1186 (2%). Demircan et al.[23] reported a rate of 3.66% and Yavuz et al.[24] a rate of 6% in their study. In our study, we found that the majority of forensic cases admitted to our hospital were male and the male/female ratio was 2.5. Although this ratio varies in similar studies in the literature, it has been observed that there are many studies in which the number of men is higher[25]. The difference in male density was thought to be due to the fact that men were more involved in the social and business environment of our country than women, and it was understood that similar opinions prevailed in other studies[26-29].

The mean age was 33.4 years (±15.7) and approximately half of the cases (49.4%) were between 21 and 40 years of age. The high number of forensic cases in this age group, which plays a greater role in the active period of the life process, was found to be consistent with similar studies in the literature[25-30].

Considering the nature of forensic events, we see that traffic accidents ranked first with 33.3%, followed by assault and battery cases with 24.1% and occupational accidents with 21.5%. In our study, it was found that physicians kept forensic reports mostly related to traffic accidents, which was consistent with similar studies in the literature[2,25,26,29,31-33]. In addition to the fact that traffic accidents and related injuries are still a major problem in our country, it was associated with the fact that our hospital was located close to the city center and intercity road junction.

In Turkey, the number of occupational accidents within the scope of Article 4-1/a of Law no. 5510 was reported as 384,262 in 2020 and 511,084 in 2021[34]. As can be seen in Table 2, our data, such as the second highest rate of forensic reports on occupational accidents, the nature of the forensic event and the mechanism of trauma, are consistent with the SSI statistics as well as the study conducted in a university hospital in the metropolitan city of İstanbul[34,35]. We are of the opinion that in large cities where industrialization and migration are increasing, there will be more occupational accident applications to emergency departments in the coming years.

When we analyzed the emergency department outcomes of the cases, it was seen that the majority (84.2%) were discharged from the emergency department after follow-up and treatment. Of the hospitalized patients, 7.5% were hospitalized in clinical wards, 2.4% were hospitalized in intensive care units, and 8 (0.7%) patients died. It was understood that the intra- and/or inter-clinical outcomes we determined were compatible with the rates of similar studies in the literature[29].

When the finalization provisions of the forensic reports were examined, it was seen that 77.2% of the cases had no life-threatening situation, but 7.4% of the cases were stated to have a life-threatening situation; 15.4% of the cases did not mention any life-threatening situation, therefore an important information was missing in some part of the investigation; 59.6% of all forensic reports were concluded as "physician's report expressing opinion", the second highest rate was "provisional physician's report" with 27.2%, 4.6% were concluded with "final report (definitive physician's report)", but 8.6% did not contain any termination statement; therefore, 98 reports in this group and 309 reports closed as "provisional physician's report" were not binding for the judicial authorities and remained open. Forensic reports are one of the important elements of the dynamics of "time in judicial proceedings" and the orderly execution of judicial workflow processes, which are important in the judicial systems of legal states. The most common problem with forensic reports is that physicians issue temporary reports without justification. There are sufficient opportunities to prepare a definitive report, but instead of stating as "it is a forensic report expressing the opinion" or "it is a definitive forensic report", the statements such as "it is a provisional report" or "it is a provisional report and a definitive report will be issued by the '.... ' clinic", "there is no danger to life for the time being, but it is a temporary report issued for the life danger that may arise in the future" unnecessarily prolong the judicial process, sometimes unnecessarily prolong the detention periods of persons who would be suspects in the incident, delay justice, and as a result, may lead to the deprivation of the rights of victims and suspects.

At the same time, it causes a defensive approach in clinics within different specialties and causes unnecessary labor and time loss[28,36]. In a study conducted by Serinken et al.[32] in Denizli, they reported that 20% of the reports were issued as "final reports". In a prospective study of forensic reports issued in the emergency departments of two different state hospitals in Mersin and Iskenderun, they reported the rates of issuing provisional reports as 58.5% and 99.6%.[28]

In many studies, it has been emphasized that physicians are reluctant to prepare forensic reports due to reasons such as lack of knowledge and experience, desire to avoid taking responsibility and not knowing the legal legislation for which they are responsible[27,36,37]. In our study, 309 of the forensic reports were "temporary physician's report" and 98 of them were "without any termination statement", which constituted a high rate of 35.8% (27.2% + 8.6%) in total, and which we think is due to the concern of being held responsible for future legal problems that might develop in the future, which are listed in the justifications mentioned in the studies, At the root of the problem is the fact that physicians' knowledge of forensic duties is limited within their general medical knowledge, and some physicians who have knowledge prefer to abstain in processes related to forensic procedures, thus suggesting that

they remain in negative defensive medical practice. There are other clinical studies that are consistent with this data of our study, as well as basic sources of information that support our conclusion[27,28,32,36-43].

In the Emergency Department of our hospital, it was observed that in the last one year, the rate of reports on the examination of entry-exit from detention was 1.6% of all forensic reports. In some literature reviews in which the İstanbul Protocol and the Regulation on Arrest, Detention and Statement Taking are emphasized in examinations and reports, it is seen that compliance with national and international legislation is emphasized[9,10]. Although the physical conditions and manpower adequacy of our hospital comply with the legislation, the relatively low rate is associated with the fact that especially in recent years, detention entry-exit examinations and reports have been made in the emergency departments of the Ministry of Health hospitals. In this context, it is thought that support is received from the city hospital, which has a campus in the central district of our province, and provincial-district state hospitals.

The number of applications to the emergency department with suspicion of sexual abuse is limited to one case. In our health institution, which is a university hospital, it is known that the applications are made to the units where forensic medicine specialist physicians are present in order to carry out the process more professionally, since there are at least 4 or more forensic medicine specialist physicians in the child follow-up centers affiliated to the nearby provinces, the Ministry of Health hospitals within the provincial-district borders and the units affiliated to the ATK organization.

Study Limitations

Since our study was a single-center retrospective file review study, the generalizability of the findings may have been limited, and problems related to the lack of data to reflect the complete population were also encountered.

CONCLUSION

The data we obtained in this study, which aimed to reveal the profile of forensic cases, are generally consistent with the results of similar studies in the literature.

Health professionals should approach forensic cases in a team-based framework and provide comprehensive treatment and care by adhering to the relevant legal processes. More regulations are needed to develop standards, protocols and training programs regarding the approach and precautions for forensic cases, which constitute an important group among emergency department visits. Since medical records are important data sources for determining the trends in the incidence of forensic cases, it is important to have complete and regular records in forensic cases, as in all medical cases, in order to prevent physician safety and patient victimization in the legal process that may occur afterwards. In many studies, it has been reported that providing regular training to emergency physicians, who frequently encounter forensic cases and have a high risk of professional errors, is important in terms of physician rights as well as the protection of patient rights. However, forensic medicine specialists have as much responsibility as emergency physicians in the management of forensic cases affecting the judicial process. In addition, we strongly recommend the establishment and expansion of forensic bureau units working with a professional team within health institutions and organizations, and the integration of a training on the workflow processes of forensic cases in health service providers into the undergraduate and graduate education curricula of medical, nursing, legal and law enforcement organizations.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (protocol number: 2023.75.04.11, date: 25.04.2023).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: S.B., N.B., N.E.S., Design: S.B., N.B., H.Ş., E.Ç., Data Collection or Processing: S.B., E.Ç., Analysis or Interpretation: S.B., N.B., H.Ş., Literature Search: S.B., N.B., N.E.S., E.Ç., Writing: S.B., N.B., H.Ş., N.E.S.

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Salivary Alpha Amylase Enzyme as a Stress Parameter: Establishment and Comparison of Laboratory Methods

Stres Parametresi Olarak Tükürük Alfa Amilaz Enzimi: Laboratuvar Yöntemlerinin Kurulumu ve Karşılaştırılması

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ABSTRACT

Aim: Salivary alpha (α)-amylase enzyme is a biomarker used to measure sympathetic nervous system activity. This study aimed to establish three existing methods for measuring salivary α-amylase enzyme activity in the laboratory and to compare these methods in terms of their usability.

Materials and Methods: α-amylase enzyme activity can be measured in ready-made kits by three methods: Starch-Iodine, 2-chloro-4-nitrophenyl maltotrioside (CNPG3) and dinitrosalicylic acid (DNS) methods. In this context, standard curves were created in the laboratory for manual study of these methods and their advantages and disadvantages were presented. The usability of these methods was tested.

Results: The starch-iodine and CNPG3 methods were successfully established. However, although a standard curve was successfully established for the DNS method, this assay was not suitable for the studies as the samples were not readable and had many disadvantages. The test sensitivities and working ranges were appropriate for the starch-iodine and CNPG3 tests, requiring 4,000-fold dilution for the starch-iodine test and 5-fold dilution for the CNPG3 test. A weak but statistically significant positive correlation was observed between the two tests (R2=0.048 for linear regression; $p<0.05$, R2=0.106 for quadratic regression; $p<0.01$).

Conclusion: The CNPG3 and starch-iodine methods were feasible, cost-effective, accessible, and time-efficient. The starch-iodine method is a cheaper but the CNPG3 method is also a practical test with fewer steps. In this respect, it has been decided that the CNPG3 method is the most effective method in studies based on salivary α-amylase enzyme method.

Keywords: α-Amylase enzyme, CNPG3, DNS, starch-iodine, saliva

ÖZ

Amaç: Tükürük alfa (α) amilaz enzimi sempatik sinir sistemi aktivitesini ölçmek için kullanılan bir biyo-belirteçdir. Bu çalışmanın amacı tükürük α-amilaz enzimi aktivitesini ölçebilmek için mevcut olan üç yöntemi laboratuvarda kurmak ve bu yöntemleri kullanılabilirliği açısından birbirleriyle karşılaştırmaktır.

Gereç ve Yöntem: α-amilaz enzim aktivitesi; nişasta-iyodin, 2-chloro-4-nitrophenyl maltotrioside (CNPG3) ve dinitrosalisilik asit (DNS) yöntemleri olmak üzere üç yöntem ile hazır kitlerde ölçülebilmektedir. Bu bağlamda bu yöntemlerin manuel olarak çalışılabilmesi için laboratuvarda standart eğrileri oluşturuldu ve avantaj ve dezavantajları ortaya konuldu. Bu yöntemlerin kullanılabilirliği test edildi.

Bulgular: Nişasta-iyodin ve CNPG3 metodları başarılı bir şekilde kuruldu, fakat DNS metodu için başarılı bir standart eğri oluşturulmasına rağmen numuneler okunamadığından ve birçok yönden dezavantajları olması sebebi ile bu test çalışmalar için uygun bulunmadı. Nişasta iyodin ve CNPG3 testleri için test hassasiyetleri ve bunların çalışma aralıkları uygun bulunmuş olup, nişasta-iyodin testinde 4.000 kat, CNPG3 testinde ise 5 kat seyreltme gerekmiştir. Kurulan iki test arasında zayıf fakat istatistiksel olarak anlamlı pozitif bir ilişki gözlenmiştir (Lineer regresyon için R2=0,048; $p<0,05$, kuadratik regresyon için R2=0,106; $p<0,01$).

