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İlhan Celil ÖZBEK

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EDITORIAL

Dear Colleagues,

We are leaving 2024 behind and entering a new year. In 2024, many joyful and upsetting events occurred that were closely related to the our country.

2024 has seen significant developments in health and medicine. The healthcare sector has opened new avenues for improving human health through advanced research, technological innovations, and groundbreaking treatment methods. Pioneering efforts in neurology, cancer treatment, and robotics have paved the way for potential therapies for lethal diseases, enhancing medical precision. Researchers and institutions around the globe have made remarkable advances in understanding complex diseases, developing targeted therapies, and leveraging artificial intelligence to transform healthcare.

The 2024 Nobel Prizes in Science have been announced. The Nobel Prize in Physiology or Medicine was awarded to Victor Ambros and Gary Ruvkun for their discovery, which introduced a new dimension to the microRNA-based regulatory mechanism of gene activity. The Nobel Prize in Physics was awarded to John Hopfield and Geoffrey Hinton for their contributions to laying the groundwork for today's robust machine learning systems. The Nobel Prize in Chemistry was awarded to David Baker for developing a computational method to design entirely new proteins. Meanwhile, Demis Hassabis and John Jumper were recognized for creating an artificial intelligence model that predicts the complex three-dimensional structures of proteins. Daron Acemoglu, Simon Johnson, and James Robinson received the Nobel Prize in Economic Sciences for their research on how institutions are formed and how they influence prosperity. Acemoglu became the third person from Turkey to receive the Nobel Prize. The 2024 Nobel Peace Prize was awarded to the Japanese organization Nihon Hidankyo for its efforts in the "denuclearization" of the world.

We have published 51 articles in 2024. One of these articles had a special place for those who set their hearts on the Istanbul Faculty of Medicine. We thank Prof. Arın Namal for her excellent article on the history of the Istanbul University, Istanbul Faculty of Medicine. We have made some changes in our editorial team. Prof. Halil Yazıcı and Prof. Bengisu Mirasoğlu, two of our section editors, took over the position of co-editors from Prof. Funda Güngör Uğurlucan and Prof. Tzevat Tefik. We are grateful to our colleagues with whom we work extensively. We bid farewell to Prof. Deniz Tuğcu, Prof. Mine Karagülle, and Assoc. Prof. Achmet Ali. We thank them for their efforts. We would also like to thank all the editorial board members for their efforts and time devoted to this task, which they have undertaken entirely voluntarily.

In the first issue of 2025, we published nine original articles, two case reports, and a letter to the editor. Duran B et al. reported an original article evaluating the clinical and pathological findings and treatment outcomes of patients with lupus nephritis. You can find the article written by Çakmak et al. about the effectiveness of SGLT-2 inhibitors on glycemic condition and liver functions in type 2 diabetes mellitus. Aydoseli et al. showed that augmented reality-based neuronavigation systems positively influence residents and have great potential for enhancing surgical education, especially regarding motivation and anatomical understanding. Sağlam et al. will also guide neurosurgeons in evaluating the morphology of the the falx cerebri. They reported an anatomical approach that may be important to increase the success rate of diagnostic and operative procedures of the falx cerebri or adjacent structures and to minimize intraoperative complications during

neurosurgical applications. Altunoğlu et al. expanded the clinical and molecular spectrum by describing novel pathogenic variants in Mowat-Wilson syndrome. Also, in this issue, you will find a very detailed article on breast milk written by Gökçay et al. Naser A et al. presented a 17-year-old patient to draw attention to the fact that gallbladder cancer can develop at a young age.

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We wish you a happy and prosperous new year in 2025 and look forward to receiving your valuable submissions.

Thank you in advance for your contributions.

Sincerely,

On behalf of the editorial board of the Journal of Istanbul Faculty of Medicine

Prof. Dr. Birsen Karaman

Prof. Dr. Ayşe Kubat Üzüm

Editors-in-Chief, Journal of Istanbul Faculty of Medicine, JMED

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EVALUATION OF CLINICAL AND PATHOLOGICAL FINDINGS AND TREATMENT OUTCOMES OF PATIENTS WITH LUPUS NEPHRITIS: A SINGLE CENTRE EXPERIENCE

LUPUS NEFRİTLİ HASTALARIN KLİNİK VE PATOLOJİK BULGULARININ DEĞERLENDİRİLMESİ VE TEDAVİ SONUÇLARI: TEK MERKEZ DENEYİMİ

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ABSTRACT

Objective: Renal involvement in systemic lupus erythematosus (SLE), also known as lupus nephritis (LN), leads to a worse prognosis than SLE without kidney involvement.

Material and Methods: Biopsy-proven LN patients diagnosed between January 2012 and January 2021 were reviewed. Complete remission (CR) was defined as a reduction in the urinary protein-to-creatinine ratio (UPCR) below 0.50 g/g. Partial response is characterised by a 24-h urine protein excretion reduction to below 3 g/day with at least a 50% decrease in proteinuria. Primary effective renal response was defined as PCR of less than 0.7 g/g and the absence of any rescue therapy for treatment failure.

Result: All patients exhibited proteinuria at diagnosis, with class IV LN being the most common (36.4%) form, and 65.9% had proliferative LN. At 12 months, CR was achieved in 16 patients (37.2%) with significant differences in systolic and diastolic blood pressure and eGFR at diagnosis (p=0.01, p=0.02, and p=0.016, respectively). CR rates were lower at 12 months in patients with proliferative LN (p=0.024) and interstitial inflammation (p=0.04). Besides, no significant difference was found in CR rates at 6 and 12 months between PLN patients treated initially with steroids and cyclophosphamide and those treated with steroids and mycophenolate mofetil (p>0.05). However, the median time to achieve CR was shorter in the mycophenolate mofetil group (p=0.048).

ÖZET

Amaç: Sistemik lupus eritematozusun (SLE) böbrek tutulumu olarak bilinen lupus nefriti (LN), böbrek tutulumu olmayan SLE'ye göre daha kötü bir prognoza yol açar.

Gereç ve Yöntem: Ocak 2012 ile Ocak 2021 arasında tanı konulan biyopsiyle kanıtlanmış LN hastaları incelendi. Tam remisyon (TR), idrar protein-kreatinin oranının (PKO) 0,50 g/g'nin altına düşmesi olarak tanımlandı. Kısmi yanıt, 24 saatlik idrar protein atılımının günde 3 g/günün altına düşmesi ve proteinüride en az %50 azalma olarak tanımlandı. Birincil etkili renal yanıt ise 0,7 g/g'dan düşük PKO ve tedavi başarısızlığı için herhangi bir kurtarma tedavisinin olmamasıdır.

Bulgular: Tanı anında tüm hastalarda proteinüri mevcut olup, en yaygın form %36,4 ile sınıf IV LN idi ve hastaların %65.9'unda proliferatif LN vardı. Oniki ayda, 16 hastada (%37,2) TR elde edildi ve tanı anında sistolik ve diyastolik kan basıncı ile eGFR'de anlamlı farklar vardı (sırasıyla, p=0,01, p=0,02 ve p=0,016). Proliferatif LN (p=0,024) ve interstisyel inflamasyonu olan hastalarda 12 aylık TR oranları daha düşük bulundu (p=0,04). Ayrıca, steroid ve siklofosfamid ile tedavi edilen PLN hastaları ile steroid ve mikofenolat mofetil (MMF) ile tedavi edilenler arasında 6 ve 12 aylık TR oranlarında anlamlı fark bulunmadı (p>0,05). Bununla birlikte, mikofenolat mofetil grubunda TR elde etme süresi daha kısaydı (p=0,048).

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Conclusion: LN remains a significant source of morbidity and mortality in patients with SLE; therefore, early diagnosis and prompt initiation of the treatment are crucial for renal and patient survival.

Keywords: Renal survival, lupus nephritis, end-stage renal disease, remission, induction therapy

Sonuç: LN, SLE hastalarında morbidite ve mortalitenin önemli bir kaynağı olmaya devam etmekte olup erken tanı ve tedavi böbrek ve hasta sağkalımı için kritik öneme sahiptir.

Anahtar Kelimeler: Böbrek sağkalımı, lupus nefriti, son dönem böbrek hastalığı, remisyon, indüksiyon tedavisi

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex, chronic, multisystemic autoimmune disease with a broad spectrum of clinical manifestations and severity (1). SLE manifests with a spectrum of clinical presentations, encompassing joint and cutaneous involvement, as well as potentially life-threatening renal, hematologic, and central nervous system manifestations. Recurrent disease flares and resulting organ damage contribute to elevated healthcare expenditures and diminished quality of life (2).

The pathogenesis of lupus nephritis (LN), the renal manifestation of SLE, involves the early formation and deposition of immune complexes containing autoantibodies in the kidneys, subsequently leading to inflammation, immune-mediated tissue damage, and fibrosis (3). The range of clinical presentations includes from mild asymptomatic proteinuria to rapidly progressive glomerulonephritis. LN, which affects up to 50% of SLE patients, significantly contributes to morbidity and early mortality, serving as an indicator of a more severe form of SLE (4). Despite recent treatment advances, only 10-58% of patients with LN achieve a complete response (CR) in the first year of treatment, and approximately 20% of patients progress to end-stage renal disease (ESRD) within five years of diagnosis (5).

LN classification based on kidney biopsy findings has shown that more than 50% of patients have class III or IV LN. Histopathological indicators determining prognosis include the presence of crescents exceeding 50%, a high chronicity index, and tubulointerstitial disease (6). The clinical factors that were associated with poor prognosis were male sex, SLE duration, and African-American ethnicity (7). In addition, elevated serum creatinine, the presence and higher titre of anti-dsDNA, high antiphospholipid antibody, and persistent hypocomplementemia influence disease prognosis (8, 9). Therefore, prompt initiation of therapy is essential in LN, as delayed treatment is related to poor prognosis and increased risk of ESRD.

In LN, complete or partial renal response should be achieved for renal survival. The current guidelines define CR in LN as inactive urine sediment, normalisation of serum creatinine levels, and uPCR of less than 500 mg (10). Renal response should be achieved within six months or, at the latest, within 12 months after the initiation of treatment (11). The primary objective of this study was to conduct a comprehensive analysis of the clinical and histological characteristics of patients diagnosed with LN based on the kidney biopsy findings obtained at our institution. In addition, our study evaluated disease remission rates and identify the key factors influencing its achievement.

MATERIALS AND METHODS

Study population

A total of 128 patients diagnosed with SLE and followed up at our hospital between January 2012 and January 2021 were identified. Of these, 47 patients (36.7%) were diagnosed with LN based on kidney biopsy findings. Patients under the age of 18, those with a follow-up period of less than six months, and those with connective tissue disorders other than SLE were excluded from the study (n=3). Consequently, the study included 44 patients diagnosed with LN. All patients with LN are routinely prescribed hydroxychloroquine and an ACE inhibitor or ARB unless there is a contraindication. The institutional review board has approved the study's design and procedures according to the principles outlined in the Declaration of Helsinki and ethical standards for human experimentation (Date: 04.10.2021, 121/07). As the study was retrospective and all procedures performed were part of routine care, no informed consent was required.

Data collection

The patient's demographic, clinical, and laboratory data at diagnosis were retrospectively reviewed. Parameters such as sex, age at diagnosis, accompanying comorbidities, and body mass index were analysed. In addition, the biochemical parameters of the patients at the time of diagnosis, such as ALT, albumin, hemoglobin, white blood cell count, platelet count, estimated glomerular filtration rate (eGFR), urea, creatinine, sedimentation rate, serum complement level, presence of anti-dsDNA positivity, 24-h urine proteinuria levels and presence of active urine sediments like hematuria or leukocyturia were analysed. LN classification was made by evaluating the pathological data of the patients' kidney biopsies according to the ISN/RPS histopathological classification (12). The distribution for LN classes I-VI was determined by considering the dominant renal findings of the patients. In addition, the patients were grouped as non-proliferative and proliferative LN (PLN) according to the proliferative findings in the kidney biopsy.

Treatment and the renal response

Patients with LN were treated according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (13, 14). In patients with PLN, induction therapy was administered with intravenous methylprednisolone 250–500 mg/day (10-15 mg/kg/day) for three days, followed by oral prednisolone therapy starting at 1 mg/kg/day according to the ideal body weight and gradually decreasing the dose. In addition, during induction therapy, patients received mycophenolate mofetil (MMF) at a dose of 2-3 g per day (for six months) or intravenous pulse cyclophosphamide (CYC) 500 mg every two weeks (for three months) according to the EURO/Lupus protocol.

In the context of response assessment, CR is defined as a reduction in 24-h urine proteinuria or urine protein-creatinine (PCR) ratio to less than 0.5 g/g within 6-12 months after treatment initiation, accompanied by stabilisation or improvement in measured renal function (within ±10-15% of baseline). In cases where the level of proteinuria does not meet the criteria for CR, partial remission (PR) is characterised by a 24-h urine protein excretion reduction to below 3 g/day with at least a 50% decrease in proteinuria. The recently updated KDIGO LN guideline included primary effective renal response (PERR) in the definition of response assessment (14). PERR is characterised by PCR of less than 0.7 g/g, an estimated eGFR no more than 20% below baseline or at least 60 ml/min per 1.73 m^2, and the absence of any rescue therapy for treatment failure. The treatments received by the patients during their follow-up and treatment responses were also examined.

Statistical analysis

Continuous data in the study are presented according to distribution as mean±standard deviation or as median with the interquartile range. Categorical data, on the other hand, are shown as numbers and percentages. To compare the baseline characteristics between different groups, the researchers used the student t-test or non-parametric tests for continuous variables based on their distribution. For categorical variables, the chi-square test was employed. In this study, statistical significance was considered for p-values less than 0.05. Statistical analyses were conducted using the SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study comprised 44 patients diagnosed with LN through renal biopsy. Among them, 32 (72.7%) were female, and the median follow-up period was 52.5 (6-117) months. At the time of diagnosis, the mean patient age was 36±10.8 years, with 12 (27.3%) patients having a history of hypertension. Notably, the serum albumin level at diagnosis was 3.0 (1.3-4.5) g/dL, and the 24-h urine proteinuria level was 4388 (594-16912) mg/day. Thirty-one (70.5%) of the patients diagnosed with LN had a known history of SLE, and the mean duration of diagnosis before biopsy was 25 months.

Because one patient died at the end of the 12-month follow-up period, the remission status was evaluated in 43 patients. At 12 months, 16 (37.2%) patients were in CR. Systolic and diastolic blood pressure values at the time of diagnosis were higher in patients who were not in CR at 12 months [131.4±12.2 vs. 120.4±12.1 mmHg (p=0.01) vs. 90.3±11.1 vs. 81.1±9.2 (p=0.02), respectively]. In addition, the eGFR level at the time of diagnosis was significantly lower in the patients who were not in CR at 12 months (p=0.02). The frequency of class II LN (43.8%) was higher in the patients who achieved CR (p=0.002), and the frequency of PLN (77.8%) was higher in the patients who did not achieve CR (p=0.024) at the end of the first year. Interstitial inflammation was also more prominent in the group that did not achieve CR (p=0.04). The patients' demographic, laboratory, and histological characteristics are shown in Table 1.

Among the eight patients diagnosed with class II LN, seven were treated with steroids, and one patient was followed with conservative management. Treatment regimens were varied in the cohort of patients with class V LN (n=8). Two patients were administered steroid monotherapy, four patients received a combination of steroids and MMF, and one patient was treated with steroids combined with CNI. While 6 (75%) of the patients with class II LN (n=8) achieved CR at six months, only one patient (12.5%) with class V LN (n=8) achieved CR. At 12 months, 7 (87.5%) patients with class II LN and 2 (25%) patients with class V LN were in CR (Figure 1). In 29 patients with PLN, the PR rate at 6 months was 69% (n=20), while the CR rate was 13.8% (n=4). At 12 months, 16 patients (57.1%) were in PR and 7 (25%) were in CR. Fourteen patients (48.3%) received steroid+CYC, and 15 (51.7%) received steroid + MMF combination for induction therapy. No difference was found between the two treatment groups regarding remission rates at 6 and 12 months. However, the median time to CR was shorter in the steroid+MMF group than in the steroid+CYC group [9 (0-27) months vs. 21.5 (0-74) months; p=0.048]. The induction treatments and response rates of the patients diagnosed with PLN are shown in Table 2.

The response status of the patients at their last follow-up was also evaluated. It was determined that 31 out of 44 patients (70.5%) were in CR, 7 (15.9%) were in PR, 4 (9.1%) were unresponsive to treatment, and 2 (4.5%) had died. The CR rate was 69% in patients with PLN and 73.3% in the non-proliferative patient group (Table 3). Moreover, we examined PERR, the latest renal response definition in patients with LN. PERR rates of the patients were found to be 46.5% at the 12 months and 72.7% at the last follow-up (Figure 2).

Table 1: Clinical, laboratory and histological findings of patients with Lupus nephritis

	Total (n=44)	Non-complete remission n=27 (62.8%)	Complete remission in the first year n=16 (37.2%)	P value
Demographic data				
Age (years)	36.0±10.8	37.5±10.6	34.1±11.1	0.32
Female, n (%)	32 (72.7)	21 (77.8)	10 (62.5)	0.31
Previous SLE diagnosis, n (%)	31 (70.5)	20 (74.1)	9 (56.2)	0.23
HT, n (%)	12 (27.3)	20 (74.1)	9 (56.2)	0.23
BMI (kg/m²)	20-34.2	26.2 (21.5-34.2)	24.6 (20-34.2)	0.13
Systolic BP (mmHg)	127.3±13.1	131.4±12.2	120.4±12.1	***0.01***
Diastolic BP (mmHg)	87.2±10.3	90.3±11.1	81.1±9.2	***0.02***
LN follow-up time (months)	52.5 (6-117)	57 (22-103)	47.5 (18-117)	0.60
Laboratory data				
WBC count (/µL)	6.9 (1.4-13.9)	6.8 (2.7-12.5)	7.5 (1.4-13.9)	0.20
Hemoglobin (g/dL)	11.2 (4.7-15.8)	11.1 (6.5-15.8)	11.7 (6.7-15.5)	0.48
Platelet count (×1000/µL)	244 (63-779)	243 (63-779)	255 (116-549)	0.13
ALT (U/L)	14.5 (3-50)	13 (3-43)	14.5 (7-50)	0.44
Serum creatinine (mg/dL)	0.8 (0.4-3.2)	1.2 (0.4-3.2)	0.8 (0.4-2.8)	0.08
eGFR (ml/min/1.73 m²)	89 (16-137)	75 (16-134)	116 (28-137)	***0.02***
Serum albumin (g/dL)	3.0 (1.3-4.5)	3.0 (1.3-4.5)	2.8 (1.5-4.5)	0.75
ESR (mm/h)	34.5 (4-123)	32 (4-123)	46 (11-93)	0.24
Low serum C3, n (%)	25 (56.8)	14 (51.9)	10 (62.5)	0.50
Low serum C4, n (%)	21 (47.7)	12 (44.4)	8 (50)	0.72
Anti-ds DNA positive, n (%)	24 (54.5)	13 (48.1)	10 (62.5)	0.36
Presence of RBC/WBC in urine (n, %)	24 (54.5)	14 (51.9)	9 (56.2)	0.78
Proteinuria (mg/24 h)	4388 (594-16912)	4582 (1476-12622)	4308 (594-16912)	0.41
Nephrotic range proteinuria, n (%)	25 (56.8)	17 (62.9)	8 (50.0)	0.74
Class of LN, n (%)				
II	8 (18.2)	1 (3.7)	7 (43.8)	***0.002***
III	12 (27.3)	10 (37)	2 (12.5)	0.16
IV	16 (36.4)	10 (37)	5 (31.2)	0.70
V	8 (18.2)	6 (22.2)	2 (12.5)	0.69
Proliferative LN	29 (65.9)	21 (77.8)	7 (43.8)	***0.02***
Histologic features				
Endocapillary proliferation, n (%)	26 (59.1)	18 (66.7)	7 (43.8)	0.14
Fibrinoid necrosis, n (%)	7 (15.9)	3 (11.1)	3 (18.8)	0.66
Hyaline wire loops (n, %)	27 (61.4)	19 (70.4)	7 (43.8)	0.08
Fibrocellular crescents, n (%)	15 (34.1)	10 (37)	4 (25)	0.42
Glomerulosclerosis, n (%)	32 (72.7)	22 (81.5)	9 (56.2)	0.09
Interstitial inflammation, n (%)	33 (75)	23 (85.2)	9 (56.2)	***0.04***
Interstitial fibrosis, n (%)	24 (54.5)	17 (63)	6 (37.5)	0.11
Tubular atrophy, n (%)	30 (68.2)	18 (66.7)	11 (68.8)	0.89

BMI: Body-mass index, BP: Blood pressure, CR: Complete remission, eGFR: estimated glomerular filtration rate, ESR: Erythrocyte sedimentation rate, HT: Hypertension, LN: Lupus nephritis, RBC: Red blood cell, SLE: Systemic Lupus erythematosus, WBC: White blood cell

In addition, the maintenance treatment regimens were reviewed. Thirty-two patients received maintenance therapy after remission. Of these, 26 (81.3%) were treated with MMF at a median dose of 1500 mg/day for an average of 39.2±25.8 months, while 6 (18.7%) received AZA at a median dose of 100 mg/day for 35.6±8.7 months (Table 4). We identified eight patients who experienced flares in the long-term follow-up. All patients with flare history had class III/IV LN. 6 of those patients were treated with CYC, and 2 of them with MMF for renal flares of LN.

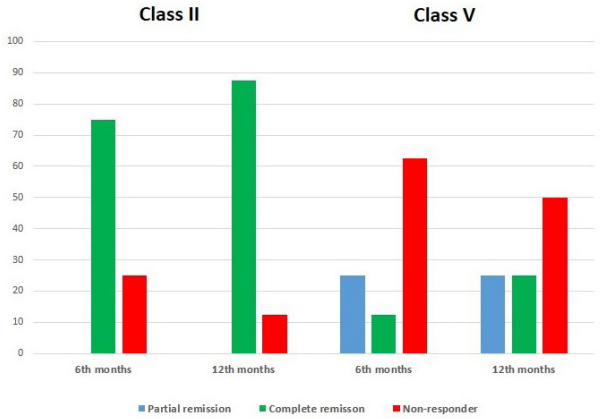

Figure 1: Partial and complete remission rates in class II and class V patients

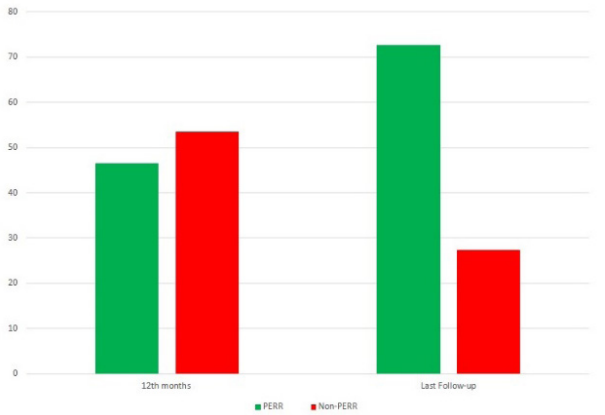

Figure 2: Primary efficacy renal response of patients with lupus nephritis

Table 2: Remission status of patients with proliferative lupus nephritis according to induction therapy

	Total n=29	Steroid+CYC n=14 (48.3 %)	Steroid+MMF n=15 (51.7%)	P value
6th months				
Partial remission, n (%)	20 (69)	9 (64.3)	11 (73.3)	0.70
Complete remission, n (%)	4 (13.8)	1 (7.1)	3 (20)	0.60
Non-responder, n (%)	5 (17.2)	4 (28.6)	1 (6.7)	0.17
12th months				
Partial remission, n (%)	16 (57.1)	9 (69.2)	7 (46.7)	0.23
Complete remission, n (%)	7 (25)	2 (15.4)	5 (33.3)	0.39
Non-responder, n (%)	5 (17.9)	2 (15.4)	3 (20)	0.57
Median time to partial remission (months)	5 (0-28)	5 (0-28)	4 (0-24)	0.47
Median time to complete remission (months)	10 (0-74)	21.5 (0-74)	9 (0-27)	***0.048***

CYC: Cyclophosphamide, MMF: Mycophenolate mofetil

Table 3: Treatment response of patients with lupus nephritis at the last follow-up

	All patients n=44	Non-proliferative LN patients n=15 (34.1%)	Proliferative LN patients n=29 (65.9%)
Partial remission, n (%)	7 (15.9)	1 (6.7)	6 (20.7)
Complete remission, n (%)	31 (70.5)	11 (73.3)	20 (69)
Non-responder, n (%)	4 (9.1)	2 (13.3)	2 (6.9)
Death, n (%)	2 (4.5)	1 (6.7)	1 (3.4)

Table 4: Maintenance treatment regimens of patients with lupus nephritis

Treatment	Total Patients (n=32)	Dosage (mg/d)	Duration (months)
MMF, n (%)	26 (81.3)	1500 (1000-2000)	39.2±25.8
AZA, n (%)	6 (18.7)	100 (100-150)	35.6±8.7

AZA: Azathioprine, MMF: Mycophenolate mofetil

DISCUSSION

This study examined patients' general characteristics and remission status followed up with LN. It was found that patients who achieved CR at 12 months had better initial eGFR and lower systolic and diastolic BP. While the frequency of class II LN was higher in the patient group that achieved CR, the frequency of PLN was higher in patients that did not achieve CR. In addition, it was seen that there was no difference in the remission rates between the induction treatment regimens in patients with PLN at 6 and 12 months. However, the median time to achieve CR was shorter in the patients with PLN receiving steroids + MMF as an induction therapy.

LN is the most common and essential visceral complication of SLE and is the leading cause of death in patients with SLE (15). Treatment response is critical in LN, and it has been shown that achieving a CR is related to the prognosis and progression to ESRD (16, 17). Studies have shown that CR rates in patients with LN vary between 10% and 40% (18). This study found that the CR rate was 37.2% at 12 months. Although the CR rates were low in the first year of our study, it should be noted that some of our patients achieved PR in the first year. Our higher CR rate (70.5%) in the long-term follow-up may be related to the fact that some patients with PR achieved CR after the first year of follow-up. In a study conducted by Gatto et al, the median time to achieve sustained clinical response was found to be 1.44 years (0.69–3.58), supporting the observation that CR in LN patients may occur even after more than one year (19).

Besides, systolic and diastolic blood pressures measured at diagnosis were higher in patients who did not achieve CR. Some studies have shown that hypertensive LN patients are associated with worse renal prognosis and mortality (20, 21). Although our results show that the frequency of HT is similar between the groups, high systolic and diastolic BPs may still influence remission. Similar to our study, a study from South Africa showed that high systolic and diastolic BPs in patients with PLN are associated with ESRD and death (22).

In addition, it was observed that patients who did not attain CR exhibited lower eGFR levels at the time of diagnosis than those who achieved remission. Similar to our study, Pirson et al. showed that patients in remission during follow-up had better baseline eGFR levels (23). This condition may be related to the more severe LN involvement in patients with low initial eGFR. In support of this finding, patients who did not achieve CR at the end of the first year had higher rates of proliferative LN and interstitial inflammation in their histopathological examinations. Many studies have shown that PLN is associated with lower CR rates and worse renal survival (24-26). Similar to our study, Lee et al. demonstrated that detecting higher interstitial inflammation in kidney biopsy increased the risk of ESRD and CKD in patients (Hazard ratio 4.67 and 3.8, respectively) (27). We also found that class II LN was more common in patients who achieved CR. Class II LN is considered a mild form of LN with a better prognosis and higher CR rates (28).

The guidelines recommend steroid therapy in combination with immunosuppressive therapy for patients with active class III/IV±V LN (13, 14, 29). In treating LN, a standard protocol involves an initial phase of intense immunosuppression lasting 3 to 6 months, followed by a long-term maintenance phase with less intensive immunosuppression to prevent renal flare. This comprehensive approach is designed to effectively manage the condition and mitigate the risk of disease recurrence. In this investigation, we assessed the remission rates at 6 and 12 months based on the induction regimen in patients with PLN. The remission rates at both 6 and 12 months were comparable between the groups that received steroid + CYC and those that received steroid + MMF. In randomised controlled trials comparing the efficacy of these two regimens, the induction remission rates were similar in both groups (30-32). Besides, the time required to achieve CR was similar between these treatments (32, 33). However, our study found that 69% of patients diagnosed with PLN achieved CR in the long-term follow-up, and the patients who received steroid + MMF treatment had a shorter median time for CR.