Sonuç: CNPG3 ve nişasta-iyodin metodlarının uygulanabilir, uygun maliyetli, kolay ve zaman açısından kısa sürmesi nedeniyle çalışmalarda kullanılabilir oldukları belirlenmiştir. Nişasta-iyodin metodu daha ucuz bir yöntemdir fakat CNPG3 metodu da daha az aşamadan oluşan pratik bir testtir. Bu açıdan tükürük α-amilaz enzimi yöntemi üzerine kurulu çalışmalardaki en etken yöntemin CNPG3 yöntemi olduğuna karar verilmiştir.

Anahtar Kelimeler: α-Amilaz enzimi, CNPG3, DNS, nişasta-iyodin, tükürük

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INTRODUCTION

Saliva is an easily obtainable fluid in our body and its importance is increasing gradually[1-3]. Salivary alpha (α) -amylase enzyme is an enzyme produced by salivary glands, released due to activation of the autonomic nervous system and breaks down starch[4]. It is also an essential parameter in the investigation of stress physiology[5,6]. Three methods can theoretically measure salivary α-amylase enzyme activity. The starch-iodine method is based on the breakdown of starch by α-amylase enzyme. The starch that α-amylase enzyme cannot break down is stained with iodine and determined by reading in a spectrophotometer[7]. The dinitrosalicylic acid (DNS) method is based on determining the amount of sugar reduced[8]. In the substrate method, 2-chloro-4-nitrophenyl maltotrioside (CNPG3) acts as a substrate and shows the enzyme's activity by forming a yellow color when bound to the enzyme[9-11].

While different methods can measure α-amylase, these methods are mainly studied with ready-made kits and no studies comparing them with each other have been found. Therefore, in this study, three methods measuring α-amylase enzyme activity were established in the laboratory and compared with each other in terms of cost, time, and practicality.

This study aimed to establish these tests in the laboratory, to determine the most appropriate test and then use it to investigate stress physiology. In addition, knowledge on this subject will be gained.

MATERIALS AND METHODS

Experimental Studies

Establishment of the Starch-Iodine Method

The starch-iodine method aims to detect the α-amylase enzyme's linkage between glucoses. It breaks down and interacts with the starch and iodine, creating a blue-violet color. This allows the activity of α-amylase to be measured.

Starch consists of two groups called amylose and amylopectin. Amylose linear is a molecule. Glucose molecules form a helix lined up one after the other, and form a double helix. Two amylose molecules are wrapped together in a double helix and can form a blue-violet color. Iodine molecules can enter these helices and form a blue-violet color. Amylopectin has a shape branching from the center like a bush[7]. It was decided that the standard starch-iodine solution, which was most suitable for the study, was the ready liquid 1% solution, and the ready solution was used in the studies.

Protocol for Establishing the Standard Curve in the Starch-Iodine Method

In the formation of the standard curve, α-amylase was diluted several times. It was decided that the most appropriate concentration was 1st Standard: 30 U/mL, 2nd Standard: 3 U/mL, 3rd Standard: 1.5 U/mL, 4th Standard: 0.6 U/mL, 5th Standard: 0.3 U/mL, 6th Standard: 0.15 U/mL and 7th Standard: 0.06 U/mL. Samples were diluted to a specific range to read the samples. When the samples were not read at the end of these trial-and-error procedures, they were re-diluted and re-run in the test. For this purpose, buffer solution (PBS) was used for dilution.

Protocol of the Starch-Iodine Test

The starch-iodine method was used to determine α-amylase activation in saliva samples. The working protocol of this method is summarized below;

- 40 µL of starch solution was pipetted into each well.
- 40 µL saliva (α-amylase) was added to all wells (shaken gently for 5-10 seconds).
- Incubated in an oven (50 degrees celsius) for 30 minutes.
- 20 µL HCl solution was added to all wells.
- 100 µL iodine solution was added to all wells.
- Read at 580 nm in a spectrophotometer with plate reader (Figure 1)[12].

Establishment of CNPG3 Method

The primary purpose of the CNPG3 method is to function by binding to the enzyme as a substrate. The enzyme binds to its substrate and creates a yellow color. Thus, the activity of α-amylase can be measured[11,13].

CNPG3 chromogen is a commercially available substrate[14]. It is directly soluble in PBS and forms a light yellow color. A

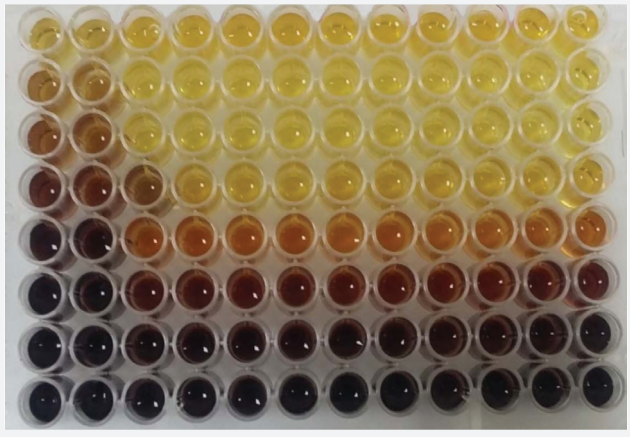

Figure 1. The starch-iodine method demonstrates the color changes caused by different concentrations of α-amylase. In the starch-iodine test, the color changes from yellow to dark brown. The enzyme's activity is measured by reading this color in a spectrophotometer (580 nm)

standard curve was created for the application of the assay. The α-amylase enzyme was diluted at different ratios to form this standard curve.

Standard Curve Generation Protocol in CNPG3 Method

In the formation of the standard curve, several dilutions were made and it was decided that the most appropriate dilutions were 1st Standard: 30 U/mL, 2nd Standard: 15 U/mL, 3rd Standard: 7.5 U/mL, 4th Standard: 6 U/mL, 5th Standard: 3.75 U/mL, and 6th Standard: 3 U/mL. Samples were diluted to a specific range so that the samples could be read. When all samples were not read, the samples were re-diluted at different ratios and read. Dilutions were made with PBS.

Protocol of the CNPG3 Test

CNPG3 method was used to determine α-amylase activation in saliva samples. The working protocol of this method is summarized below;

- 175 µL of PBS solution was pipetted into each well.
- 5 µL saliva (α-amylase) was added to all wells (shaken gently for 5-10 seconds).
- Incubated for -1 hour (at 37 degrees celsius).
- 20 µL of CNPG3 solution was added to all wells.
- Read at 405 nm in a spectrophotometer with plate reader (Figure 2)[14-16].

Setting up the DNS Method

The DNS method is used to measure α-amylase activity[17] and is based on measuring the amount of reducing sugar. The DNS method is used to estimate the concentration of reducing sugars in a sample. Reducing sugars contain the free carbonyl group, which can reduce most reagents. All monosaccharides and some disaccharides are reducing sugars[8]. When 3,5-DNS reacts with reduced sugars, orange colored 3-amino-5 nitrosalicylic acid is formed.

Standard Curve Generation Protocol in DNS Method

In forming the standard curve, several dilutions were made and it was decided that the most appropriate dilutions were 1st Standard: 30 U/mL, 2nd Standard: 3 U/mL, 3rd Standard: 0.3 U/mL, and 4th Standard: 0.03 U/mL. The appropriate standard curve was created for the test, but although the samples were diluted, no color intensity was obtained in the range of the standard curve. There is also a boiling step in the DNS test. It seems easy for a few samples but impractical when many must be read. Although boiling occurs in sealed tubes, water can get into the tubes.

Protocol of the DNS Test

The DNS method was used to determine α-amylase activation in saliva samples. The study protocol of this method is summarized below;

- 0.9 mL substrate and 0.1 mL enzyme were added to each tube.
- Incubated at 37 degrees for 5 minutes.
- 1 mL of DNS was added.
- Boiled for 10 minutes and allowed to cool.
- It was read at 540 nm in a spectrophotometer with plate reader, (Figure 3)[18,19].

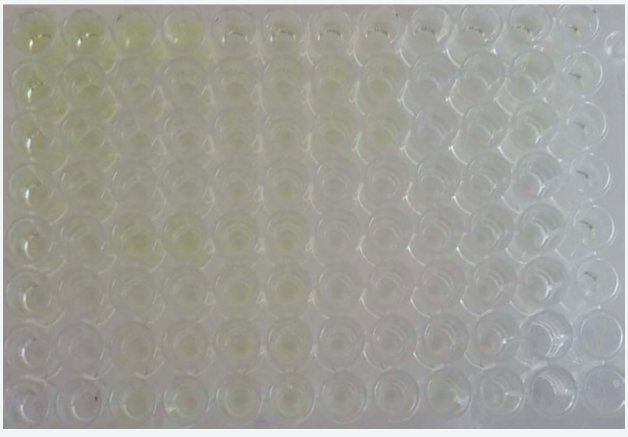

Figure 2. CNPG3 method demonstrating the color changes caused by using different concentrations of α-amylase. In the CNPG3 assay, a light yellow color is formed with the addition of CNPG3 and α-amylase activity is determined by reading in a spectrophotometer (405 nm)

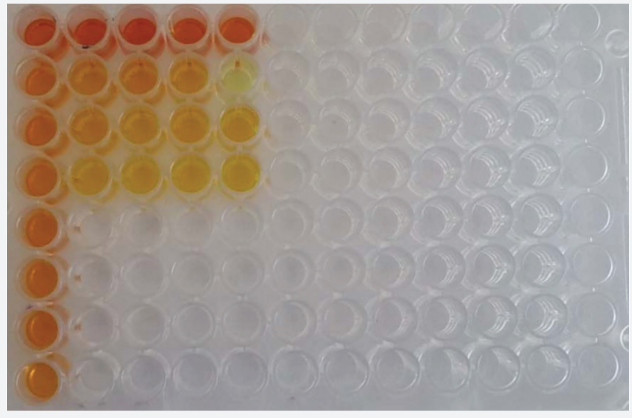

Figure 3. Demonstration of the color changes caused by using different concentrations of α-amylase by DNS method. In the DNS assay, yellow color is formed with the addition of DNS and α-amylase activity is determined by reading in a spectrophotometer (540 nm)

Statistical Analysis

MINITAB (USA) statistical program was used for data analysis. Data are presented as mean±standard error. A 4-parameter logistic curve was used to construct standard curves (Gen 5, BioTek Synergy, USA). The relationship between the Starch-iodine test and CNPG3 test was demonstrated by the Pearson correlation.

RESULTS

Starch-Iodine Standard Curve

Standard curves of α-amylase obtained by starch-iodine assay were run in 5 tests and average standard curve values were obtained (Figure 4).