The study's limitations should be noted, given its single-centre, retrospective design, which could limit the generalizability of the results. Second, the study's reliance on a localised population and its relatively small sample size may constrain the broader applicability of the findings. In addition, the absence of SLE activity indices, such as SLEDAI or BILAG, has prevented us from comprehensively addressing the extrarenal manifestations of lupus. Finally, some missing parts in the biopsy data limited our analysis of the activity and chronicity indices. These limitations should be carefully considered when interpreting the study's outcomes and implications.

CONCLUSION

In conclusion, despite advances in LN treatment, CR rates are still low in the first year of treatment. Although the CR rates of induction treatments applied to patients with PLN are comparable in the first year, the median time to achieve CR is shorter in patients receiving steroids and MMF. Our results need to be supported by prospective and multicenter studies. New treatment regimens with more effective and fewer side effects must increase the success rate of LN treatment.

Ethics Committee Approval: Ethics committee approval was

received for this study from the University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital (Date: 04.10.2021, No: 121/07).

Informed Consent: As the study was retrospective and all procedures performed were part of routine care, no informed consent was required.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.G.O., M.D.A., B.D.; Drafting Manuscript- Ö.F.A., B.D., E.G.O., A.S.; Final Approval and Accountability- E.G.O., Ö.F.A., M.D.A.; Technical or Material Support – A.S.

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RESEARCH / ARAŞTIRMA
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IMPACT OF AUGMENTED REALITY-BASED NEURONAVIGATION ON NEUROSURGICAL EDUCATION AND TRAINING

ARTIRILMIŞ GERÇEKLİK TABANLI NÖRONAVİGASYON SİSTEMİNİN NÖROŞİRÜRJİ EĞİTİMİNE ETKİSİ

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ABSTRACT

Objective: Integrating technology into surgical training enhances learning experiences. Affordable extended reality (XR) equipment, like the Illumetry XR screen with ArSurgeon software, allows segmentation of preoperative MRI and CT scans in a digital environment, presenting 3D visualisations of tumours and surrounding anatomy. This improves the spatial orientation and understanding of surgical cases, benefiting neurosurgery residents.

Material and Methods: This study utilized preoperative MRI and CT scans of two patients, one with a right frontal mass and the other with a pituitary adenoma. The scans were segmented to isolate the brain, mass, and vascular structures. The 3D models were integrated with MRI and CT data and examined on the Illumetry XR screen with ArSurgeon software. Surgical procedures were recorded and edited into 5-minute videos. Forty neurosurgery residents, split into two groups based on the training year, were provided with the scans and 3D models. After viewing the surgical video, the participants completed a 20-item survey. The survey results were analysed using IBM SPSS Statistics version 29.0.

Result: Among the 40 participants (28 male, 12 female), half were in the first three years of training, and half had 3-5 years of experience. The AR-based neuronavigation system received an average motivation score of 8.4/10, an ease of use rating of 7.6/10, and an ergonomic design rating of 7.9/10. Participants also rated the system's contribution to anatomical understanding and mastery at 8.3/10.

Conclusion: The study showed that AR-based neuronavigation systems effectively enhance surgical education by motivating

ÖZET

Amaç: Teknolojiyi cerrahi eğitime entegre etmek öğrenme deneyimlerini geliştirir. ArSurgeon yazılımına sahip Illumetry XR ekranı gibi uygun fiyatlı genişletilmiş gerçeklik (XR) ekipmanı, ameliyat öncesi MR ve BT taramalarının dijital bir ortamda segmentasyonuna olanak tanıyarak tümörlerin ve çevresindeki anatominin 3D görselleştirmelerini sunar. Bu, uzamsal oryantasyonu ve cerrahi vakaların anlaşılmasını geliştirerek nöroşirürji asistanlarına fayda sağlar.

Gereç ve Yöntem: Bu çalışmada sağ frontal kitle ve hipofiz adenomu olan iki hastanın preoperatif MR ve BT taramaları kullanıldı. Taramalar beyin, kitle ve vasküler yapıları izole etmek için segmentlere ayrıldı. 3D modeller MR ve BT verileriyle entegre edildi ve ArSurgeon yazılımı ile Illumetry XR ekranında incelendi. Cerrahi prosedürler kaydedildi ve beş dakikalık videolar halinde düzenlendi. Eğitim yılına göre iki gruba ayrılan 40 beyin cerrahisi asistanına taramalar ve 3D modeller verildi. Cerrahi videoyu izledikten sonra katılımcılar 20 maddelik bir anket doldurdu. Anket sonuçları IBM SPSS Statistics version 29.0. kullanılarak analiz edilmiştir.

Bulgular: Kırk katılımcının (28 erkek, 12 kadın) yarısı eğitimlerinin ilk üç yılında, yarısı ise 3-5 yıllık deneyime sahipti. AR tabanlı nöronavigasyon sistemi ortalama 8,4/10 motivasyon puanı, 7,6/10 kullanım kolaylığı puanı ve 7,9/10 ergonomik tasarım puanı almıştır. Katılımcılar ayrıca sistemin anatomik anlayış ve ustalığa katkısını 8,3/10 olarak değerlendirmiştir.

Sonuç: Çalışma, AR tabanlı nöronavigasyon sistemlerinin öğrencileri motive ederek ve anatomik bilgiyi geliştirerek cerrahi eğitimi etkili bir şekilde geliştirdiğini göstermektedir. Bununla

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learners and improving anatomical knowledge. However, further improvements in ergonomics and design could enhance their utility in medical training.

Keywords: Augmented reality, neurosurgery, neuronavigation, education

birlikte, ergonomi ve tasarımdaki daha fazla iyileştirme, tıp eğitimindeki faydalarını artırabilir.

Anahtar Kelimeler: Artırılmış gerçeklik, beyin cerrahisi, nörona-vigasyon, eğitim

INTRODUCTION

Conventional navigation and imaging technologies have advanced considerably in recent years, offering crucial three-dimensional (3D) images that have played a pivotal role in training and guiding specialised surgeons worldwide. The integration of preoperative and intraoperative imaging, such as magnetic resonance imaging (MRI), computed tomography (CT), tractography, and angiography, into an augmented reality (AR) environment allows surgeons to visualise diverse data in 3D formats. Research has shown that 3D visualisations improve surgeons' spatial understanding and orientation and reduce operation time (1).

AR technology superimposes computer-generated images onto the user's real-world view, enhancing or modifying the visual experience. In neurosurgery, AR combines preoperative and intraoperative imaging data to optimise the surgical process. It improves surgical planning, enhance neuronavigation, and decrease operative time. Moreover, AR offers significant potential as an educational tool for neurosurgeons, reducing complication risks while providing a comprehensive training platform (2).

The advantages of AR technology have led to its successful adoption in various medical applications, which can be categorised into two main areas. The first category includes treatment programs that support patients or healthcare providers in clinical or hospital environments, such as therapies, rehabilitation, or surgical procedures. The second category focuses on educational programs aimed at improving teaching and learning outcomes in academic settings (3).

Before the introduction of computers in medical education, training primarily relied on textbooks, lectures, cadavers, anatomical models, and live patients. However, the integration of computers into medical education has significantly reduced the reliance on these traditional tools. Computer-based education programs have made information more accessible and improved learning methods. These programs enhance visualisation, making information easier to retain for extended periods. Basic computer-based education programs first emerged in the early 1990s, marking the beginning of a trend that has continued to evolve to the present day (4).

In response to increasing budgetary constraints and the need for standardisation, extended reality (XR) has emerged as a cost-effective alternative to traditional simulation methods. XR is a broad term that includes immersive technologies such as virtual reality (VR), AR, augmented virtuality (AV), mixed reality, and other computer-generated environments using head-mounted displays (5). The introduction of XR has created new opportunities for immersive learning, particularly in mastering complex medical concepts. These technologies address the financial, ethical, and regulatory challenges associated with traditional training resources, such as cadavers and specialised laboratory equipment, used for skill development (6).

Training in neurosurgery requires substantial investment due to the extensive theoretical and procedural knowledge and practical skills that residents must acquire. This learning process continues throughout a neurosurgeon's career. However, the complexity of the neurosurgical procedures and the sensitivity of the anatomical regions involved limit the training opportunities, further prolonging the learning curve. Similar to the field of aviation, simulation in neurosurgery offers a trial-and-error learning approach without risking patient safety (7).

The complexity of neurosurgery largely stems from the intricate anatomy, making a deep understanding of neuroanatomy crucial for neurosurgeons. Research has shown that studying anatomy in a virtual environment improves the retention and recall of both topographic and operative anatomy (7-9). This study aimed to assess the effect of using 3D images, generated from preoperative MRI and CT scans, displayed in an XR environment, on surgeons' surgical orientation.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of İstanbul Faculty of Medicine (Date: 12.07.2024, No:13). All patients provided written informed consent to participate in this study. Preoperative MRI and CT scans were obtained from two patients, one with a right frontal mass and the other with a pituitary adenoma, who both underwent surgery at the İstanbul Faculty of Medicine. These images were used to segment the brain, masses, and vascular structures separately using the Illumetry XR screen integrated with the ArSurgeon software developed by SimBT. Surgical procedures were recorded and edited by two surgeons into a 5-min video. The study included 40 neurosurgical residents. Participants were first presented with the preoperative MRI and CT images of

the patient. They were then shown the segmented 3D images displayed in the XR environment, along with the edited video of the surgical procedure (Figure 1, 2). Following this, the participants completed a 20-item questionnaire (Figure 3). The survey responses were analysed descriptively using IBM SPSS Statistics version 29.0 (IBM SPSS Corp., Armonk, NY, USA).

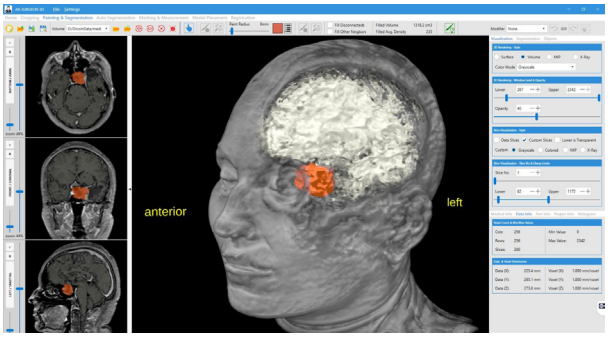

Figure 1: 3D image of preoperative MR and CT images of a patient with pituitary adenoma segmented on an Illumetry XR screen integrated with ArSurgeon software developed by SimBT

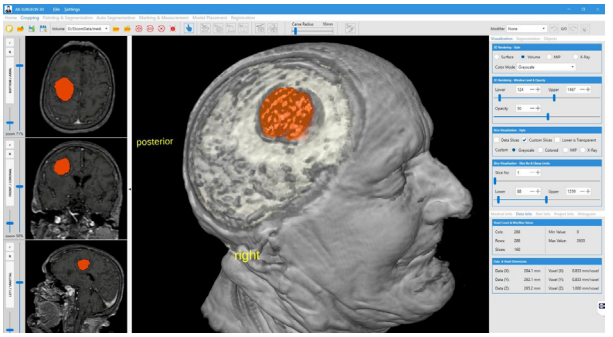

Figure 2: 3D image of preoperative MR and CT images of a right frontal mass patient segmented on the Illumetry XR screen integrated with the ArSurgeon software developed by SimBT

Name-Surname:

Age:

Sex:

Educational background:

Please rate the following questions between 1 and 10

1. How would you evaluate the overall user-friendliness of the augmented reality-based neuronavigation system?
2. Did using the augmented reality-based neuronavigation system enhance your mastery of anatomy?
3. How effective was the augmented reality-based neuronavigation system in improving the understanding of anatomical structures?
4. Would you recommend this system to others?
5. Do you believe that using this system in education is beneficial?
6. Are you satisfied with the visual and technical features of the augmented reality-based neuronavigation system?
7. How motivating do you think it would be to work with the augmented reality-based neuronavigation system?
8. Does the augmented reality-based neuronavigation system have any advantages over other educational materials?
9. Were you satisfied with the graphical interfaces of the augmented reality-based neuronavigation system?
10. How helpful do you think the augmented reality-based neuronavigation system would be in your learning process?
11. Were you satisfied with the accuracy of the information provided by the augmented reality-based neuronavigation system?
12. How comfortable does it feel to use the augmented reality-based neuronavigation system?
13. How suitable is the integration of the augmented reality-based neuronavigation system with educational materials?
14. What are your thoughts on the ergonomic design of the augmented reality-based neuronavigation system?
15. How would you rate the quality of the visual materials provided by the augmented reality-based neuronavigation system?
16. To what extent does the augmented reality-based neuronavigation system enhance your learning motivation?

Figure 3: Questions of the 20-question questionnaire asked neurosurgeons

RESULTS

Forty neurosurgery residents participated in the study, evaluating the contribution of an AR-based neuronavigation system to their training. Among the participants, 28 were male and 12 were female. Half of the residents were in the first three years of their neurosurgical residency, while the other half had completed 3–5 years of training.

Participants rated the system's impact on their motivation to learn with an average score of 8.4/10, indicating a positive influence on their engagement in surgical education. The system's overall ease of use received a rating of 7.6/10, and its ergonomic design was rated at 7.9/10. Additionally, the system was found to enhance the understanding of anatomical structures and improve anatomical mastery, earning an average score of 8.3/10.

Biostatistical analyses revealed that gender did not significantly influence the ease of use or anatomical mastery of the AR-based neuronavigation system. Analysis of variance (ANOVA) test results showed no significant gender differences in ease of use ($F=1.50$; $p=0.23$) or anatomical mastery ($F=0.46$; $p=0.50$). Similarly, a chi-square test examining the relationship between educational level and ease of use found no significant association ($\chi^2=1.30$; $p=0.86$). The educational level did not significantly impact the participants' evaluations of the system's usability.

These findings indicate that AR-based systems positively influence residents regardless of gender or education level. Nonetheless, the need for improvements in ergonomics and the quality of visual materials was highlighted to further enhance the user experience.

DISCUSSION

Neurosurgery is a challenging field that demands several skills and qualities from its practitioners. To succeed, neurosurgeons must undergo extensive training, devel-

op strong manual dexterity and hand–eye coordination, and make sound decisions (10, 11). Technological tools are essential in this educational process. The 3D visualisation of segmented MRI or CT images provides a clearer understanding of the surrounding anatomical structures, aiding in the selection of the best approach for treating the tumoral tissue (2, 12).