Standard curve showing the changes that occurred. Color changes caused by changes in α-amylase concentrations (units/mL) were measured spectrophotometrically at a wavelength of 580 nm, and the standard curve graph above was obtained. Values are presented as the mean and standard error values obtained in 5 tests. The standard curve was linear between 0.06 U/mL and 1.5 U/mL.

CNPG3 Standard Curve

The α-amylase standard curves obtained with the CNPG3 assay were run in 13 assays and average standard curve values were obtained (Figure 5).

DNS Test Standard Curve

Standard curve showing changes in density. Standard curve, 3-30 U/mL between the concentration difference of approximately 0.300 units in the optical density. Change has occurred (Figure 6).

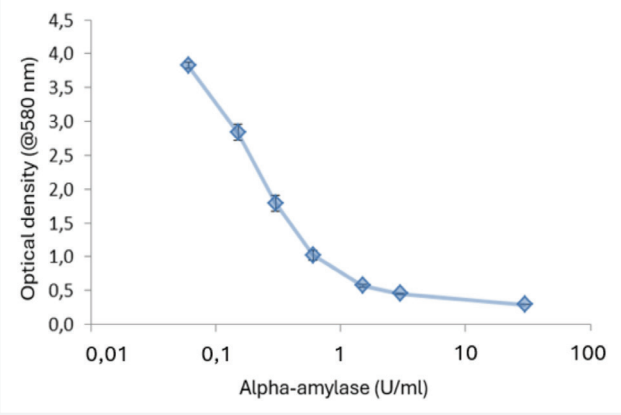

Figure 4. Optical density at increasing α-amylase concentrations according to the starch-iodine method

DISCUSSION

In the present study, the measurement methods of salivary α-amylase activity were investigated and the methods of measuring salivary α-amylase activity were compared for the first time. α-amylase starch-iodine compared to measure the enzyme activity in saliva method, CNPG3 method and DNS method are discussed.

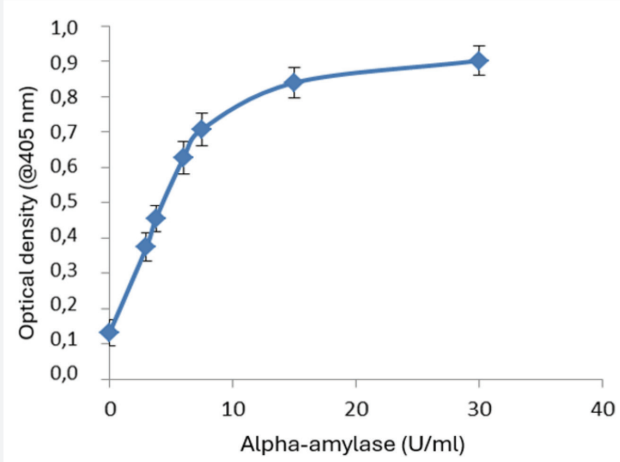

Figure 5. CNPG3 optical response at increasing α-amylase concentrations according to the chromogen substrate method. A standard curve showing changes in density. Measurements made at 405 nm is presented as the mean of 13 standard curves (±SEM). The curve was linear in the range 0-7.5 U/mL

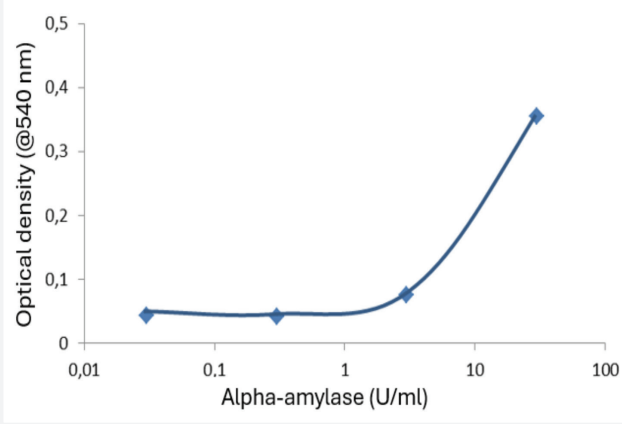

Figure 6. The standard curve in the DNS test shows changes in optical density with increasing α-amylase concentrations. In the standard curve, there was a change of approximately 0.300 units in optical density in response to the concentration difference between 3-30 U/mL

Determination of α-amylase by Starch-Iodine Method

The starch-iodine test was successfully established, and the dynamic range of the standard curve was found to be between 0.05-3 U/mL and its sensitivity was 0.05 U/mL. Additionally, the optical density range of the test is approximately 0 to 4,000, which shows a dynamic change. In other words, it is a test susceptible to α-amylase activity. In this form, the test can detect α-amylase when diluted approximately 4,000x in saliva samples. However, this level of dilution relatively increases the workload and causes a loss of time. On the other hand, it seems to be a preferable method because the incubation period is very short, and the materials are easy and cheap to obtain.

However, the necessity of separate dilutions for each saliva sample has a slight negative impact on the practical applicability of the test. The starch-iodine test is a test that is cheap, easy to set up and apply, and whose materials are readily available. However, it requires many dilutions and relatively increases the workload. On the other hand, the most crucial feature of the test is that the optical density values vary between 0 and 4,000. Therefore, it is a dynamic test, which is an essential reason for preference.

Determination of α-amylase by CNPG3 Method

The CNPG3 assay was successfully established, and the dynamic range of the standard curve was found to be between 3-15.75 U/mL with a sensitivity of 0.100 U/mL. Furthermore, the optical density range of the assay also shows a dynamic range of approximately 0.1 to 0.8. As such, the assay can detect α-amylase in saliva samples at approximately 5x dilution. The single reconstitution minimizes the workload and wasted time, but the large amount of saliva used and the length of the incubation period have a negative impact. The CNPG3 assay is an easy to set up and implement method that reduces the workload but is relatively expensive.

Determination of α-amylase by DNS Method

The DNS method is known as a method used for the determination of urea and sugar in the biochemical field. DNS material was prepared under laboratory conditions, and a standard curve was created. It was determined that the dynamic range of the DNS standard curve was between 0.3-0.03 U/mL and its sensitivity was 0.4 U/mL. Additionally, the optical density range of the test varies slightly between approximately 0.1 and 0.4. For this reason, the probability of being preferred is very low, and the fact that the test consists of many stages is considered a negative factor. The presence of a problematic step, such as boiling, and the fact that the samples are not within the optical range of the standard curve are also noted as essential shortcomings. Additionally, although the test works as intended, it is not suitable for practically examining large numbers of samples.

Study Limitations

In our study, there may be some limitations in revealing the advantages and disadvantages of the methods established in the laboratory.

CONCLUSION

The starch-iodine and CNPG3 chromogen tests were successfully developed. The standard curve of the DNS test was successfully established, but the samples were not compatible with the optical density. The sensitivities and working ranges of the established tests were appropriate; 4000-fold dilution was required for the starch-iodine test and 5-fold dilution was required for the CNPG3 test. Both tests appear to be inexpensive, easy to perform, and of short duration. Although the starch-iodine test is 4-5 times cheaper than the CNPG3 test, the CNPG3 test is a more practical test with fewer steps.

Ethics

Ethics Committee Approval and Informed Consent: Since this study was a laboratory study, ethical committee approval and informed consent were not required.

Authorship Contributions

Concept: Ö.B., Design: Ö.B., Data Collection or Processing: Ö.B., S.Y., Analysis or Interpretation: Ö.B., S.Y., Literature Search: Ö.B., S.Y., Writing: Ö.B.

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Evaluation of Factors Associated with Adult Sepsis Prognosis

Erişkin Sepsis Prognozu ile İlişkili Faktörlerin Değerlendirilmesi

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ABSTRACT

Aim: The aim of this study was to evaluate the factors affecting the prognosis of sepsis in patients admitted to the intensive care unit (ICU).

Materials and Methods: We retrospectively included all adult patients admitted to the ICU, who were diagnosed with sepsis according to the Sepsis 3 criteria between September 2013 and February 2021. Demographic, clinical data and laboratory results were recorded.

Results: Of the 245 patients in the ICU, 100 (40.8%) died during the 30-day follow-up. In univariate logistic regression analysis, Sequential Organ Failure Assessment (SOFA) score, vasopressor need, immunosuppressive treatment, neutropenic fever, hematological malignancy, pneumonia, urinary tract infection, lactate, ferritin, lactate dehydrogenase (LDH), and albumin levels were found to be independently associated with mortality. When evaluated in terms of prognostic significance in ROC curve, the optimal cutoff values for 30-day mortality were 7 for SOFA score (AUC=0.713, p<0.001), 1518 µg/L for ferritin (AUC=0.732, p<0.001), 324 U/L for LDH (AUC=0.593, p=0.035), and 2.9 g/dL for albumin (AUC=0.632, p=0.001), in mortality. Using these values, in multivariate logistic regression analysis, we determined that a SOFA score >7 [odds ratio (OR): 95% confidence interval (CI): 9.66 (1.16-80.82), p=0.036], history of immunosuppressive treatment [OR 95% CI: 12.41 (1.45-106.17), p=0.021], and ferritin levels >1518 µg/L [OR 95% CI: 9.46 (1.36-65.79), p=0.023] were independent risk factors for 30-day mortality.

Conclusion: In our single-center study, serum ferritin level was determined to be a valuable prognostic biomarker in patients with sepsis.

Keywords: Sepsis, prognosis, mortality, ferritin, critical care

ÖZ

Amaç: Yoğun bakım ünitesinde (YBÜ) takip edilen sepsis tanılı erişkin hastalarda prognozu etkileyen faktörleri değerlendirmektir.

Gereç ve Yöntem: Eylül 2013 ile Şubat 2021 arasında Sepsis-3 kriterlerine göre sepsis tanısı almış tüm yetişkin hastalar retrospektif olarak değerlendirildi. Demografik, klinik veriler ve laboratuvar sonuçları kaydedildi.

Bulgular: YBÜ'de 245 hastadan 100'ü (%40,8) 30 günlük takip süresi içinde öldü. Tek değişkenli lojistik regresyon analizinde; ardışık organ yetmezlik değerlendirme (SOFA) skoru, vazopresör ihtiyacı, immünsupresif tedavi öyküsü, nötropenik ateş, hematolojik malignite, pnömoni, üriner sistem enfeksiyonu, laktat, ferritin, laktat dehidrogenaz (LDH) ve albümin seviyelerinin 30 günlük mortalite ile ilişkili olduğu saptandı. ROC eğrisinde; 30 günlük mortalite için optimal kesme değerleri SOFA skoru için 7 (AUC=0,713, p<0,001), ferritin için 1,518 µg/L (AUC=0,732, p<0,001), LDH için 324 U/L (AUC=0,593, p=0,035), ve albümin için 2,9 g/dL (AUC=0,632, p=0,001) olarak belirlendi. Bu değerleri kullanarak yapılan çok değişkenli lojistik regresyon analizinde; SOFA skoru >7 [odds oranı (OR) %95 güven aralığı (GA): 9,66 (1,16-80,82), p=0,036], immünsüpresif tedavi öyküsü [OR %95 GA: 12,41 (1,45-106,17), p=0,021] ve ferritin seviyesinin >1,518 µg/L [OR %95 GA: 9,46 (1,36-65,79), p=0,023] olması 30 günlük mortalite için bağımsız risk faktörleri olarak saptandı.

Sonuç: Tek merkezli çalışmamız sonucunda, serum ferritin seviyesi, sepsis hastalarında değerli bir prognostik biyobelirteç olarak saptandı.

Anahtar Kelimeler: Sepsis, prognoz, mortalite, ferritin, yoğun bakım

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INTRODUCTION

Sepsis is characterized by an excessive immune response to an infectious agent, leading to multiple organ failure with a high mortality rate[1]. Sepsis is a major concern in public health due to its widespread impact, with over 19 million individuals diagnosed each year globally[2]. Despite advances in supportive care and intensive care technologies, the mortality rate in septic shock remains approximately 40%[3]. Sepsis involves the release of high levels of proinflammatory cytokines due to microbial agents, accompanied by the release of anti-inflammatory cytokines. The initial hyperinflammatory phase can lead to early mortality[4]. Numerous studies have been conducted on various laboratory parameters to evaluate the severity of the increased inflammatory response and to predict mortality. Mortality predictor parameters can help identify patients who, in addition to supportive therapies, may benefit from potentially effective additional treatments by predicting early mortality. Recently, in addition to commonly used parameters such as C-reactive protein (CRP), procalcitonin, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR), studies have been conducted on numerous new markers such as N-terminal pro b-type natriuretic peptide (NT-proBNP), ferritin, and presepsin. Some of these studies have yielded significant results for these parameters[5-10]. However, none of these parameters have been accepted as additional mortality predictive markers in scoring systems such as Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE) according to international guidelines[11]. In this study, we aimed to evaluate the capability of certain inflammatory markers to predict prognosis independently of general patient characteristics and parameters already associated with disease severity and mortality, such as the SOFA score and serum lactate levels.

MATERIALS AND METHODS

Patient Selection and Study Design

This retrospective study was conducted in a single-center internal medicine intensive care unit (ICU) between September 2013 and February 2021. The Institutional Ethical Review Board of Ege University Hospital approved the study (decision no: 21-6.1T/54, date: 10.04.2016). This study was conducted in accordance with good clinical practice guidelines and adhered to the principles of the Declaration of Helsinki. Patients or their relatives provided written informed consent. We included all adult patients (≥18 years old) diagnosed with sepsis. The diagnosis of sepsis and septic shock was made according to the Sepsis-3 criteria[12].

Demographic characteristics and clinical features were extracted from patients' medical records. In addition, the following laboratory parameters were obtained on admission and 48 h after hospitalization: neutrophil count, lymphocyte count, platelet count, CRP at admission and 48 h, procalcitonin, albumin, ferritin, NT-proBNP, troponin-T, lactate, and LDH. The primary endpoint of the study was 30-day mortality. Secondary endpoints included factors associated with prognosis.

Statistical Analysis

Descriptive statistics were used to summarize the data. For continuous (numerical) variables, depending on the distribution, either mean±standard deviation or median, minimum, and maximum values were presented. Categorical variables are summarized as counts and percentages. The normality of the numerical variables was assessed using the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests. For comparisons between two independent groups, the Independent Samples t-test was used when numerical variables were normally distributed, and the Mann-Whitney U test was used when they were not. Risk factors affecting 30-day mortality were investigated using univariate and multivariate logistic regression models. To identify the ideal cutoff level to evaluate 30-day mortality, receiver operating characteristic curve (ROC), in which the Youden J index was considered in determining the threshold value, was performed. Statistical analyses were performed by Jamovi project (2020), Jamovi (version 1.8.4.0) [computer software], and JASP with a significance level set at 0.05 (p value).

RESULTS

During the study period, 245 patients diagnosed with sepsis were followed up in the ICU. The 30 day-mortality rate was 40.8%. Demographic information, comorbid conditions, and infection sites, laboratory data along with the differences between the survivors and non-survivors are presented in Table 1. Within comorbidities, vasculitis, hematologic malignancy, and the use of immunosuppressive therapy were significantly more frequent in the non-survivor group (p values 0.009, <0.001, 0.004 respectively). Among the sources of infection, pneumonia was more commonly observed in the non-survivor group (p=0.001), whereas urinary tract infections were more frequent in the survivor group (p=0.003). Neutropenic fever, high SOFA scores, and the need for vasopressors were more common in the non-survivor group (p values <0.001, <0.001, 0.004 respectively). Among baseline data, albumin, LDH, lactate, and ferritin levels at admission as well as the changes in CRP and LDH levels at the 48 h of hospitalization compared with their admission values were found to be statistically different between the non-survivor and survivor groups (p values 0.002, 0.034, 0.005, <0.001, 0.002, 0.012, respectively).

Logistic regression analysis was performed to determine the parameters that were significantly different between the survivor and non-survivor groups. In the evaluation based

Table 1. Patient characteristics and laboratory parameters of surviving and non-surviving septic patients

	30 days mortality		p value
	Survivors (n=145)	Non-survivors (n=100)	
Age	60.0±17.8	62.2±15.9	0.306
Gender (%)			
Male	71 (49.0)	50 (50.0)	0.977
Female	74 (51.0)	50 (50.0)	
Comorbidity	100 (69.0)	70 (70.0)	0.975
Diabetes mellitus	51 (35.2)	32 (32.0)	0.705
Chronic heart failure	24 (16.6)	21 (21.0)	0.474
Cardiovascular disease	23 (15.9)	23 (23.0)	0.215
COPD/asthma	11 (7.6)	11 (11.0)	0.489
Chronic renal disease	12 (8.3)	12 (12.0)	0.456
Vasculitis	1 (0.7)	7 (7.0)	**0.009**
Connective tissue disease	7 (4.8)	8 (8.0)	0.455
Hematological malignancy	16 (11.0)	35 (35.0)	**<0.001**
Solid organ malignancy	8 (5.5)	2 (2.0)	0.206
History of immunosuppressive therapy	10 (6.9)	20 (20.0)	**0.004**
Site of infection			
Pneumonia	33 (22.8)	44 (44.0)	**0.001**
Urinary tract	51 (35.2)	17 (17.0)	**0.003**
Biliary system	12 (8.3)	3 (3.0)	0.155
Abdomen (other than biliary system)	14 (9.7)	5 (5.0)	0.273
Catheter	21 (14.5)	19 (19.0)	0.445
Infective endocarditis	4 (2.8)	2 (2.0)	0.999
Meningitis	0 (0.0)	1 (1.0)	0.408
Skin and soft tissue	10 (6.9)	10 (10.0)	0.526
Septic arthritis	3 (2.1)	1 (1.0)	0.647
Primary bacteremia	1 (0.7)	4 (4.0)	0.162
Spondylodiscitis	1 (0.7)	0 (0.0)	0.999
Neutropenic fever	15 (10.3)	35 (35.0)	**<0.001**
Vasopressor need	69 (47.6)	66 (66.0)	**0.004**
SOFA score	6.0 [2.0-15.0]	9.0 [2.0-16.0]	**<0.001**
Biochemical results			
CRP mg/L	240.0 [7.0-619.0]	230.0 [18.0-471.0]	0.402
CRP D2-D0	-6.4 [-87.5-564.3]	7.9 [-86.4-566.7]	**0.002**
Albumin g/dL	3.1±0.6	2.8±0.7	**0.002**
CRP/albumin ratio mg/g	79.6 [1.5-238.1]	81.2 [4.4-261.7]	0.397
LDH U/L	277.0 [85.0-3798.0]	320.5 [99.0-4730.0]	**0.034**
LDH D2-D0	-4.5 [-48.2-258.5]	6.4 [-76.0-1344.3]	**0.012**
Procalcitonin µg/L	19.5 [0.3-100.0]	7.7 [0.3-100.0]	0.069
NT-proBNP ng/L	8299.5 [113.0-70000.0]	5536.0 [262.0-70000.0]	0.941
Troponin-T ng/L	51.0 [13.0-604.0]	61.0 [13.0-1824.0]	0.090
Lactate mmol/L	2.3 [0.6-24.0]	3.0 [0.6-11.0]	**0.005**
Ferritin µg/L	705.0 [104.0-76893.0]	2337.0 [291.0-44258.0]	**<0.001**

COPD: Chronic obstructive pulmonary disease, SOFA score: Sequential Organ Failure Assessment Score, CRP: C-reactive protein, LDH: Lactate dehydrogenase, NT-proBNP: N-terminal pro b-type natriuretic peptide

on 30-day mortality outcomes, univariate regression analysis identified SOFA score, use of immunosuppressive therapy, neutropenic fever, pneumonia, urinary tract infection, lactate, albumin, ferritin, and LDH as statistically significant factors. CRP D2-D0, LDH D2-D0, NLR, PLR, and NPAR were not found to be statistically significant in univariate analysis. Because the SOFA score and lactate levels are already established parameters used in determining the severity of sepsis and in diagnosing septic shock, model-1 was formed incorporating these along with other patient characteristics found to be associated with mortality. Ferritin, LDH, and albumin were added to the existing model to perform a multivariate regression analysis. After the modeling yielded inconclusive results, ROC curve was conducted on the relevant parameters (Figure 1). The cutoff values obtained from the ROC curve are shown in Table 2. Using the cutoff values obtained from the ROC curve, logistic regression analysis was repeated (Table 3). Initially, the univariate logistic regression model revealed that the following factors individually had a significant impact on 30-day mortality: a SOFA score above 7, the need for vasopressors at admission, administration of immunosuppressive therapy, the presence of neutropenic fever, hematologic malignancy, pneumonia, urinary tract infection, an albumin level below 2.9 g/dL, an LDH level over 324 U/L, and a ferritin level exceeding 1.518 µg/L. When examining the results of the multivariate logistic regression analysis, it was observed that a SOFA score

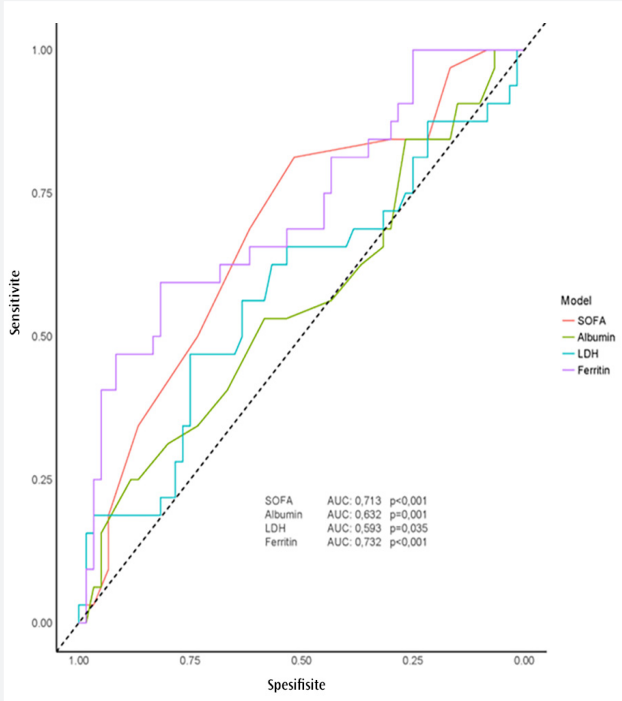

Figure 1. ROC curve analysis

SOFA score: Sequential Organ Failure Assessment Score, LDH: Lactate dehydrogenase