The findings of this study emphasise the positive effect of XR-based neuronavigation systems on medical education, particularly in boosting residents' motivation to learn and enhancing their understanding of anatomy. The high average scores for motivation (8.4/10) and anatomical mastery (8.3/10) show that AR-based systems are valuable tools in neurosurgical training, helping residents better visualise and understand complex anatomical structures. This supports existing research that highlights the benefits of 3D visualisation technologies in improving cognitive retention and practical skills in surgical education (12, 13).

Despite the clear advantages, the ergonomic design and ease of use of the system received moderate scores (7.6/10 for ease of use and 7.9/10 for ergonomics), indicating areas for improvement. Some participants raised concerns about the visual quality and comfort while using the system, that improving these aspects could enhance the overall educational experience. These results align with previous studies emphasising the importance of user-centred design in medical technologies, where both functionality and user comfort are crucial for optimal learning outcomes.

The biostatistical analysis revealed no significant gender differences in the system's ease of use or its contribution to anatomical understanding. This that XR-based systems are equally effective across different demographic groups, making them versatile tools in various educational environments. The ANOVA test showed no significant differences in ease of use ($F=1.50$; $p=0.23$) or anatomical mastery ($F=0.46$; $p=0.50$) between male and female participants, indicating that the system's benefits apply broadly.

In recent years, technology has become an increasingly significant factor in medicine, particularly with the rise of artificial intelligence applications. These systems support doctors by aiding in the interpretation of diagnostic images, such as identifying bleeding in a CT scan from the emergency room or detecting acute ischaemia in a diffusion MRI (14, 15). Furthermore, advancements in medical education have gained attention, with traditional fresh cadavers in anatomy laboratories being replaced by virtual cadavers in VR environments. Studies have shown that these 3D virtual environments improve the understanding of anatomical structures, reduce the reliance on cadavers, and lower costs due to their accessibility from any location (16-18). Technological advancements are expected to continue expanding, particularly in resident and medical education.

Although the present study is methodologically thorough, it has some limitations. The survey methodology is prone to inherent bias, particularly with the use of Likert scales, which may increase the risk of non-response and consent bias, potentially leading to inaccurate responses. These biases could affect the validity of the data, so caution is needed when interpreting the results.

CONCLUSION

In recent years, technology has become increasingly integrated into surgical assistant and postgraduate training. It has been demonstrated that 3D simulation of complex pathologies, using devices like the cost-effective Illumetry XR screen with ArSurgeon software developed by SimBT greatly enhances resident training by improving case orientation. This allows for better visualisation of tumours or pathological structures in relation to the surrounding anatomy, offering a more thorough understanding of the case.

In conclusion, while XR-based neuronavigation systems show great potential for enhancing surgical education, especially in terms of motivation and anatomical understanding, there are areas that still need improvement, particularly in ergonomics and visual design. Addressing these issues could further strengthen AR's role as a key educational tool in medical training. Future research should investigate the long-term impact of AR on learning retention and practical skill development, as well as its broader clinical applications.

Ethics Committee Approval: Ethics committee approval was received for this study from the İstanbul Faculty of Medicine (Date: 12.07.2024, No:13).

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

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Author Contributions: Conception/Design of Study- A.A., S.Ö., E.A., M.S.Ş., T.K., Ç.Ü.; Data Acquisition- A.A., S.Ö., E.A., M.S.Ş.; Data Analysis/Interpretation – A.A., S.Ö., E.A., M.S.Ş.; Drafting Manuscript- A.A., S.Ö., E.A., M.S.Ş.; Critical Revision of Manuscript- A.A., S.Ö., E.A., M.S.Ş., T.K., Ç.Ü.; Final Approval and Accountability- A.A., S.Ö., E.A., M.S.Ş., T.K., Ç.Ü.; Technical or Material Support – T.K., Ç.Ü.; Supervision- A.A., S.Ö.

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

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ROLE OF THE DUCTUS THORACICUS IN PULMONARY FAT EMBOLISM AFTER SUBARACHNOID HAEMORRHAGE: A PRELIMINARY EXPERIMENTAL STUDY

SUBARAKNOİD KANAMA SONRASI GELİŞEN PULMONER YAĞ EMBOLİSİNDE DUCTUS THORACICUS'UN ROLÜ: ÖN DENEYSEL ÇALIŞMA

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ABSTRACT

Objective: Subarachnoid haemorrhage is a multisystemic disease due to its effects on the autonomic nervous system. Pulmonary tissues may be affected due to its effects on the intestinal system, lipid metabolism, and ductus thoracicus in subarachnoid haemorrhage. This study demonstrate the pathophysiology of pulmonary fat embolism after sympathetic activation of the ductus thoracicus with lipid absorption disorders after subarachnoid haemorrhage.

Material and Methods: In the study, 24 male rabbits were used, of which five were selected as the control group (GI) and five as the SHAM group (GII). The SHAM group was injected with a 0.7 mL isotonic solution into the cisterna magna. The study group (GIII) was injected with 0.7 mL autologous blood into the cisterna magna. The lymphatic vessels in the middle sections of the duodenum, the thoracic duct portions opening into the jugular vein, and the terminal branches of the pulmonary arteries were examined and counted.

Results: The thoracic duct vasospasm index (VSI) values and numbers of branches occluded by fat particles in the pulmonary arteries were: 1.65±0.22/3.21±0.54 in GI, 1.97±0.34/7.3±2.4 in GII, and 2.54±0.56/14.53±14.53 in GIII. In the six subjects with severe thoracic duct spasm in GIII, the number of pulmonary arteries occluded with chylomicrons was higher (p>0.00001). P values among groups were: p > 0.05 in GI/GII; p<0.005 in GII/GIII and p<0.0001 in GI/GIII.

Conclusion: This is the first experimental study conducted on animals investigating the lipid metabolism disorder associated with subarachnoid haemorrhage affecting the cervical ganglia, thoracic duct spasm, and pulmonary fat embolism.

Keywords: Subarachnoid haemorrhage, thoracic duct, chylomicrons, pulmonary fat embolism

ÖZET

Amaç: Subaraknoid kanama, otonom sinir sistemi üzerindeki etkileri nedeniyle multisistemik bir hastalıktır. Subaraknoid kanamada intestinal sistem, lipid metabolizması ve duktus torasikus üzerindeki etkileri nedeniyle pulmoner dokular etkilenebilir. Bu çalışma, subaraknoid kanamadan sonra lipid emilim bozuklukları ile birlikte duktus torasikusun sempatik aktivasyonundan sonra pulmoner yağ embolisinin patofizyolojisini göstermeyi amaçlamıştır.

Gereç ve Yöntem: Çalışmada kullanılan 24 erkek tavşandan beşi kontrol grubu (GI), beşi SHAM (GII) olarak seçildi ve SHAM grubunda sisterna magnaya 0,7 mL izotonik enjekte edildi. Çalışma grubunda (GIII) ise sisterna magnaya 0,7 mL otolog kan enjekte edildi. Deneklerin duodenum orta kısımlarındaki lenf damarları, juguler vene açılan torasik duktus kısımları ve pulmoner arterlerin terminal dalları incelendi ve sayıldı.

Bulgular: Duktus torasikus vazospazm indeks (VSI) değerleri ve pulmoner arterlerde yağ parçacıkları tarafından tıkanan dal sayıları: GI'de 1,65±0,22/3,21±0,54, GII'de 1,97±0,34/7,3±2,4 ve GIII'te 2,54±0,56/14,53±14,53 idi. GIII'te şiddetli duktus torasikus spazmı olan altı denekte, şilomikronlarla tıkanmış pulmoner arter sayısı daha yüksekti (p>0,00001). Gruplar arasındaki p değerleri: GI/GII'de p>0,05; GII/GIII'te p<0,005 ve GI/GIII'te p<0,0001 idi.

Sonuç: Çalışmamız; subaraknoid kanama sonrası gelişen lipid metabolizması bozukluğunun, servikal ganglionlar, duktus torasikus spazmı ve pulmoner yağ embolisi ile ilişkisini araştıran ilk deneysel çalışmadır.

Anahtar kelimeler: Subaraknoid kanama, torasik duktus, şilomikronlar, pulmoner yağ embolisi

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INTRODUCTION

Subarachnoid haemorrhage is an important disease with intracranial and extracranial complications causing increased mortality and morbidity. Subarachnoid haemorrhage triggers an inflammatory process and causes multisystemic pathologies (1, 2). Subarachnoid haemorrhages affect the autonomic nervous system through the activation of the sympathetic system and cause extracranial complications (2). The most common site of extracranial complications is the pulmonary system, often presenting with neurogenic pulmonary oedema (3). Pulmonary oedema may present with non-specific findings such as dyspnoea, tachypnoea, tachycardia, and fever, which are commonly observed in the clinical findings of pulmonary fat embolism (4, 5). Fat embolism is a non-traumatic systemic disease with multiple causes, often occurring after orthopaedic trauma. Fat embolism results from the pathological processes of lipid production and transport in the intestinal tract. In the pathophysiology of pulmonary fat embolism, chylomicrons and the ductus thoracicus, which allows chylomicrons to enter the systemic circulation, are involved (5, 6). The ductus thoracicus is structurally 38-45 cm long and 2-5 mm in diameter, located between L2 and the lower cervical region. The sympathetic nervous system predominantly innervates this region. In a pathology such as subarachnoid haemorrhage, where the sympathetic response is dominant, an increase in the contraction of the ductus thoracicus is expected (7, 8). Pulmonary fat embolism may occur with the involvement of the ductus thoracicus accompanied by lipid metabolism disorders (5). In our study, we histopathologically examined the effect of subarachnoid haemorrhage on the cervical ganglia, lipid absorption changes in the intestines, and its impact on the ductus thoracicus and pulmonary tissues.

MATERIAL AND METHODS

A total of 24 rabbits, aged 2.5±0.1 years and weighing 4±0.5 kg, were used in the study. The principles of the "Guidelines for the Care and Use of Laboratory Animals" were applied. This study received ethics committee approval from the Atatürk University Local Ethics Council of Animal Experiments (Date: 18.08.2022, No: 166). Five subjects were randomly selected as the control group (GI), five as the SHAM group (GII), and 14 as the study group (GIII).

The SHAM group and the subjects included in the study group were first anaesthetised with 0.2 mL/kg isoflurane and subcutaneously with 50 mg/1.5 mL Ketamine HCL. General anaesthesia was maintained with the injection of 30 mg/1.5 mL Xylazine HCL and 1 mL distilled water. After the occipital-cervical region was prepared for the surgical conditions, the subject's head was hyperflexed, and the cisterna magna was accessed after the posterior notch of the foramen magnum was identified. Once it was confirmed that there was no bleeding by aspirating 1 mL of cerebrospinal fluid, 0.5 mL of isotonic solution was administered to the SHAM group, and 0.5 mL of autologous blood taken from the ear arteries was administered to the cisterna magna in the study group. Vital signs were monitored twice daily during the experiment. After three weeks of follow-up, the animals were euthanized under general anaesthesia with an intracardiac injection of 2 mL of 10% formalin solution. They were then decapitated at the level of the 7th cervical vertebra to examine the third vagal nerve networks and cervical sympathetic ganglia. The cervical, duodenal, and lung tissues were incubated in formalin solution for three days. To analyse the thoracic ducts, the supraclavicular regions were excised along with the surrounding tissues, including the subclavian vessels, axillary nerves, and thoracic ducts. The paravertebral deep soft tissues on both the right and left sides were removed together. The sympathetic ganglia and thoracic ducts were fixed in formalin and dissected with the help of an operating microscope for histological examination. Twenty consecutive 5-m sections were prepared from the tissue samples. They were stained with haematoxylin-eosin, oil red, and aldehyde fuchsin and examined under a light microscope. Data were analysed using a commercially available statistical software package SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Data analysis consisted of the Kruskal-Wallis and Mann–Whitney U tests. Differences were considered significant at $p<0.05$.

RESULTS

Histopathological analysis revealed stellate ganglion degeneration in the subjects of the subarachnoid haemorrhage model group (Figure 1). In this group, fat and protein droplets of lymphatic content were observed on the duodenal wall, particularly under the serosa, in subjects with spasms in the thoracic duct (Figure 2). Dilatation of lymphatic vessels, hydropic degeneration of endothelial cells, and enlargement of Peyer's patches were observed. Fluid accumulation was also detected around the blood vessels and within the lymphatic vessels. Swelling of the lymph nodes in these areas and dilatation of the distal lymphatic ducts were noted (Figure 3). In subjects who developed spasms in the region where the ductus thoracicus opens into the subclavian vein, luminal narrowing of the thoracic ducts, contraction of myocyte, and degeneration of endothelial cells were observed. High fatty components were detected in the thoracic ducts with spasms (Figures 4, 5). Finally, pulmonary tissue examination revealed lipid droplets in the lung tissues of the haemorrhage group (Figure 6). The thoracic duct vasospasm index (VSI) values and fat particle density counts in the pulmonary arteries were as follows: 1.65±0.22/3.21±0.54 in GI, 1.97±0.34/7.3±2.4 in GII, and 2.54±0.56/14.53±14.53 in GIII. Severe thoracic duct spasm was more common in GIII, affecting six subjects where fat cells were densely present in the pulmonary arteries. The P-values between the groups were as follows: $p>0.05$ between GI and GII, $p<0.005$ between GII and GIII, and $p<0.0001$ between GI and GIII (Table 1).