Table 2. ROC curve results

	AUC	Sensitivity	Specificity	Cutoff value	95% CI	p value
SOFA score	0.713	73	64.83	>7	0.652-0.769	**<0.001**
Albumin	0.632	64.95	58.27	≤2.9	0.567-0.694	**0.001**
LDH	0.593	50	71.58	>324	0.517-0.667	**0.035**
Ferritin	0.732	61.54	80.9	>1518	0.646-0.806	**<0.001**

SOFA score: Sequential Organ Failure Assessment Score, ROC: Receiver operating characteristic, LDH: Lactate dehydrogenase, CI: Confidence interval

Table 3. Results of the logistic regression analysis following the ROC curve

	Univariate LR		Multivariate LR	
	OR [95% CI]	p value	OR [95% CI]	p value
SOFA score: >7 vs ≤7	4.98 [2.85-8.70]	**<0.001**	9.66 [1.16-80.82]	**0.036**
Vasopressor need yes vs no	2.14 [1.26-3.62]	**0.005**	0.14 [0.02-1.16]	0.068
History of immunosuppressive therapy: yes vs no	3.37 [1.50-7.57]	**0.003**	12.41 [1.45-106.17]	**0.021**
Neutropenic fever: yes vs no	4.67 [2.38-9.16]	**<0.001**	4.94 [0.60-40.51]	0.137
Hematological malignancy: yes vs no	4.34 [2.24-8.42]	**<0.001**	1.21 [0.12-11.97]	0.872
Pneumonia: yes vs no	2.67 [1.53-4.64]	**<0.001**	0.95 [0.16-5.68]	0.954
Urinary tract infection yes vs no	0.38 [0.20-0.70]	**0.002**	1.58 [0.23-10.86]	0.639
Lactate mmol/L	1.13 [0.97-1.30]	0.111	1.51 [0.92-2.49]	0.103
Albumin: >2,9 vs ≤2,9 g/dL	0.39 [0.23-0.66]	**<0.001**	0.47 [0.12-1.90]	0.288
LDH: >324 vs ≤324 U/L	2.52 [1.35-4.71]	**0.004**	3.81 [0.89-16.18]	0.070
Ferritin >1518 vs ≤1518 µg/L	6.78 [2.94-15.60]	**<0.001**	9.46 [1.36-65.79]	**0.023**

SOFA score: Sequential Organ Failure Assessment Score, LDH: Lactate dehydrogenase, LR: Logistic regression, OR: Odds ratio, CI: Confidence interval

above 7 [odds ratio (OR): 95% confidence interval (CI): 9.66 (1.16-80.82) p=0,036], history of immunosuppressive therapy [OR 95% CI: 12.41 (1.45-106.17) p=0.021], and a ferritin level over 1.518 µg/L [OR 95% CI: 9.46 (1.36-65.79) p=0.023] were independent risk factors for 30-day mortality (p=0.036, p=0.021, and p=0.023).

DISCUSSION

In the current study, which included patients with sepsis, the 30-day mortality was 40.8%. High SOFA score (>7), history of immunosuppressive therapy, and ferritin levels >1.518 µg/L were significantly associated with 30-day mortality.

Ferritin is a protein composed of heavy and light chain structures that are responsible for iron binding and storage. It prevents the free circulation of iron in the body, thereby protecting proteins, lipids, and DNA structures from potential iron toxicity. Serum ferritin is also known as an acute phase reactant, which is regulated by proinflammatory cytokines. An increase in ferritin levels is often observed in inflammatory processes following the stimulation of heme oxygenase-1. This rise in ferritin levels plays a protective role aimed at preventing oxidative damage that occurs during inflammatory processes[13-15]. In adults, hyperferritinemia can be seen in various conditions, including hemophagocytic lymphohistiocytosis, in patients undergoing hemodialysis, having hemochromatosis, receiving frequent transfusions, having liver failure, antiphospholipid antibody syndrome, adult-onset still's disease, and patients with sepsis. Each of these conditions has unique mechanisms and implications related to elevated ferritin levels, emphasizing the importance of ferritin as a marker in diverse clinical scenarios[16]. High ferritin levels are increasingly being recognized as valuable biomarkers for the prognosis of several conditions, including cancer, connective tissue diseases, and notably, Coronavirus disease-2019 infection[17,18].

Studies on the relationship between ferritin levels and mortality in sepsis have particularly been conducted in the pediatric patient group. In a study conducted on pediatric patients, it was found that a ferritin level greater than 500 µg/L significantly increased the mortality risk by 3.2 times[6]. In another study conducted with pediatric patients, it was found that a ferritin level above 3.000 µg/L was associated with a 4.32-fold increase in the risk of mortality[19]. Studies conducted on elderly inpatients with ferritin levels above 1.000 µg/L have demonstrated the prognostic significance of high ferritin levels in sepsis and their importance in the recognition of malignancies[20]. In another study conducted on sepsis patients, ferritin levels exceeding 4.420 µg/L g/ml were found to be associated with a diagnosis of macrophage activation-like syndrome and related to 28-day mortality. Furthermore, a reduction of less than 15% in ferritin levels within three days compared to the initial value has been found to be associated with 28-day mortality[21]. In a recent study among adult septic patients, high ferritin levels were found to be associated with mortality. In this study, the cutoff value for ferritin was determined to be 591 µg/L[22]. In our study, the cutoff value for ferritin was found to be 1.518 µg/L. The different cutoff values obtained in these studies may be attributable to variations in the comorbid conditions of the patients. As previously mentioned, patients with malignancies, those undergoing hemodialysis, and those receiving frequent transfusions tend to have higher ferritin levels compared to other patients[16].

In addition to ferritin, univariate analysis in our study also found that low serum albumin levels and high LDH levels were predictors for mortality. In studies conducted on patients with abdominal sepsis, albumin levels below 2.9 g/dL were associated with high SOFA and APACHE scores, although no direct relationship with mortality was established[23]. In a prospective study investigating the relationship between low albumin levels and 28-day mortality, albumin levels below 2.9 g/dL were identified as an independent risk factor[24]. In our study, similar to other studies, the cutoff value for albumin was determined to be 2.9 g/dL in the ROC curve; however, in the multivariate analysis, it was not identified as an independent risk factor. In previous studies, LDH levels exceeding the upper limit of local laboratory standards were reported as to be associated with mortality[25]. In our study, the ROC curve identified a cutoff value of 324 U/L for LDH. However, LDH was not determined to be an independent predictive factor in the multivariate analysis.

Study Limitations

The present study has some limitations. First, this was a retrospective and observational study. Second, it is a single-center study and has limited generalizability. Serial determination of biomarkers will be more useful than single measurements. However, changes were not evaluated in this study.

CONCLUSION

In conclusion, many biomarkers have been studied in the prognosis of sepsis. How to guide the therapy is still a question for the clinicians. The findings of our study suggest that ferritin can be a useful bedside prognostic biomarker with clinical evaluation.

Ethics

Ethics Committee Approval: The Institutional Ethical Review Board of the study center (Ege University Faculty of Medicine), approved the study (decision no: 21-6.1T/54, date: 10.04.2016). The study was conducted in accordance with Good Clinical

Practice guidelines and adhered to the principles of the Declaration of Helsinki.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: C.A., Concept: C.A., Ş.M.K.B., D.B., Design: C.A., Data Collection or Processing: C.A., Analysis or Interpretation: C.A., Ş.M.K.B., D.B., Literature Search: C.A., Writing: C.A., Ş.M.K.B., D.B.

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ORIGINAL ARTICLE

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Evaluation of Tp-e/QTc Ratio in Obesity

Obezitede Tp-e/QTc Oranının Değerlendirilmesi

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ABSTRACT

Aim: We aimed to detect simple findings that might predict sudden cardiac death in electrocardiography recordings in obese patients.

Materials and Methods: Patients were included in our study retrospectively. Two groups with body mass index (BMI) ≥30 kg/m^2 (Group 1) and BMI <30 kg/m^2 (Group 2) were sampled from the study population with similar baseline characteristics, biochemical and echocardiographic features. Ventricular repolarization parameters were compared between the two groups. The Tp-e interval was defined as the period of time between the T waves' peak and their end. Tp-e/QTc ratio was calculated.

Results: This study included 190 participants. There were no differences between the two groups in terms of age (p=0.42), diabetes (p=0.238), hypertension (p=0.877), smoking (p=1.000), medical treatment used, laboratory parameters, left ventricular ejection fraction (p=0.673), and left ventricular mass index (p=0.089). The QTc interval was similar between the groups (416.4±11.6 ms, and 422.1±14.8 ms; p=0.081). Tp-e, and Tp-e/QTc ratio were greater in Group 1 (93.1±6.2 ms, and 67.7±2.5 ms; p=0.00; 0.22±0.02, and 0.15±0.01; p=0.001). Twelve months after the first examinations, six deaths were noted in the obese group (p=0.001).

Conclusion: Our study results showed that the Tp-e interval and Tp-e/QTc ratio were significantly increased, and sudden cardiac death was more common in patients with BMI ≥30 kg/m^2.

Keywords: Arrhythmias, ventricular tachycardia, electrocardiography, sudden cardiac death, Tp-e interval, Tp-e/QTc

ÖZ

Amaç: Obez hastalarda elektrokardiyografi kayıtlarında ani kardiyak ölümü öngörebilecek basit bulguları saptamayı amaçladık.

Gereç ve Yöntem: Çalışmamıza hastalar retrospektif olarak dahil edildi. Hastalar vücut kitle indeksi (VKİ) ≥30 kg/m^2 (Grup 1) ve VKİ <30 kg/m^2 (Grup 2) olacak şekilde iki gruba ayrıldı. İki grup da benzer temel özelliklere, biyokimyasal ve ekokardiyografik özelliklere sahipti. İki grup ventriküler repolarizasyon parametreleri açısından karşılaştırıldı. Tp-e aralığı, T dalgasının zirvesi ile sonu arasındaki süre olarak tanımlandı. Tp-e/QTc oranı hesaplandı.

Bulgular: Çalışmaya 190 hasta dahil edildi. İki grup arasında yaş (p=0,42), diyabet (p=0,238), hipertansiyon (p=0,877), sigara kullanımı (p=1,000), kullanılan medikal tedavi, laboratuvar parametreleri, sol ventrikül ejeksiyon fraksiyonu (p=0,673), sol ventrikül kitle indeksi (p=0,089) açısından anlamlı fark saptanmadı. QTc aralığı gruplar arasında benzerdi (416,4±11,6 ms ve 422,1±14,8 ms; p=0,081). Tp-e ve Tp-e/QTc oranı Grup 1'de daha yüksekti (93,1±6,2 ms ve 67,7±2,5 ms; p=0,00; 0,22±0,02 ve 0,15±0,01; p=0,001). İlk muayenelerden 12 ay sonra obez grupta altı ölüm kaydedildi (p=0,001).

Sonuç: Çalışma sonuçlarımız VKİ ≥30 kg/m^2 olan hastalarda Tp-e aralığı ve Tp-e/QTc oranının anlamlı olarak arttığını ve ani kardiyak ölümün daha sık olduğunu gösterdi.

Anahtar Kelimeler: Aritmiler, ventriküler taşikardi, elektrokardiyografi, ani kardiyak ölüm, Tp-e aralığı, Tp-e/QTc

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INTRODUCTION

A higher incidence of cardiac arrhythmias and sudden cardiac death (SCD) has been associated with obesity[1]. The risk of arrhythmias rises with obesity. The most common arrhythmias with obesity are premature atrial and ventricular contractions, ventricular and supraventricular tachycardia[2]. Obese patients may experience cardiac arrhythmias due to hypoxia, hypercapnia, obstructive sleep apnea, electrolyte imbalances, coronary heart disease, elevated catecholamine levels, and left ventricular hypertrophy[3]. Repolarization in the myocardium can be assessed using variables including the QT interval, corrected QT interval, and QT dispersion. The Tp-e interval, the time interval between the T wave peak and the endpoint, is considered as the distribution index of repolarization. Compared to other measurements, the ratio of the Tp-e interval to the QT interval is considered a more accurate predictor of arrhythmogenesis. Tp-e/QT is unaffected by changes in heart rate and can be used as a reliable indicator[4-6]. This study aimed to evaluate the risk of arrhythmias in obese patients using the Tp-e interval and Tp-e/QT ratio.

MATERIALS AND METHODS

Study Population

Body mass index (BMI) was calculated with the formula of body weight in kilograms/height in meters squared. In Group 1, 44 men and 52 women with a BMI more than 30 kg/m^2 were included. Group 2 (BMI less than 30 kg/m^2) included 42 men and 52 women. A BMI of 18.5 to 24.9 kg/m^2 was regarded as the range for normal, and ≥30 kg/m^2 was considered obese.

Patients were classified as hypertensive if they were taking antihypertensive drugs or if blood pressure was ≥140/90 mmHg. Diabetes was defined as having fasting blood glucose more than 126 mg/dL or the use of anti-diabetic drugs or insulin. To rule out systemic disorders, blood biochemistry studies, medical histories of patients, and physical examinations were reviewed in each group. Patients with coronary artery disease, recent acute coronary syndrome, severe valvular disease, chronic renal failure, ventricular systolic dysfunction, electrolyte imbalance, and bundle branch block were excluded. None of the patients were on any antiarrhythmic, tricyclic antidepressant, antihistamine, or antipsychotic drugs, and all were in sinus rhythm.

Electrocardiography (ECG) and echocardiography procedures were performed at the first examination. Twelve months after the first examinations, the death status of the patients and the cause of death were noted with ID number interrogation. Ethical committee approval was received from the University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Local Ethics Committee (approval no: 2022/03-18, date: 15.03.2022).

Electrocardiography

ECG was performed at 50 mm/s (Nihon Kohden, Tokyo, Japan). ECGs were taken using a 10-mm 1 mV calibration electrode. The patient's resting heart rate was calculated. The QT dispersion (QTd) refers to the difference between the maximal and minimal QT intervals in ECG[7]. QT duration and Tp-e were calculated using the precordial lead V5 in all patients. The Bazett formula was used to determine the QTc interval, calculated between the beginning of the QRS complex and the termination of the T wave and adjusted for heart rate. The Tp-e interval was defined as the period between the T waves' peak and their end. Precordial V5 ECG lead was used to measure the Tp-e interval. Calculations were made for the Tp-e/QTc ratio.

Echocardiography

The patients underwent echocardiographic evaluation (Philips EP-Q 7) and the calculation of each parameter involved average of three subsequent cycles. The left ventricular end-diastolic diameter, interventricular septal end-diastolic thickness, and left ventricular posterior and anterior wall end-diastolic thickness were measured from the left sternal margin, and apical four-chamber sections under M mode. Body surface area was calculated as [0.0061 × height (cm) + 0.0128 × body mass (kg) - 0.1529]. LV mass of patients was calculated with the Devereux formula.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 24.0 for Windows was used to perform the statistical analysis (SPSS Inc., Chicago, IL). The distribution of the variables was evaluated using the Kolmogorov-Smirnov test. Comparison of parametric data was performed using the Student's t-test, non-parametric variables were evaluated using the Mann-Whitney U test, and categorical variables were compared using the chi-square test. For non-parametric variables, the median (minimum-maximum) represents the data, but the mean and standard deviation are used for parametric variables. Statistical significance was defined as a p value of <0.05.

RESULTS

Data from patients in our study population of 321 patients were reviewed. Since the cardiac mass index is associated with mortality in obese patients, patients with similar cardiac mass index were included in the study and 79 patients were excluded from the study. Fifty two patients were excluded from the study to reduce confounding factors, and patients with similar basic clinical features and laboratory measurements were included in the study. As a result, 190 patients were included in the study.

The obese group included 96 patients and the mean age was 54.2±3 years. The control group included 94 patients and the mean age was 53.1±2 years. No significant difference was observed between the two groups in terms of antihypertensive medications, hypertension, age, gender distribution, diabetes mellitus, and smoking status (Table 1). Laboratory analyses of the groups except total cholesterol were similar. Total cholesterol level was significantly higher in the obese group (p=0.029) (Table 2). There were no significant differences in left ventricular dimensions and ejection fraction (p>0.05) (Table 2).

The ECG parameters are summarized in Table 3. The QTc interval was similar between the groups (416.4±11.6, and 422.1±14.8; p=0.081). Tp-e, and Tp-e/QTc ratios were greater in the obese group (93.1±6.2 ms, and 67.7±2.5 ms; p=0.00; 0.22±0.01, and 0.15±0.02; p=0.001). There was no difference in cardiac mass index between the two groups.

Twelve months after the first examinations, six deaths were noted in an obese group with ID number interrogation (p=0.001). The non-cardiac cause of death was not noted in the death reporting system.

DISCUSSION

The BMI ≥30 kg/m^2 group in our study had a higher Tp-e interval and Tp-e/QTc ratio. The literature review indicates

Table 1. Baseline characteristics of the patients

Baseline clinical features	Group 1 n=96	Group 2 n=94	p value
Age (years)	54.2±3	53.1±2	0.42
Sex (female), n (%)	52 (52)	52 (52)	1.000
Body mass index (kg/m^2)	35.6±2.5	22.3±1.5	0.001
Smoking, n (%)	5 (5)	5 (5)	1.000
Hypertension, n (%)	44 (45)	43 (44)	0.877
Diabetes mellitus, n (%)	24 (25)	17 (18)	0.238
Angiotensin-converting enzyme inhibitors, n (%)	31 (32)	33 (34)	0.564
Angiotensin receptor blockers, n (%)	11 (12)	11 (12)	0.912
Diuretics, n (%)	24 (25)	31 (33)	0.413
Calcium channel blockers, n (%)	10 (11)	5 (6)	0.198
Sudden cardiac death at 12 month, n(%)	6 (6)	0	0.001

Table 2. Biochemical and echocardiographic features

	Group 1	Group 2	p value
Fasting glucose, (mg/dL)	106.7±24.1	102.4±2.3	0.155
Creatinine, (mg/dL)	0.9±0.1	0.9±0.2	0.134
Total cholesterol, (mg/dL)	208.1±38.6	201.5±27.3	0.049
Hemoglobin, (g/dL)	13.4±1.4	12.5±1.1	0.625
Na, (mmol/L)	139.5±2.0	137.7±2.3	0.347
K, (mmol/L)	4.4±0.4	4.5±0.5	0.350
Ca, (mg/dL)	9.8±0.5	9.7±0.4	0.097
Mg, (mg/dL)	2.0±0.2	2.0±0.1	0.452
TSH, (mIU/mL)	1.8±0.8	1.7±0.8	0.511
AST, (U/L)	20.7±5.5	19.2±5.0	0.761
ALT, (U/L)	20.9±7.3	20.2±7.1	0.432
LV ejection fraction, (%)	64.5±1.9	64.5±1.3	0.673
Left ventricular end-diastolic diameter, (mm)	48.0±1.5	47.2±2.1	0.249
Left ventricular end-systolic diameter, (mm)	29.3±1.1	27.8±1.2	0.837
LVPWT, (mm)	10.3±2.8	10.4±2.1	0.911
LVAWT, (mm)	11.2±2.3	10.1±1.8	0.892
LV mass index, (g/m^2)	92.2±9.2	89.4±8.5	0.089

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TSH: Thyroid-stimulating hormone, LV: Left ventricular, LVPWT: Left ventricular posterior wall end-diastolic thickness, LVAWT: Left ventricular anterior wall end-diastolic thickness

Table 3. Electrocardiographic features

	Group 1	Group 2	p value
Heart rate, beats/min	74.2±7.1	73.2±9	0.064
QRS duration, ms	91.4±5.2	84.6±6.4	0.071
QT interval, ms	363.2±18.8	379.2±14.4	0.591
QTc interval, ms	416.4±11.6	422.1±14.8	0.081
Tp-e, ms	93.1±6.2	67.7±2.5	0.001
Tp-e/QTc ratio	0.22±0.01	0.15±0.02	0.001

that this study is the first to demonstrate how ventricular depolarization and repolarization are out of balance in obese individuals. The recording of three cardiac deaths in the obese group in the 12-month follow-up period in our study may suggest that ECG parameters may be clinically important in obesity.

Patients with morbid obesity have a higher risk of SCD before heart disease develops[8]. In SCD patients with anatomically normal hearts, obesity is a significant comorbidity[9]. The main causes of arrhythmia and SCD in obese people are cardiomyopathies, which include myocyte hypertrophy, mononuclear cell infiltration, abnormal cardiomyocyte lipid deposits, and cardiac fibrosis[10,11]. Fatty infiltration alters the parallel orientation of cardiac bundles; thus, affecting ventricular activation and leading to uneven repolarization[9]. Increased intracellular lipid content can cause ventricular tachycardia and abrupt cardiac death due to a decrease in potassium channel levels and impaired repolarization[12]. Adipocytokines from the epicardial fat of cardiomyocytes lengthen action potentials and increase triggered activity immediately after depolarization by decreasing delayed rectifier outward currents[13].