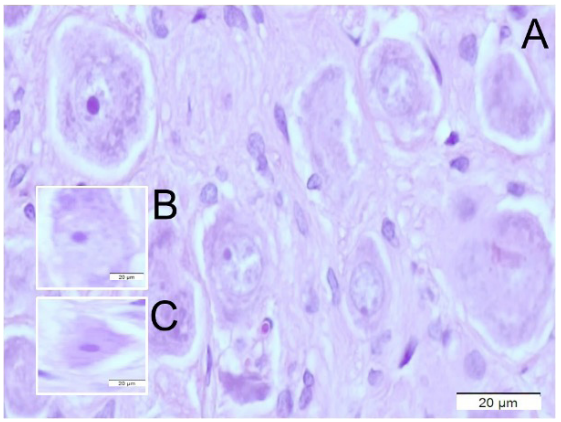

Figure 1: Normal stellate ganglion neurones belong to GI (A), moderately degenerated neurones in GII (B), and severely degenerated neurones are seen (C). Degenerated neurones have neurodegeneration criteria such as cellular angulation, shrinkage, nuclear halo formation and condensation (LM, H&E, x40)

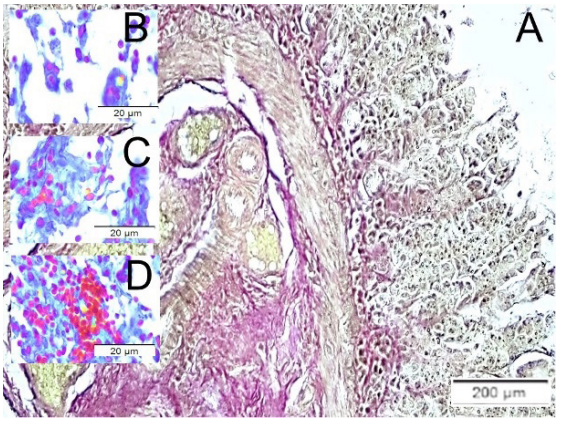

Figure 2: Duodenal histomorphology (A) and chylomicron droplets (Yellow/red) in GI (B); GII (C) and GIII (D) animals (LM, Oil Red, x4/A, x40/B-D)

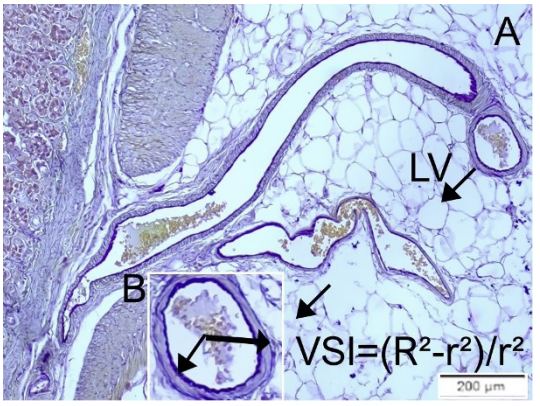

Figure 3: The calculation method of duodenal lymph vessels (LV) and vasospasm index (VSI) in a normal subject is followed (LM, Aldehyde Fuchscine, x4)

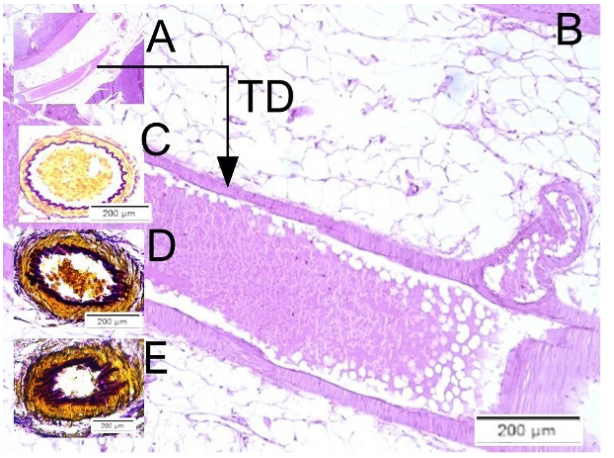

Figure 4: The longitudinal (A-B) and vertical section (C) of the thoracic duct of a normal subject, the vertical section of the thoracic duct of a subject belonging to the SHAM group (D), and the vertical section of the thoracic duct of a subject belonging to the study group are observed. Degenerated endothelial cells have neurodegeneration criteria such as cellular angulation, shrinkage, nuclear halo formation and condensation (LM, H&E/A; Aldehyde Fuchscine/C-E, x4)

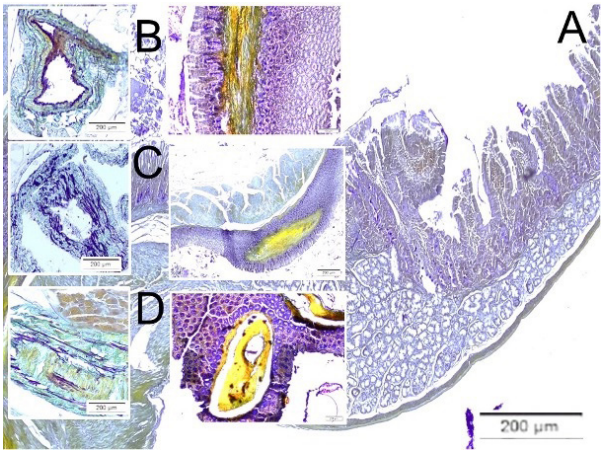

Figure 5: The histopathological structures of the thoracic duct sections (left column) and duodenal lymph channels (right column) in the GI (A-B), GII and GIII groups are observed. While minimal toric canal spasm and an LNF fluid lake in the duodenum wall are observed in GII (C); There is marked spasm in GIII and a significant lymphoid fluid collection in the duodenal wall (LM, Aldehyde fuchscine, x4)

DISCUSSION

Lymph fluid drains into the venous system through the lymphatic ducts, specifically the thoracic and right lymphatic ducts. The thoracic duct is the largest and is responsible for the lymphatic drainage of the entire body, except the head, neck, and right side of the chest. Its function is to return the lymph to the circulatory system. Structurally, the thoracic duct is 38–45 centimetres long and 2–5 millime-

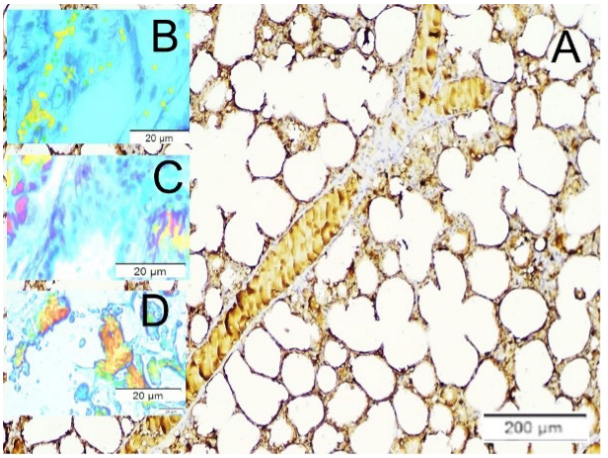

Figure 6: Pulmonary tissue (A) and chylomicron droplets (Yellow/Red) in GI (A-B); GII (C)and GIII (D) animals (LM, H&E/A; Oil Red/B-D, x40)

stimulation predominates (7, 8). In Telinius et al., electrical field stimulation increased contractions in the ductus thoracicus. Using a muscarinic receptor blocker (atropine) and an α-adrenoceptor blocker (phentolamine), they showed that sympathetic innervation has a significant effect on the ductus thoracicus, almost completely eliminating contractions caused by stimulation (8). The lungs are exposed to particles or toxic agents in the environment, and a robust lymphatic system is essential for protecting them. The lymphatic system is complex, consisting of collateral branches, lymphatic capillaries, local lymph nodes, lymph trunks, and larger ducts like the ductus thoracicus, all working together. Inflammatory processes can disrupt the lymphatic flow, causing impairment (11, 12). Chylomicrons are lipoprotein particles formed during the absorption of fats from the intestines. Fatty acids and monoglycerides absorbed from the intestine are converted into chylomicrons by enterocytes, which then enter the lymphatic system (13, 14).

Table 1: Comparison of thoracic duct vascular severity index (VSI), pulmonary artery fat particle density, and severe thoracic duct spasm results among the control (GI), SHAM (GII), and study (GIII) groups

	Thoracic duct VSI	Pulmonary artery fat particle density	Severe thoracic duct spasm (Number/Total)	P values compared to GI	P values compared to GII
GI (Control group)	1.65±0.22	3.21±0.54	0/5	-	-
GII (SHAM group)	1.97±0.34	7.3±2.4	0/5	p>0.05	-
GIII (Study group)	2.54±0.56	14.53±14.53	6/11	p<0.0001	p<0.005

VSI: Thoracic duct vasospasm index

tres in diameter, located between L2 and the lower cervical region. A typical anatomical description of the thoracic duct is found in approximately 50% of individuals, with the remainder displaying variations in anatomy. The thoracic duct most commonly (95%) terminates in the internal jugular vein, the subclavian vein, or at the junction between the two. It is often composed of a single vessel, although variations involving two or more vessels may occur (9). Lymph from the thoracic duct returns to the bloodstream through the lymphovenous junction. The lymphovenous valve regulates and protects the lymph flow, often being bicuspid to prevent the lymph from escaping back and the blood from entering the lymphatic system. However, a cadaveric study by O'Hagan et al. found this valve to be absent in approximately 30% of individuals, raising questions about its function (10). The ductus thoracicus is activated by both adrenergic (predominantly) and cholinergic components. It is known to carry lymph to the venous system through spontaneous contractions of the lymphatic vessel wall, independent of systemic effects, but the mechanisms of this coordination and modulation are not yet understood. In pathologies where the sympathetic system is triggered, increased venous flow in the lymph vessels is expected in the ductus thoracicus, where adrenergic

Chylomicrons travel from the small intestine through the ductus thoracicus into the lymphatic system, eventually entering the bloodstream. This process is critical for the efficient transport and distribution of fats and lipids throughout the body (15, 16). Fat embolism occurs when fat droplets or particles enter the bloodstream. This condition is often associated with trauma, surgical operations, adipose tissue damage, or inflammatory processes and can be fatal. Excessive fat in chylomicrons can lead to a pathological process, increasing free fatty acids and fat droplets, which may trigger the formation of fat emboli. These emboli, which are the remnants of chylomicrons or other lipid particles, can enter the bloodstream. Although chylomicrons play an essential role in fat transport, pathological conditions can link their metabolism to fat embolism. Although the mechanisms remain under investigation, they require further study (6, 17). Regardless of the pathophysiological mechanism, fat entering the circulation can result in fat embolism, causing clinical signs in various systems, including neurological, cardiac, and pulmonary. Fat embolism has many causes, including orthopaedic trauma (most common), liver injury, cardiopulmonary resuscitation, transplantation, liposuction, poisoning, and caesarean section

(6, 18, 19). Symptoms include respiratory (the most common), cardiac, neurological, and dermatological issues. The lack of specific laboratory tests and clinical findings makes diagnosing fat embolism challenging. Radiologically, it presents with capillary occlusion in the pulmonary and brain systems, leading to non-specific findings like petechial haemorrhages, oedema, and ground-glass opacities, which are common in other conditions affecting these systems. Fat embolism triggers an inflammatory process, but each underlying cause induces a unique inflammatory response. While the exact mechanism is unclear, it is likely that a fat embolism develops because of this inflammation (5, 6). Despite the complexity of fat embolism's pathophysiology and the lack of definitive diagnostic methods, some criteria have been proposed. Among these, the Gurd and Wilson criteria are the most widely used. The major criteria included respiratory distress, cerebral symptoms in non-head injury patients, petechial rash, renal and retinal changes, haemoglobin drop, new-onset thrombocytopenia, elevated erythrocyte sedimentation rate, and fat macroglobulinemia. Minor criteria included tachycardia (>110 bpm), fever (>38.5°C), and jaundice. The diagnosis can be made with two major criteria or one major and three minor criteria (6, 20). Pulmonary capillaries and small blood vessels are often the first to be affected, resulting in hypoxia, increased capillary permeability, and pulmonary oedema. However, the exact mechanism of this oedema remains unclear (5, 21). Subarachnoid haemorrhage is a well-known condition with significant morbidity and mortality. The most common extracranial complication is pulmonary oedema caused by sympathetic hyperactivity, but its pathophysiology and clinical presentation are not well understood (22). Neurogenic pulmonary oedema shares non-specific findings with pulmonary fat embolism, such as dyspnoea, tachypnoea, hypoxia, tachycardia, and fever (3). The prevalence of pulmonary embolism secondary to subarachnoid haemorrhage is unknown. However, according to Davidson et al., the clinical prevalence is 31%, while the autopsy prevalence is 78% (3). The most emphasised pathophysiological mechanism is the pulmonary venule response to adrenergic hypersensitivity (22). Sympathetic hyperactivity affects the cervical ganglia, increasing catecholamine levels, diverting blood from the systemic to the pulmonary circulation, and increasing pulmonary blood volume and capillary permeability. This dual impact on the central and pulmonary systems releases inflammatory mediators, triggering systemic immune responses (22-24). Studies have highlighted cervical ganglia's role in systemic inflammation following subarachnoid haemorrhage (25, 26). Zhang et al. showed that stellate ganglion blockade in subarachnoid haemorrhage patients reduced middle cerebral artery vasospasm and inflammatory mediator release (26). Limited studies exist on lipid profile changes in subarachnoid haemorrhage patients. Dhandapani et al. reported lipid peroxidation disorders and elevated triglyceride levels (27). Pilitsis et al. showed increased free fatty acid levels and vasospasm following subarachnoid haemorrhage (28).

This study has several limitations. First, although the sample size of 24 rabbits is sufficient for an experimental model, it is relatively small and may limit the generalizability of the findings. Second, while the rabbit model provides valuable insights, it may not fully replicate human physiological responses to subarachnoid haemorrhage and pulmonary fat embolism, limiting the direct applicability of the results to clinical settings. Third, the follow-up period was restricted to three weeks, and the longer-term effects of subarachnoid haemorrhage on lipid metabolism and pulmonary fat embolism were not assessed, leaving potential chronic changes unexplored. In addition, although the study demonstrated the pathophysiological role of the ductus thoracicus and sympathetic activation, no direct intervention, such as sympathetic blockade, was performed. Such interventions could have provided further clarity on the mechanisms behind the observed changes. Finally, the study primarily focused on histological findings, without incorporating functional assessments such as pulmonary function tests or direct measurements of lipid metabolism, which limits the clinical relevance of the histopathological outcomes.

CONCLUSION

Subarachnoid haemorrhage is an intracranial pathology, but it is also a systemic disease because it affects the autonomic nervous system and triggers inflammation. In our animal study, we demonstrated that the effects of the sympathetic system on the lipid profile and the ductus thoracicus after subarachnoid haemorrhage can influence pulmonary tissues, as observed in histological sections. This study on the pathophysiological mechanism should be further validated with studies involving cervical sympathetic ganglion blockade, whether through electrical, pharmacological, or surgical methods.

Availability of Data and Materials: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Committee Approval: Ethics committee approval was received for this study from the the Atatürk University Local Ethics Council of Animal Experiments (Date18.08.2022, No: 166).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- H.K., M.E.; Data Acquisition- H.K.; Data Analysis/Interpretation – H.K., M.E.; Drafting Manuscript- H.K.; Critical Revision of Manuscript- M.E.; Final Approval and Accountability- H.K., M.E.