Premature ventricular contractions are common in obese patients, and this is unrelated to hypertension or concentric ventricular hypertrophy. Conduction system problems in obese people are uncommon[12]. The conduction system may play a part in sudden death in obese young people, according to a study by Bharati and Lev[14] These researchers have found enlarged and hypertrophied hearts, focal mononuclear cells in and around the conduction system, fibrosis of the left bundle branch and atrioventricular bundle, and fibrosis in the interventricular septum[15]. Patients who were mild to moderately obese had a higher amount of fibrosis/fat than those who were very obese. Because of the irregularities in sympathovagal balance, obese people have their heart rate variable between faster and lower, which is a factor increasing the risk of myocardial infarction and SCD[16]. Resting heart rate was higher in patients with BMI ≥30 kg/m^2 in our study.

Obese women who lost weight had a significantly shorter QTc interval and QT dispersion which was linked to a regression of ventricular hypertrophy. The risk of potentially lethal arrhythmias and sudden death may be reduced by shortening the QT interval and increasing the cardiac parasympathetic activity[16]. Three months following sleeve gastrectomy in patients with morbid obesity, the QT interval was shorter. The ventricular depolarization and repolarization periods are included in the QT and QTc intervals, and their lengthening is linked to malignant ventricular arrhythmias. The dispersion of QT and QTc represents electrical heterogeneity in the myocardium. These could be useful in predicting obesity in patients. QTc dispersion, a marker of a significantly increased risk of ventricular arrhythmia, is associated with obesity. Longer QT interval was linked to higher sympathetic and lower parasympathetic tone in obese people.

In normal and obese women, the QTc interval was associated with a free fatty acid level, and fatty infiltration could enhance the dispersions of action potential length, thus, increasing the chance of reentry circuits[11,16]. Plasma epinephrine and norepinephrine concentrations were all shown to be correlated with QTc intervals by Corbi et al.[16] suggesting that autonomic nervous system dysfunction may be the cause of prolonged QTc intervals in visceral obesity. The sympathetic nervous system is stimulated by higher plasma-free fatty acid levels.

Finally, it is possible to identify the elevated risk of unfavorable cardiovascular events linked to obesity using the Tp-e interval and Tp-e/QT ratio measurements. We discovered that obese patients had higher Tp-e intervals and Tp-e/QTc ratio than non-obese patients. Our findings, which point to higher ventricular repolarization heterogeneity in obese patients, may help us better understand the pathophysiological causes of the higher prevalence of arrhythmias. Prolonged transmural dispersion may explain the increased ventricular arrhythmia frequency.

Study Limitations

Patients could be followed with a long-term rhythm Holter or loop recorder for ventricular arrhythmic events. To assess the predictive ability of the longer Tp-e interval and higher Tp-e/QTc ratio in this population, large-scale prospective investigations are needed.

CONCLUSION

Obesity has a higher risk of ventricular arrhythmogenesis because obese patients have higher Tp-e/QTc ratio and longer Tp-e intervals. In the twelve-month follow-up, SCD was found to be higher in the obese group.

Ethics

Ethics Committee Approval: Ethical committee approval was received from the University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Local Ethics Committee (approval no: 2022/03-18, date: 15.03.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: M.K., Concept: A.D., Design: U.U., Ş.A., Data Collection or Processing: U.U., M.K., Analysis or Interpretation: C.A., A.D., Ş.A., Literature Search: A.D., Writing: U.U., C.A., A.D., M.K., Ş.A.

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CASE REPORT

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Multipl MRSA Absesses Following Intramuscular Injection a Case Report

İntramüsküler Enjeksiyon Sonrası Gelişen Çoklu MRSA Apsesi Olgu Sunumu

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ABSTRACT

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) cases are rarely reported. In this article, we present a patient who had no history of disease or risk factors for MRSA but developed multiple MRSA abscesses after receiving intramuscular injections outside the hospital. With this case report, we aimed to emphasize the importance of attention to community-acquired MRSA infections and highlight the need to maintain aseptic conditions to reduce potential complications after intramuscular injections.

Keywords: Methicillin-resistant *Staphylococcus aureus*, abscess, intramuscular injection

ÖZ

Toplumdan kazanılmış metisiline dirençli *Staphylococcus aureus* (TK-MRSA) olguları nadir de olsa karşımıza çıkmaktadır. Bu yazıda öyküsünde bilinen bir hastalığı ve MRSA için risk faktörleri olmayan, hastane dışında yaptırdığı intramüsküler enjeksiyonlar sonrası çoklu MRSA apsesi gelişen bir olgu sunulmuştur. Bu olgu örneğinde toplum kökenli MRSA enfeksiyonlarına dikkat çekmeyi ve intramüsküler enjeksiyonlar sonrası istenmeyen komplikasyonları azaltmak için asepsi koşullarına dikkat edilmesi gerekliliğini vurgulamayı amaçladık.

Anahtar Kelimeler: Metisiline-dirençli *Staphylococcus aureus*, apse, intramüsküler enjeksiyon

INTRODUCTION

Widespread and unnecessary use of antibiotics causes the emergence of resistant strains and the spread of these strains. *Staphylococcus aureus* is one of the bacteria that can develop resistance due to inappropriate antibiotic use and is one of the factors that cause mortality. While methicillin-resistant *Staphylococcus aureus* (MRSA) causes hospital-acquired infections, it has also caused community-acquired infections since the 1990s[1]. Although community-acquired-MRSA (CA-MRSA) most commonly occurs with skin and soft tissue infections, it can also cause life-threatening infections such as liver abscess, bone and joint infections, and bloodstream infections[2-4]. In this article, a case with multiple community-acquired MRSA abscesses that occurred as a complication of intramuscular injections was presented.

CASE REPORT

A thirty-eight-year-old female patient applied due to back and hip pain that had been going on for twenty days. It was learned that the patient's lower back pain started after falling to the ground twenty days ago, and during the examinations, nonsteroidal anti-inflammatory treatment was started due to soft tissue damage caused by trauma. It was learned that

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the patient had her intramuscular injections administered by non-medical personnel and was referred to us after contrast enhancement was detected in the paravertebral muscle planes and left iliac fossa in the lumbar magnetic resonance imaging (MRI) taken at the center to which she applied due to an increase in her complaints despite the treatment. The patient had no known medical history other than panic disorder. On the physical examination, there was hip movement limitation and a painful lesion approximately 10 cm in diameter was palpated in the left gluteal region. In laboratory tests performed at admission, the values were as follows: leukocyte: 11.870/µL, C-reactive protein (CRP): 218 mg/L, erythrocyte sedimentation rate (ESR): 150 mm/hour, procalcitonin (PCT): 0.167 µg/L, and 2 erythrocytes and 24 leukocytes in complete urinalysis. Blood and urine cultures were taken at the time of hospitalization. The patient, who had no previous history of antibiotic use or hospitalization, was started on ampicillin-sulbactam 4x3 g intravenously (iv) and ciprofloxacin 2x400 mg intravenously with the preliminary diagnosis of soft tissue infection-abscess. In the lumbar MRI, a lesion measuring approximately 10x6 cm in size and extending inferiorly towards the pelvis was observed in the left lumbar region. In abdominal computed tomography, a lesion that was initially thought to be an abscess, reaching 9 cm in size anterior to the iliac muscle in the left lower quadrant (Figure 1), and an abscess measuring approximately 8 cm in the gluteal area, as well as edema and fluid densities, were observed (Figure 2). Thereupon, percutaneous drainage was performed by interventional radiology for the abscess areas in both the gluteal and anterior iliac muscles. There was no growth in the blood culture taken during hospitalization. MRSA growth was detected in the abscess cultures taken with drainage (sensitive to vancomycin, teicoplanin, linezolid and trimethoprim/sulfamethoxazole). The patient's treatment was changed to vancomycin 2x1 gr iv. No pathology was detected in the echocardiography. On the 7th day of treatment, leukocyte count decreased to 5.900/µL, CRP to 13.3 mg/L, ESR to 104 mm/hour and PCT to 0.04 µg/L. In the control lumbar MRI taken on the 20th day of hospitalization, a significant decrease in the size of the abscess was detected and an appearance compatible with sacroiliitis was reported. The patient, whose intravenous antibiotic treatment was completed in 4 weeks, was discharged with trimethoprim/sulfamethoxazole 2x800/160 mg tablets to be completed for 12 weeks. The patient's pain decreased and laboratory findings returned to normal during outpatient clinic follow-ups, and her treatment was discontinued at the 12th week. Written informed consent was obtained from the patient to present this case.

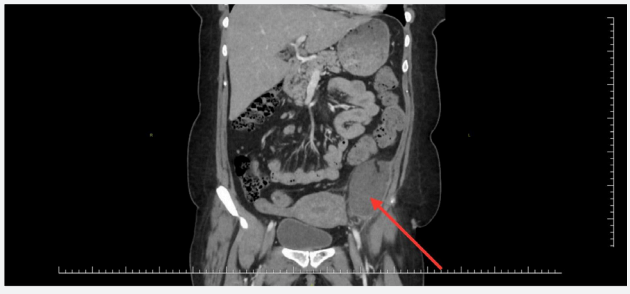

Figure 1. 11*6 cm abscess in the iliopsoast on contrast-enhanced abdominal CT

CT: Computed tomography

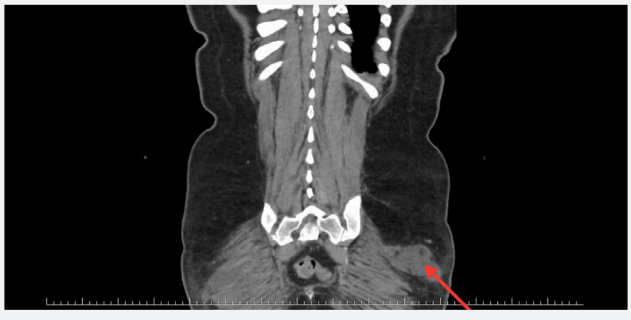

Figure 2. 8*5 cm abscess in the left gluteal area on contrast-enhanced abdominal CT

CT: Computed tomography

DISCUSSION

Intramuscular injection is used as a preferred technique in many treatment protocols to obtain a rapid and effective response in the administration of drugs. Complications of this procedure include bleeding at the injection site, hematoma, sciatic nerve damage, pain, abscess formation, and tissue necrosis. Complications such as abscess formation and sepsis after intramuscular injection were reported to be extremely rare, with a rate of 1.9% in a study[5].

For the diagnosis of community-acquired MRSA infection, there must be no previous MRSA infection, no hospitalization in the last year, no stay in a nursing home, no permanent catheter or medical device[6]. Although our patient had no known risk factors, multiple abscesses developed due to intramuscular drug injections administered outside the hospital.

It has been reported that CA-MRSA is transmitted through physical and sexual contact. At the same time, in addition to nasal colonization, colonization of the genital area was also found to be significant as a reservoir[7].