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

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EVALUATION OF THE FALX CEREBRI FROM THE PERSPECTIVE OF THE FENESTRA AND ITS POSSIBLE CLINICAL OUTCOMES

FALX CEREBRİ'NİN FENESTRA PERSPEKTİFİNDEN DEĞERLENDİRİLMESİ VE OLASI KLİNİK SONUÇLARI

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ABSTRACT

Objective: The human falx cerebri is an important anatomical structure due to the hemispheres it is adjacent to and the dural venous sinuses it contains. It is also an important landmark in determining the midline in the interhemispheric transcallosal approach for lateral and third ventricular tumours in neurosurgical practises. Thus, the goal of this cadaveric study was to investigate the existence, number, and topography of fenestra on the falx cerebri in the Turkish population.

Material and Methods: For this study, 60 adult Turkish cadaveric dura maters were examined. The number of falx cerebris and the existence and topography of fenestra on the falx cerebri was determined. The length and width of the fenestra were measured using a digital compass.

Result: All falces cerebrum were single, and no double or triple falx cerebri were observed. There was fenestra on the falx cerebri in five cases (8.3% of all cases), and two of them included multiple foramina (%40 of all fenestrae). In addition, one fenestra was on the middle part of the falx cerebri, whereas the other was placed on the posterior part of this partition. The mean length and width of these fenestrae were 23.3x7.5 mm.

Conclusion: The novel findings documented in this study may be important to increase the success rate of diagnostic and operative procedures of the falx cerebri or adjacent structures and to minimise intraoperative complications during neurosurgical applications.

Keywords: Falx cerebri, fenestra, topography, variations, neurosurgical applications

ÖZET

Amaç: İnsan falx cerebri'si, komşu olduğu hemisferler ve içerdiği dural venöz sinüsler nedeniyle önemli bir anatomik yapıdır. Ayrıca, nöroşirürji uygulamalarında lateral ve üçüncü ventrikül tümörleri için interhemisferik transkallozal yaklaşımda orta hattın belirlenmesinde önemli bir landmarktır. Bu nedenle, mevcut kadavra çalışmasının amacı, Türk toplumunda falx cerebri üzerindeki fenestra varlığını, sayısını ve topografyasını araştırmaktı.

Gereç ve Yöntem: Bu çalışma için 60 yetişkin Türk kadavra dura mater'i incelendi. Falx cerebri sayısı, falx cerebri üzerinde fenestra varlığı ve fenestranın topografyası belirlendi. Fenestranın uzunluğu ve genişliği dijital kaliper kullanılarak ölçüldü.

Bulgular: Falces cerebrorum'un tamamı tekli olup, ikili veya üçlü falx cerebri gözlemlenmedi. Beş olguda (tüm olguların %8,3'ü) falx cerebri üzerinde fenestra vardı ve bunların ikisinde çoklu foramenler (tüm fenestraların %40'ı) vardı. Ayrıca fenestralardan biri falx cerebri'nin orta kısmında, diğeri ise bu bölümün arka kısmında yer alıyordu. Bu fenestraların ortalama uzunluğu ve genişliği 23,3x7,5 mm idi.

Sonuç: Bu çalışmada belgelenen yeni bulgular, falx cerebri veya komşu yapılara yönelik tanısal ve operatif prosedürlerin başarısını artırmak ve nöroşirürji uygulamaları sırasında intraoperatif komplikasyonları en aza indirmek için önemli olabilir.

Anahtar Kelimeler: Falx cerebri, fenestra, topografya, varyasyonlar, nöroşirürji uygulamaları

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INTRODUCTION

The falx cerebri, a sickle-shaped partition of the meningeal cranial dura mater, lies in the longitudinal fissure between the two cerebral hemispheres, with a narrow anterior end and a broad posterior part (1). This large fold is anteriorly fixed to the crista galli of the ethmoid bone and blends posteriorly with the upper surface of the tentorium cerebelli (1, 2). The convex superior margin of the falx cerebri is attached to the internal cranial surface on either side of the midline, and this attachment runs as far posteriorly as the internal occipital protuberance (1, 2). The superior sagittal sinus runs along a slight cranial groove in the midsagittal plane, and the falx cerebri is attached to the lips of this groove. The lower free edge of the falx lying on the corpus callosum is concave and contains the lower sagittal sinus (1, 3).

Anatomical variations of the falx cerebri are very rare. These variations include complete ossification of the human falx cerebri, absence or total agenesis of the falx cerebri, agenesis of the anterior falx cerebri, and fenestrated falx cerebri (4-10).

The falx cerebri is an important neurosurgical landmark that helps to find the midline of the cranial cavity in the anterior interhemispheric transcallosal approach in the excision of cerebral tumours (10, 11). Therefore, fenestrations of the falx cerebri may confuse in finding the midline (7). Additionally, fenestrations in the falx cerebri, for example after head trauma, may be misinterpreted by radiologists or neurosurgeons as resulting from head trauma (11). Another beneficial function of the falx cerebri is thought to act as a barrier for subdural haemorrhage to cross the midline (8). Hence, a large fenestration may allow potential subdural haematomas to cross the midline.

Because it restricts the movement of the brain and reduces deformation along the midline, the shape, rigidity, and position of the falx cerebri are important (12). It has been reported that the falx cerebri may cause strain, especially on the corpus callosum, during impact (13). Therefore, it has been emphasised that changes in the dimensions of the falx can change the stress distribution within the skull (14).

Although previous studies provide a baseline for the understanding and identification of fenestrations on the falx cerebri, research studies investigating fenestrated falx cerebri are very scarce (15, 16). In that context, the aim of this anatomical study was to analyse the existence, number, and topography of the fenestra on the falx cerebri in the Turkish population, which could be important for neurosurgeons and neuroradiologists.

MATERIAL AND METHODS

The present study included a sample of 60 adult Turkish cadaveric dura maters (age and sex were unknown) fixed with a formaldehyde-phenol-glycerine-ethanol mixture in the Department of Anatomy at İstanbul University, İstanbul Faculty of Medicine. None of the cases had any evidence of gross pathology or previous surgical procedures. The exclusion criteria were visibly injured, ruptured falx cerebri, and falx cerebri with pathology or deformity. The number of falx cerebri, the presence of fenestra and the topography of fenestra on the falx cerebri was determined. The length and width of the fenestra were calculated. Additionally, the falx cerebri height (FCH) was measured as the shortest perpendicular distance from the distal point of the great cerebral vein to the lateral border of the superior sagittal sinus (Figure 1). A single author performed the measurement three times to provide intraobserver reliability, and the mean value per parameter was recorded in the final calculation of the statistics. A digital calliper accurate to 0.01 mm (INSIZE Co., Ltd., Taiwan) was used for the measurement. The morphometric parameters were noted in mm. The Clinical Research Ethical Committee of İstanbul Faculty of Medicine approved the study (IRB (Date: 09.08.2024, No: 15).

Statistical analysis

IBM SPSS Statistics version 21.0 was employed for the acquired data evaluation and analysis (IBM SPSS Corp., Armonk, NY, USA). Descriptive statistics for categorical variables were presented as frequencies (n) and percentages (%), while continuous variables were summarised using means and standard deviations (SD) or medians with corresponding ranges (minimum-maximum), depending on the distribution of the data. Since there were no two independent group variables such as gender in our data set, comparisons of the falx cerebri measurements could not be made.

RESULTS

No duplications or abnormalities in the falx cerebri were found, and the dural venous sinuses did not exhibit struc-

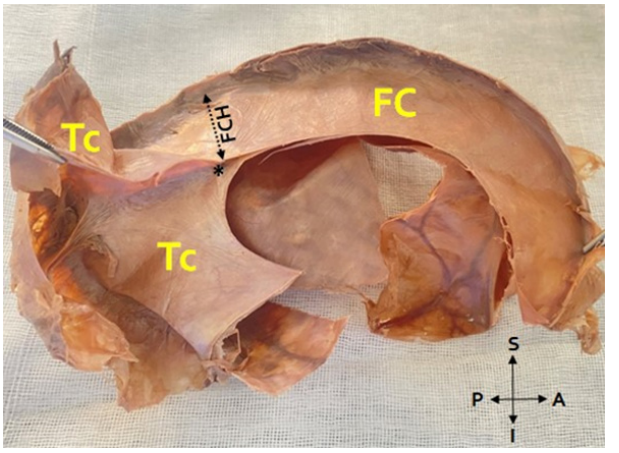

Figure 1: The falx cerebri height (FCH), falx cerebri (FC), tentorium cerebelli (Tc), anterior (A), posterior (P), superior (S), inferior (I), and distal point of the great cerebral vein (*)

tural anomalies, such as hypoplastic transverse sinuses, variations in the superior sagittal sinus (SSS), or occipital sinus abnormalities. The mean FCH was 53±5.02 mm in 60 dura maters. Fenestra were observed on the falx cerebri in five cases (8.3% of all cases) (Figure 2 a, b; Figure 3 a, b, c), and two of these fenestrae had multiple foramina (Figure 3 b, c) (40% of all fenestrae). One fenestra was located on the middle part of the falx cerebri (Figure 3 a), while the other was situated on the posterior part of this fold (Figure 2 b). The mean length and width of these fenestrae were 23.3x7.5 mm (ranged from 7.8x2.8 mm to 40.3x5.9 mm). The detailed morphological and morphometric data of the falx cerebri are shown in Table 1.

DISCUSSION

Isolated variations of the falx cerebri are extremely rare findings. These variations are usually in the form of fenestration or hypoplasia developing in the anterior part of the falx cerebri. In this paper, all falces cerebrum were single. We did not find publications that reported double or triple falx cerebri in a population in our country or elsewhere. On the other hand, numerous cases, such as bifrontopolar subdural haematoma and absence of the falx cerebri, complete ossification of the human falx cerebri, total agenesis of the superior sagittal sinus and falx cerebri, missing falx, agenesis of the anterior falx cerebri, total agenesis of the

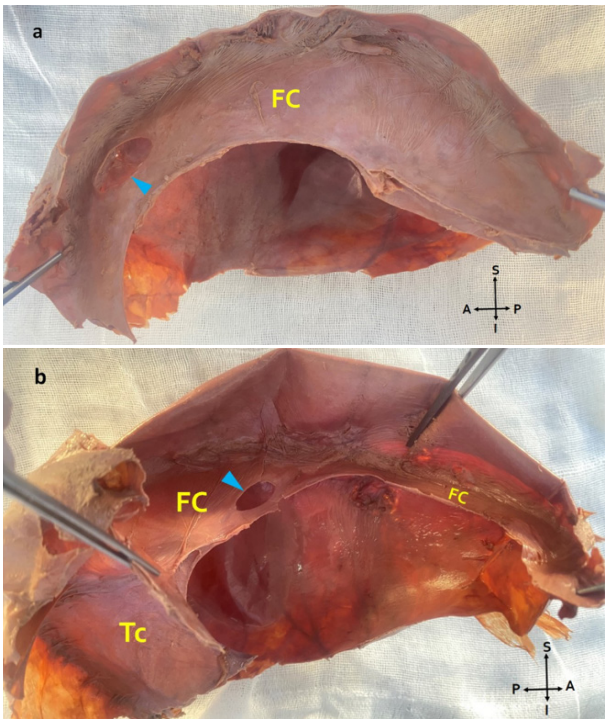

Figure 2: a) Blue arrowhead shows a fenestration located on the anterior part of the falx cerebri **b)** Blue arrowhead indicates a fenestration located on the posterior part of the falx cerebri: falx cerebri (FC), anterior (A), posterior (P), superior (S), inferior (I)

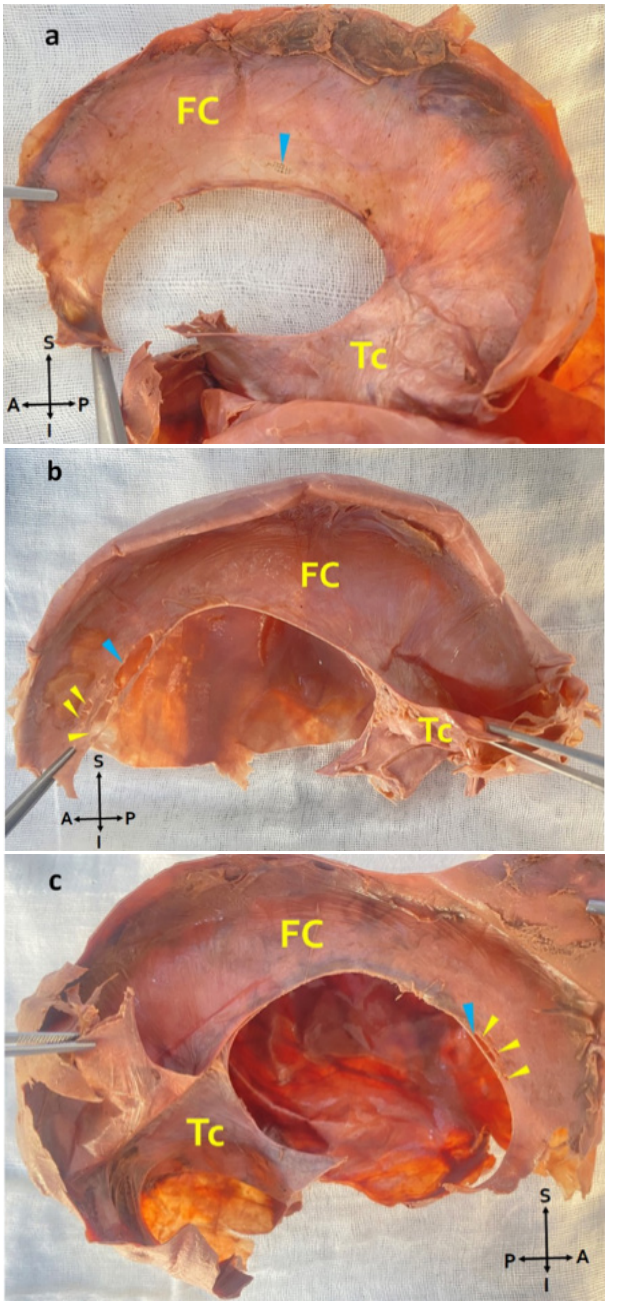

Figure 3: a) Blue arrowhead shows a fenestration located on the middle part of the falx cerebri. **b** and **c)** Blue and yellow arrowheads indicate a fenestration including multiple foramina located on the anterior part of the falx cerebri: falx cerebri (FC), tentorium cerebelli (Tc), anterior (A), posterior (P), superior (S), inferior (I)

falx cerebri with narrowing of the superior sagittal sinus, and fenestrated falx cerebri and additional sinuses in the tentorium cerebelli, have been previously reported in the literature (4-10). Mossman has well shown that falx cerebri and superior sagittal sinus anomalies coexist, as their embryological development is interlinked (17).

Table 1: Detailed morphologic and morphometric properties of the falx cerebri

Cadaver No	The number of falx cerebri	Fenestra(s) on the falx cerebri	Height of the falx cerebri (mm)
1	single	no	46.9
2	single	no	45.1
3	single	no	36.9
4	single	no	46.8
5	single	no	40.4
6	single	no	48.5
7	single	no	41.3
8	single	yes	48.5
9	single	no	43.4
10	single	no	53.3
11	single	no	48
12	single	no	49.9
13	single	no	43.6
14	single	no	44.9
15	single	no	41.9
16	single	no	39.4
17	single	no	49.2
18	single	no	42
19	single	no	40.5
20	single	no	40.1
21	single	no	51.2
22	single	no	46.7
23	single	no	46.5
24	single	no	48.5
25	single	no	41.9
26	single	no	42.6
27	single	no	49.7
28	single	no	45.3
29	single	no	40.6
30	single	no	52.3
31	single	no	39.2
32	single	no	46
33	single	no	55.5
34	single	no	55.1
35	single	no	50.5
36	single	no	52.1
37	single	Yes, included multiple foramina	44.1
38	single	yes	46.2
39	single	yes	57
40	single	no	47.9
41	single	no	53.2
42	single	no	47.3
43	single	no	54.4
44	single	no	41.6
45	single	no	40.2
46	single	no	49
47	single	no	49.5
48	single	no	39
49	single	no	51.2
50	single	no	55.4
51	single	no	41.4
52	single	no	47.8
53	single	no	49.5
54	single	no	50.6
55	single	Yes, included multiple foramina	46.4
56	single	no	50
57	single	no	48
58	single	no	44.1
59	single	no	35.1
60	single	no	48.6

mm: millimetre

Galligioni et al. evaluated 200 selected adult carotid angiograms in which the inferior longitudinal sinus was visualised (18). Because this sinus was located in the posterior two-thirds of the free margin of the falx, they used the position of this sinus to measure the height of the falx cerebri at different points. They determined three lines, A, B, and C, starting from the tuberculum sellae and extending to the inner side of the skull from front to back. Line A defined the linear line extending to the inside of the skull at the bregma. Line B represents the

linear line drawn from line A at a 30-degree angle and extending to the inside of the skull. Line C refers to the linear line crossing the proximal end of the great cerebral vein extending to the inside of the skull. They named the distances (height of the falx cerebri) extending from the points where these lines intersect the inferior longitudinal sinus (the free lower edge of the falx cerebri) to the inside of the skull as lines a, b, and c from anterior to posterior. They reported that these heights varied from 28 to 48 mm at the anterior (a), 41 to 62 mm at the middle (b), and 40 to 62 mm at the posterior points (c).

In their study performed on 52 adult Turkish cadaver specimens, Kayalioglu et al. classified the height of the falx cerebri into 3 types based on the study of Jiang and Jia (15, 16). They found that the mean falx cerebri height from its free edge towards the splenium of the corpus callosum was 45.6±3.6 mm in type I, 47±4.7 mm in type II, and 44.1±2.7 mm in type III. Their findings are close to those presented in research by Dausacker, performed on cadaveric specimens (19).

Staquet et al. investigated the height of the falx cerebri in 40 anonymous brain CT scans (14). They determined this height as the part of a linear line starting from the midpoint of the clinoid process, passing through the tentorial apex, and extending to the upper edge of the falx cerebri, between the tentorial apex and the upper edge of the falx cerebri. Consequently, they reported that the height of the falx cerebri varied between 29 and 48 mm. The average height (FCH) was 46.53±5.07 mm in our study, and the measurement is compatible with the results reported by Galligioni et al., Kayalioglu et al., and Staquet et al. (14, 16, 18).

The height of the falx cerebri is important in the resection of tumours of the region (16). Consequently, we believe that our mean FCH value may guide a safe surgical approach to the great cerebral vein, the beginning of the straight sinus, or the end of the inferior sagittal sinus. In addition, FCH may be beneficial for neurosurgeons for treating lesions involving the relevant region.

The last edition of Gray's Anatomy stated that the anterior part of the falx cerebri may have some irregular perforations (1). Jiang and Jia, in their study, conducted on 100 Chinese foetus cadaveric specimens, observed natural defects in 31% of their cases. The length and width of the defects varied from 0.6x0.3 to 2.7x1.4 cm, most often approximately the size of a bean. They also pointed out that other types consisting of smaller and scattered peasized spaces or even sieve-like spaces might be detected (15).

Kayalioglu et al. reported natural defects in 12 (23%) of their specimens (16). In their study on Turkish specimens, the sizes of the defects ranged from 0.31x0.18 cm to 1.7x0.7 cm. In both studies, most defects were on the anterior part of the falx cerebri, and most were round or oval in shape. Nayak and Vasudeva, in their report on a case of fenestrated falx cerebri and additional sinuses in the tentorium cerebelli, determined that the anterior part of the falx cerebri (4 cm behind the crista galli) had fenestrations and looked like a mesh (10). We observed fenestra in five cases (8.3% of all cases) on the falx cerebri (Figure 2 a, b; Figure 3 a, b, c), and two of the fenestrae included multiple foramina (40% of all fenestrae) (Figure 3 b, c). The vast majority of fenestrations (60%) were located on the anterior part of the falx cerebri, with two exceptions, one on the middle (20%) (Figure 3 a) and the other on the posterior part (20%) (Figure 2 b). The mean length and width of the fenestrae were 23.3x7.5 mm (ranged from 7.8x2.8 mm to 40.3x5.9 mm). Our results do not correspond to those presented in the investigations by Jiang and Jia and Kayalioglu et al. (15, 16). The different number of specimens and/or ethnicity may influence the different results.

Although the rate of fenestrae in this study was less than that in previous studies, it was obtained at a significant rate (8.3%). Interestingly, unlike previous studies, the shape of nearly half of the fenestrae (40%) resembled a mesh-like structure. In this context, radiologists may misinterpret fenestrae and that knowledge of the unusual topography of the fenestrae in the posterior part of the falx cerebri may prevent diagnostic confusion. For example, a patient with a fenestration who presents to the emergency room with a head injury may have their radiographs mistakenly considered as being due to head trauma and may be subject to unnecessary applications.

Crucial vascular networks and functionally significant neural structures surround tumours in the lateral and third ventricles. The microsurgical excision of these tumours necessitates meticulous preoperative planning. The interhemispheric transcallosal approach is also one of the surgical options for the excision of these tumours. The falx cerebri serves as an important landmark in determining the midline in the interhemispheric transcallosal approach for lateral and third ventricular tumours (11). The fenestrations may challenge the identification of the midline, both at the onset and during surgery, increasing the risk of inadvertent injury to critical neurovascular structures and vital brain regions (7, 9). Because the sizes of the fenestrae in this study were larger than those in previous studies, we believe that larger fenestrae may make midline orientation seriously difficult, and accordingly, the risk of developing surgical complications may increase. In addition, as larger fenestrae on the falx cerebri were observed in the current study, subdural haemorrhage may easily cross the midline, and potential misdiagnosis may occur.

This study has some limitations; There was no data on the clinical presentation of our cases. If they had, we can show whether fenestration was associated with a particular clinical presentation.

CONCLUSION

In this study, we investigated the morphology of the falx cerebri and documented its isolated variations in the Turkish population. The sizes of the fenestrae were larger, and one fenestra was located posteriorly on the falx cerebri, which was different from those of previous studies. Fenestrations may complicate midline identification in the transcallosal approach for lateral and third ventricular tumours, heightening the potential for unintentional damage to key neurovascular structures and essential brain areas. Additionally, radiologists or neurosurgeons may misinterpret it as developing due to head trauma.

Ethics Committee Approval: Ethics committee approval was received for this study from the İstanbul Faculty of Medicine (Date: 09.08.2024, No: 15).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- L.S., Ö.G.; Data Acquisition- L.S., M.B.; Data Analysis/Interpretation – O.C., A.K.; Drafting Manuscript- L.S.; Critical Revision of Manuscript- A.K.; Final Approval and Accountability- L.S., M.B., Ö.G., O.C., A.K., A.Ö.; Technical or Material Support- L.S., M.B.; Supervision- A.Ö., A.K.

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

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MOWAT-WILSON SYNDROME: DEEP PHENOTYPING AND MOLECULAR CHARACTERISATION OF TWELVE NEW INDIVIDUALS

MOWAT-WİLSON SENDROMU: ONİKİ YENİ OLGUNUN FENOTİPİK VE MOLEKÜLER KARAKTERİZASYONU

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ABSTRACT

Objective: Mowat-Wilson syndrome (MOWS) is a rare multisystem malformation syndrome characterised by distinctive facial features, moderate to severe intellectual disability, and variable findings including callosal anomalies, ocular features, genital anomalies, congenital heart defects, and Hirschsprung's disease. Pathogenic variants in the *ZEB2* gene are implicated in the aetiology, with nearly all cases arising sporadically due to *de novo* variants. In addition to its low prevalence, the broad clinical spectrum observed among patients can make the diagnostic process challenging. This study aims to expand the clinical and molecular spectrum of MOWS by elucidating the characteristics of a new cohort.

Material and Methods: Twelve patients with a clinical diagnosis of MOWS were included in the study. Following obtaining normal karyotype results, molecular analysis of *ZEB2* was performed using Sanger sequencing.

ÖZET

Amaç: Mowat-Wilson sendromu (MOWS); tanınabilir yüz özellikleri, orta-ağır zihinsel yetersizlik ve korpus kallozum anomalileri, oküler tutulum, genital anomaliler, konjenital kalp defektleri ve Hirschsprung hastalığı gibi multisistemik bulgularla karakterize nadir bir malformasyon sendromudur. Etiyolojide *ZEB2* genindeki patojenik varyantlar rol oynamakta olup, neredeyse bütün vakalar sporadik olarak *de novo* varyantlardan kaynaklanmaktadır. Hastalığın düşük prevalansının yanı sıra etkilenmiş olgularda gözlenen geniş klinik spektrum tanı sürecini zorlaştırabilmektedir. Bu çalışmada yeni bir MOWS kohortu tanımlayarak bu hastalığın klinik ve moleküler spektrumunu genişletmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya MOWS klinik tanısı almış 12 olgu dahil edildi. Normal karyotip sonuçlarının elde edilmesinin ardından *ZEB2* geni Sanger dizi analizi yöntemiyle incelendi.

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Results: Anthropometric measurements at birth and subsequent visits largely aligned with the national and MOWS growth charts, respectively. All patients exhibited moderate to severe intellectual disability and shared a characteristic facial gestalt. In addition to the well-described features, very rare or previously undescribed abnormalities comprising persistent left superior vena cava, choanal stenosis, shawl scrotum, and ocular anomalies were observed. Skin pigmentation defects were noted at significantly higher frequencies than those previously reported. Two patients displayed atypical features overlapping with CHARGE and Aicardi syndromes. We identified 12 heterozygous variants in *ZEB2*, five of which were novel.

Conclusion: Deep phenotyping data of 12 patients enabled the identification of previously uncertain clinical associations and underrepresented features. The novel pathogenic variants identified here expand the molecular spectrum of *ZEB2*.

Keywords: Mowat-Wilson syndrome, *ZEB2*, intellectual disability, CHARGE syndrome, Aicardi syndrome

Bulgular: Doğum ve tekrarlayan klinik değerlendirmelerde alınan antropometrik ölçümlerin ulusal ve MOWS'a özgü büyüme eğrileriyle büyük ölçüde uyumlu olduğu gözlendi. Tüm hastalarda orta ila ağır zihinsel yetersizlik ve karakteristik yüz görünümü mevcuttu. Hastalığın iyi bilinen bulgularına ek olarak; persistan sol süperior vena kava, koanal stenoz, şal skrotum ve atipik oküler anomaliler gibi çok nadir veya daha önce MOWS spektrumunda tanımlanmamış bulgular izlendi. Pigmentasyon bozukluklarının literatür verisine kıyasla belirgin şekilde daha yüksek sıklıkta olduğu gözlendi. İki hastada, CHARGE ve Aicardi sendromları ile örtüşen atipik klinik bulguların varlığı dikkat çekiciydi. Hastalarda, *ZEB2* geninde beşi daha önce tanımlanmamış olmak üzere 12 heterozigot varyant tespit edildi.

Sonuç: On iki hastanın derin fenotipleme verileri, daha önce MOWS ile klinik ilişkisi net şekilde ortaya konulmamış veya hastalık spektrumunda çok nadir olduğu düşünülen klinik bulguların tanımlanmasını sağladı. Ayrıca, bu çalışma kapsamında ilk kez bildirilen patojenik *ZEB2* varyantları ile hastalığın moleküler spektrumu da genişletilmiş oldu.

Anahtar Kelimeler: Mowat-Wilson sendromu, *ZEB2*, zihinsel yetersizlik, CHARGE sendromu, Aicardi sendromu

INTRODUCTION

Mowat-Wilson syndrome (MOWS, MIM #235730) is a rare, dominantly inherited malformation syndrome characterised by recognisable facial features, moderate-to-severe intellectual deficit, and variable multisystem involvement comprising corpus callosum anomalies, ocular features, genital anomalies, congenital heart defects, and Hirschsprung's disease (1). MOWS is caused by heterozygous loss-of-function variants in the "zinc finger E-box binding homeobox 2 (*ZEB2*)" gene (MIM# 605802) (2, 3). The majority of causative *ZEB2* variants identified to date are truncating variants or large deletions resulting in haploinsufficiency (4). Although nearly all cases arise sporadically due to *de novo* variants, familial recurrence has been described in four families, suggesting low-level somatic or putative germline mosaicism (5-8). The prevalence of MOWS is estimated to be 1 in 50,000 to 70,000 live births, although some authors propose that the prevalence may be higher because milder cases may remain undiagnosed (9, 10).

The phenotypic spectrum of MOWS is well characterised through genetically confirmed patient series (9, 11-17). A comprehensive review of 344 molecularly confirmed cases by Ivanovski et al. revealed that the most common clinical features observed in patients with MOWS are seizures, microcephaly, callosal anomalies, hypospadias, and congenital heart defects (4). Although Hirschsprung's disease and postnatal-onset short stature were initially considered cardinal features, they have been reported in less than half of the affected individuals (4, 18, 19).