In a study conducted in the USA, the frequency of CA-MRSA was reported as 8-20%[8]. In a study conducted in France, the prevalence of MRSA was found to be 1-3%[9]. In another study conducted at the community level in the Asia Pacific region, the prevalence of CA-MRSA carriage among the population was found to be between 0% and 23.5%[10].

In a comprehensive multicenter study conducted in Turkey, CA-MRSA was found to be at a very low rate (0.7%)[11]. In a study evaluating primary and high school students in Manisa, CA-MRSA carriage was found to be at the rate of 2.6%[12]. In an epidemiological study conducted in the Turkish Cypriot community in 2019, the prevalence of nasal CA-MRSA carriage was reported as 6.98%[13]. Günal et al.[14] evaluated infections due to *S. aureus* for 5 years and found that 64.4% of CA-*S. aureus* strains developed due to MRSA. In a multicenter study conducted in the pediatric population in our country, the frequency of CA-MRSA was reported as 17.4% and the authors emphasized that this rate was higher in the refugee population[15].

A case of MRSA infection resulting in gluteal compartment syndrome after intramuscular injection has been presented in the literature. In this presentation, it was emphasized that the risk of developing soft tissue infection and gluteal compartment syndrome increased after intramuscular injection in the presence of anticoagulant use, bleeding diathesis, immune deficiency and diabetes[16]. Another case with a history of recurrent gluteal abscess, intravenous drug use, and diabetes mellitus, in which MRSA septicemia was reported to occur 2 months after the drainage and treatment of the gluteal abscess, was admitted to the emergency department with sudden onset vision loss. MRSA was detected in the blood culture of this patient and it was reported to be complicated by tricuspid valve endocarditis, multiple septic pulmonary embolism and endogenous endophthalmitis[17].

CONCLUSION

The most common cause of community-acquired skin and soft tissue infections is *Staphylococcus aureus*, but recently soft tissue infections due to MRSA strains have also started to be seen. Therefore, culture antibiogram results will guide the treatment plan in community-acquired infections. Application of intramuscular injections after appropriate technique and necessary asepsis conditions will reduce the risk of possible MRSA infection.

Ethics

Informed Consent: Written informed consent was obtained from the patient to present this case.

Authorship Contributions

Surgical and Medical Practices: Ö.G., S.S.K., B.B.Ö., Concept: Ö.G., H.Ü., Design: Ö.G., H.Ü., Data Collection or Processing: B.B.Ö., Analysis or Interpretation: S.S.K., Literature Search: B.B.Ö., H.Ü., Writing: B.B.Ö.

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

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LETTER TO THE EDITOR

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Hemochromatosis as a Preventable Cause of Heart Failure: A Rare Case

Kalp Yetmezliğinin Önlenebilir Bir Nedeni Olarak Hemokromatozis: Nadir Bir Olgu

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Keywords: Hemochromatosis, thalassemia major, heart failure, ventricular tachycardia, global longitudinal strain, cardiac magnetic resonance

Anahtar Kelimeler: Hemokromatozis, talasemi majör, kalp yetmezliği, ventriküler taşikardi, global longitudinal strain, kardiyak manyetik rezonans

To the Editor,

A 29-year-old male patient presented to the emergency department with palpitations and fainting sensations. Ventricular tachycardia was detected on electrocardiography and sinus rhythm was achieved with cardioversion. The patient's medical history revealed a diagnosis of thalassemia major (TM) at the age of 1 year, splenectomy at the age of 5 years, and blood transfusions at external centers. Upon examination of the patient's hospitalization records at our hematology clinic, it was reported that the T2* value in the interventricular septum was 20.3 msec in the cardiac magnetic resonance (CMR) examination with T2* sequence performed for iron accumulation on October 6, 2015, and clinical follow-up was recommended. During the 8-year period after this date, the patient underwent T2* sequence CMR examinations to assess myocardial iron loading. The results showed the values of 6 msec and 4.5 msec, respectively. Despite recommendations from the hematology clinic, the patient did not attend cardiology check-ups and did not continue the deferiprone 500 mg 3x2 treatment prescribed for iron chelation. Furthermore, the patient has not visited our hospital for the past 1.5 years. Echocardiography was performed on the patient who was admitted to the coronary intensive care unit. The results showed an ejection fraction of 40%, left ventricular (LV) dilatation, and global hypokinesia. Strain echocardiography was also performed on recorded images, which revealed a decrease in the LV global longitudinal strain value compared to normal, with a value of -17.7%. The interventricular septum revealed a more pronounced decrease in the strain value (Figure 1B). Coronary angiography was performed to determine the cause of VT. The coronary arteries were found to be normal (Figure 1C, 1D). The patient's serum ferritin level was greater than 2000 µg/L. In the cardiology council, with the participation of a hematology specialist, the patient's current and previous CMR findings were evaluated. The clinical condition was determined to be due to cardiomyopathy/heart failure caused by secondary hemochromatosis. Another CMR imaging was performed to investigate the cardiac iron load. CMR imaging revealed LV enlargement and decreased systolic function on 4-chamber cine images. The mean T2* value in the interventricular septum was less than 5 milliseconds (Figure 2). Based on these results, the patient was diagnosed with heart failure due to secondary hemochromatosis caused by cardiac iron accumulation resulting from frequent transfusions due to thalassemia. The patient, who received follow-up and treatment in the cardiology clinic, was prescribed ramipril 2.5 mg once daily, spironolactone 25 mg once daily, carvedilol 6.25 mg twice daily, and furosemide 40 mg twice weekly. Additionally, amiodarone 200 mg was

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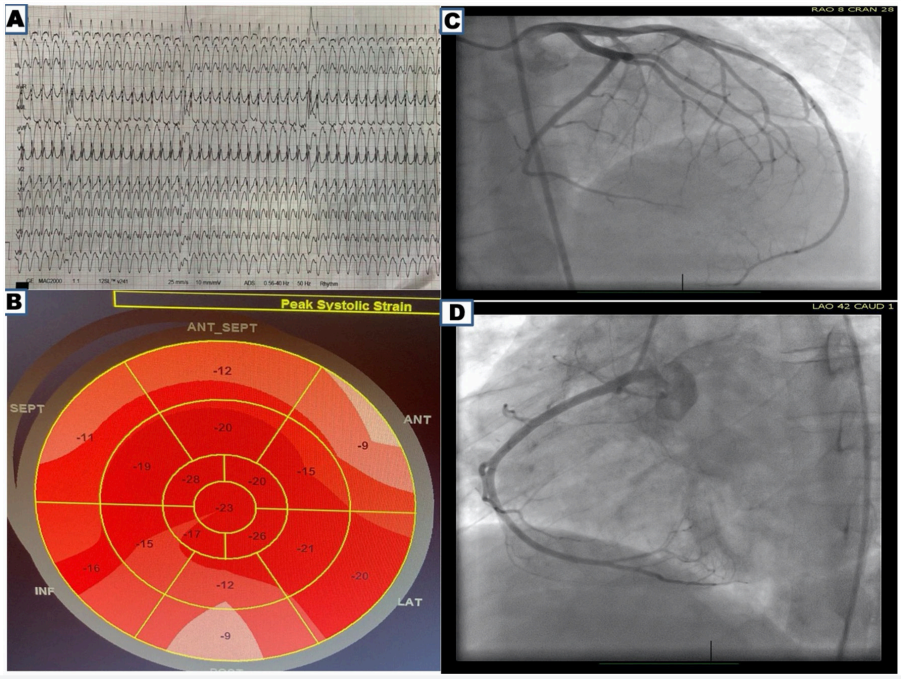

Figure 1. A) Ventricular tachycardia on ECG. B) Strain echocardiography shows a decrease in strain values, especially in the interventricular septum. C) Left system is normal in coronary angiography. D) Right system is normal in coronary angiography

ECG: Electrocardiography

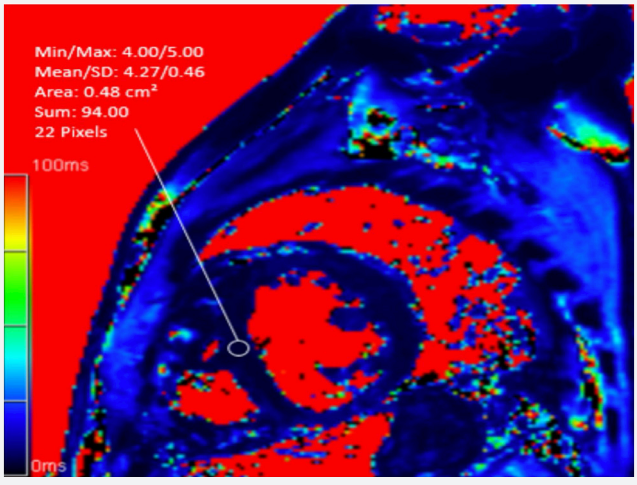

Figure 2. T2-star (T2*) mapping to show iron deposition in CMR imaging

CMR: Cardiac magnetic resonance

prescribed twice daily to prevent sudden cardiac death. The council performed cardioverter-defibrillator implantation for secondary prevention. Following a hematology consultation, iron chelation therapy was initiated with deferiprone 500 mg taken three times daily. The patient was discharged with a recommendation for outpatient clinic follow-up.

This case highlights the potential development of cardiac dysfunction due to myocardial iron loading in patients with frequent blood transfusions, such as those with TM. Therefore, it is important for patients to undergo periodic checks for cardiac hemochromatosis, a preventable cause of heart failure. Myocardial biopsy is an invasive and impractical method for patient follow-up, making it necessary to explore alternative diagnostic methods[1]. Instead, if possible, CMR should follow the T2* value[2,3]. Studies have reported that the decrease in T2* detected in CMR is associated with LV dysfunction and an increased risk of cardiac events[1-3]. T2* measurements in CMR are obtained from a single section passing through the midventricular level using a multi-echo T2* sequence on short-axis images[3,4]. The measurement is based on the change in signal intensity of the interventricular septum according to the echo time[3]. Research has shown a correlation between myocardial T2* values and myocardial iron concentration. Loading begins when this value falls below 20 ms, and the risk of developing heart failure is very high when it falls below 10 ms[2-4]. Additionally, there is a good correlation between the LV GLS value obtained from strain echocardiography and T2* values determined by CMR imaging, as reported in the literature. Therefore, in centers without access to CMR imaging, echocardiography and GLS examination can be considered as alternatives to detect subclinical dysfunction caused by iron accumulation[5].

In conclusion, iron chelation therapy should be applied to patients with TM who undergo frequent recurrent blood transfusions. Investigation of LV function and iron deposition using T2* sequence, along with echocardiographic examination including strain echocardiography and/or CMR examination, can guide the clinician in the early detection of heart failure due to secondary hemachromatosis.

Ethics

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: M.E., E.Y., Concept: M.E., M.G., Design: M.E., M.G., Data Collection or Processing: M.G., F.E.U., Y.G., Analysis or Interpretation: F.E.U., Y.G., Literature Search: M.E., E.Y., M.G., Writing: M.E., M.G.

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

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