ZEB2 encodes the 1214-amino acid-long Smad-interacting protein 1 (SIP1), an evolutionarily conserved member of the two-handed zinc finger and homeodomain-containing protein family. It functions as a transcriptional repressor by interacting with activated SMAD proteins (20). SIP1 plays a critical role in various stages of vertebrate embryogenesis, with knockout mouse model studies revealing several functions, including signal regulation during corticogenesis and the modulation of BMP-Smad and Wnt-β-catenin pathways involved in central nervous system (CNS) myelination (21-23). In humans, *ZEB2* is localised to the 2q21-q23 region, and the canonical transcript (NM_014795.4) consists of 10 exons spanning 136 kb. Nearly half of the known *ZEB2* pathogenic variants are found in exon 8. The N-terminal region of the ZEB2 protein (NP_055610.1) contains five C2H2-type zinc finger domains (ZnF_C2H2), four of which are encoded by exon 6 and one by exon 8. These are followed by a homeobox domain also encoded by exon 8 and a cluster of three ZnF_C2H2 domains encoded by exons 9 and 10 (24).

No standardised diagnostic criteria have been established for MOWS to date. The recognisable facial appearance is the most reliable handle for the clinical diagnosis, except in mildly affected patients harbouring missense or specific splice-site (5'UTR) variants (25-27). The distinctive facial features associated with MOWS include hypertelorism, medial flaring of the eyebrows, low-hanging columella, deeply-set eyes, long and pointed chin, an open mouth, and centrally depressed-uplifted earlobes. While facial appearance tends to evolve with age, the unique ear configuration, likened to orecchiette pasta or red blood cells, remains a constant finding throughout life (15).

In this study, we investigated a cohort of 12 MOWS patients with deep phenotyping and *ZEB2* sequencing to

further expand the clinical and molecular spectrum of MOWS.

MATERIAL AND METHODS

Twelve patients with MOWS were included in the study based on clinical evaluations by medical geneticists or paediatric geneticists from four national centres. Growth centiles were assessed following both the national guidelines and MOWS growth charts (28, 29). In all cases, high-resolution chromosome analysis was performed to exclude gross chromosomal abnormalities.

DNA isolation for the sequencing of *ZEB2* (NM_014795.4) was performed using a commercial kit according to the instructions of the manufacturer (DNA Isolation Kit for Mammalian Blood, Roche). Nine coding exons (exon 2-10) of *ZEB2* and the flanking intronic regions were amplified by PCR and sequenced using the Sanger technique (ABI3500). The human genome assembly GRCh38/hg38 was used as the reference genome.

Ethical approval for this study was provided by the Istanbul Faculty of Medicine, Clinical Research Ethics Committee (Date: 07.10.2022, No: 18), in accordance with the Helsinki Declaration. Informed written consent for genetic testing and the use of clinical photographs was obtained from the legal guardians.

RESULTS

This cohort included 12 unrelated patients diagnosed with MOWS, comprising eight males and four females. The age at the last clinical assessment ranged between 12 months and 10 years 3 months. The clinical data are described below and summarised in Table 1.

Antenatal and natal findings

The prenatal period was unremarkable in the majority, except for abortus imminens in two cases, severe hyperemesis gravidarum in one case, and polyhydramnios of unknown aetiology in another. One individual was born at the late preterm period, 36 weeks of gestation, and the remaining were born at an average of 38^{+3} weeks. The standard deviations (SDs) corresponding to the mean birth weight and birth length of the cohort were 0.20±0.6 and 0.47±0.31, respectively. Occipitofrontal circumference (OFC) measurements at birth were within normal limits in the three patients where it was available. One patient developed prolonged neonatal jaundice. No neonatal complications requiring neonatal intensive care unit support were reported in any of the patients.

Growth

At the last clinical evaluation between 12 months and 10 years 3 months (mean: 47.8 months), the mean SD values for weight, length/height, and OFC according to the national growth charts were -1.37±0.46 (range -2.43 to 0.53), -1.31±0.59 (range -3.49 to 0.32), and -2.89±0.59 (range -0.60 to -4.58), respectively. Two patients had OFC values within the normal range, and the remaining patients developed postnatal microcephaly, borderline (between -2/-3 SD) in four and true (below -3 SD) in six. Short stature was observed in two patients.

Craniofacial features

The dysmorphic features observed in all patients included a long face, medially flared and broad eyebrows, and a long chin (Figure 1). Other consistent facial features were characteristic ear shape (n=11/12), hypertelorism (n=10/12), deeply-set eyes (n=10/12), wide nasal root (n=10/12), low-hanging columella (n=10/12), prominent or bulbous nasal tip (n=7/12), open-mouth appearance (n=7/12), thick or prominent lower lip vermilion (n=7/12), and short philtrum (n=7/12).

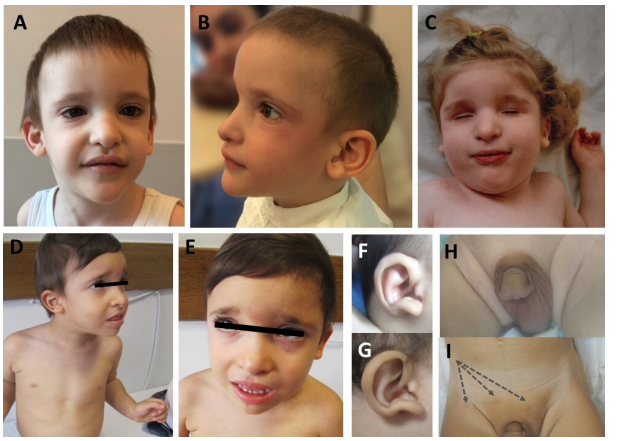

Figure 1: Clinical photographs of individuals with Mowat-Wilson syndrome. Facial features of Patient 8 at 2 years 10 months (A, B), Patient 9 at 3 years 1 month (C), and Patient 4 at 3 years 7 months (D, E). All three patients exhibited an elongated face, broad and medially flared eyebrows, deeply set eyes, low-hanging columella, full lips, short philtrum, and prominent chin. Notably, Patient 8 also displayed a prominent forehead (B), and both Patient 8 and Patient 9 had ears with uplifted lobules (B, C). Ear photographs of Patient 12 taken at 2 months (F) and 19 months (G) illustrate the characteristic ear configuration, likened to orecchiette pasta or red blood cells. Atypical findings observed in Patient 12 included hyperpigmented shawl scrotum (H), and hyperpigmented spots along with wide hypopigmented patches of skin (dashed arrows) (I). Note that the shawl scrotum appearance regressed over time, while the pigmentation anomalies became more pronounced

Neurological findings, development, and behavioural features

All 12 patients exhibited global developmental delay and/or intellectual deficit, severe in five patients, moderate to severe in four, and moderate in three. The mean

Table 1: Clinical and molecular overview of the cohort

Patient	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Antenatal and natal history												
Prenatal findings	-	-	abortus imminens	-	abortus imminens	hyperemesis gravidarum	-	polyhydramnios	-	-	-	-
Gestational week at birth	39	40	37	40	39	39	38	38	40	36	39	37
Length at birth [SD]	50 cm [-0.05]	NA	49 cm [0.35]	51 cm [0.28]	50 cm [-0.05]	NA	50 cm [0.21]	51 cm [0.74]	NA	50 cm [1.1]	NA	51 cm [1.15]
Weight at birth [SD]	3450 g [0.28]	3000 g [-0.87]	3150 g [0.72]	3150 g [-0.84]	3350 g [0.02]	3000 g [-0.82]	3700 g [1.24]	3250 g [0.15]	2750 g [-1.58]	3350 g [1.68]	3400 g [0.43]	3820 g [2.02]
OFC at birth [SD]	NA	NA	NA	33 cm [-1.68]	34 cm [-0.73]	NA	NA	NA	NA	NA	NA	34 cm [-0.08]
Neurological and behavioural features												
Epilepsy (onset)	+ (2 yrs 6 mos)	-	-	-	+ (2 yrs)	-	-	-	+ (2 yrs)	+ (9 mos)	-	-
Pathological EEG pattern	epileptiform activity	epilep-tiform activity	diffuse spike and slow wave discharges	NA	bilateral central spike discharges with a normal background	-	multifocal spike-and-wave discharge	-	bilateral frontotemporal spike and wave discharges	slowing of background activity with frequent generalised bursts	-	-
Callosal anomalies	ACC	-	DCC	-	-	ACC	-	-	CH	-	NA	CH
Additional structural anom-alies on MRI	-	-	-	-	-	-	CSP	benign tonsillar ectopia	-	Reduced hippocampal volume on the left, deep pa-rieto-occipital white matter hyperinten-sities on T2 and FLAIR sequences	NA	-

Table 1: Continued

Patient	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Neurological and behavioural features												
Additional neurological anomalies	-	ataxic gait	-	-	-	-	-	-	-	ataxic gait	-	Oral motor dysfunction
Abnormal behavioural features	oral behaviours, motor stereotypies (hand biting, chin slapping)	stereotypic hand movements, hyperorality	-	happy demeanour	happy demeanour	lack of eye contact	-	-	oral behaviours, motor stereotypies (hand biting), episodic laughter	-	-	-
Gastrointestinal findings												
Hirschprung disease (HSCR)	+	-	-	-	+	+	+	-	-	-	-	-
Chronic constipation without HSCR	-	+	-	+	-	-	-	-	-	+	-	-
Congenital cardiac defects	PPS	-	ASD	-	small perimembranous VSD	PPS	ASD	PDA, VSD, PLSVC, PHT	-	bicuspid aorta	-	bicuspid aorta PDA, PFO
Genitourinary findings												
Renal abnormalities	-	NA	-	-	-	-	-	-	-	transient pelviectasis	grade I pelviectasis (L)	pelvicalicial ectasia (L)

Table 1: Continued

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Genitourinary findings												
Genital abnormalities	hypospadias, cryptorchidism (R)	-	-	hypospadias, cryptorchidism (blt)	hypospadias	Cryptorchidism (blt)	-	hypospadias	-	hypospadias	-	hypospadias, cryptorchidism (R), minimal hydrocele (L), chordee, shawl scrotum with severe hyperpigmentation
Ophthalmologic abnormalities	microphthalmia (L), microcornea	-	-	-	astigmatism, myopia	-	-	peripapillery atrophy	microphthalmia and aniridia (blt), congenital cataracts (L), retinal atrophy	esotropia (L), congenital retinal atrophy	-	iris coloboma (R), nasolacrimal duct obstruction
History of recurrent infections	frequent otitis media	-	-	frequent upper respiratory tract infections and otitis media		-	transient hypogammaglobulinemia of infancy	-	-		-	-
Other	fibroma in the occipital subcutaneous region	hypersalivation	prolonged neonatal jaundice	hypopigmented spots, low-set ears, delayed eruption, genu valgum	precocius puberty, accessory nipple (R), hypopigmented skin lesions	nail hypoplasia, positional anomalies of toes	hypopigmented macules around the abdomen	broad and deep philtrum, pes valgus	-	torticollis	-	1 CAL spot, choanal stenosis, hypopigmented macules
Age at last examination	3 yrs 6 mos	5 yrs 6 mos	3 yrs 5 mos	3 yrs 7 mos	10 yrs 3 mos	24 mos	22 mos	2 yrs 10 mos	3 yrs	9 yrs 4 mos	12 mos	19 mos
Height (cm) [SD]	97.5 [-0.73]	103 [-1.93]	99 [0.14]	98 [-0.74]	118 [-3.49]	81 [-1.86]	83 [-0.93]	92 [-0.89]	85 [-2.60]	125 [-1.58]	76 [0.32]	79 [-1.37]
Weight [SD]	14 [-1.0]	14.5 [-2.07]	16 [0.53]	14.5 [-0.79]	28 [-1.03]	9.55 [-2.43]	10.5 [-1.32]	13.2 [-0.74]	11.4 [-1.74]	20.9 [-2.39]	8.05 [-1.26]	9.02 [-2.14]
Occipitofrontal circumference [SD]	47 cm [-2.49]	45 cm [-4.03]	47 cm [-1.64]	47 cm [-2.52]	47 cm [-4.58]	45 cm [-2.81]	45.7 cm [-2.19]	49 cm [-0.60]	44 cm [-3.11]	48 cm [-3.60]	41.5 cm [-3.35]	43 cm [-3.7]

Table 1: Continued

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Genitourinary findings												
Height, weight and OFC centiles according to MOWS charts (Ivanovski et al., 2020)	75p, 50-75p, 25-50p	25-50p, 25p, 5-25p	75-95p, 75-95p, 50-75p	50-75p, 50-75p, 25-50p	5-25p, 50-75p, 25-50p	25-50p, 5-25p, 25-50p	50-75p, 25-50p, 50p	75p, 50-75p, >95p	5-25p, 25p, 5p	50-75p, 25p, 25p	75-95p, 25-50p, 5-25p	25-50p, 5-25p, 5-25p
Achievement of developmental milestones												
Unsupported sitting	21 mos	NA	9 mos	11 mos	11 mos	20 mos	10 mos	9 mos	25 mos	NA	8 mos	14 mos
Unsupported walking	-	4 yrs	-	2 yrs	3 yrs	-	-	18 mos	-	4 yrs	-	-
Speech and language skills	none	vocalises random sounds	2-3 words	babbles	builds 2-3 word sentences	none	babbles	3-4 words in total	babbles	none	none	none
Bowel/bladder control	-	-	-	-	-	-	-	-	-	-	-	-
Intellectual Disability/Global Developmental Delay	severe	moderate to severe	moderate to severe	severe	moderate	severe	moderate to severe	moderate	severe	moderate	moderate to severe	severe
Dysmorphic facial features												
Elongated face	+	+	+	+	+	+	+	+	+	+	+	+
Broad or medially flared eyebrows	+	+	+	+	+	+	+	+	+	+	+	+
Hypertelorism	+	+	+	+	-	+	+	+	-	+	+	+
Deeply set eyes	+	+	-	+	+	+	-	+	+	+	+	+
Downslanting palpebral fissures	-	-	-	-	-	+	-	-	-	-	+	-
Wide nasal bridge	+	+	+	+	-	+	+	+	+	+	-	+
Prominent or bulbous nasal tip	+	+	-	+	+	+	-	+	-	+	-	-
Columella extending below the ala nasi	+	-	+	+	+	-	+	+	+	+	+	+
Open-mouthed expression	+	+	+	+	+	+	-	-	-	+	-	-

Table 1: Continued

Patient	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Dysmorphic facial features												
Thick or everted lower lip vermillion	+	+	-	+	+	-	+	+	-	-	-	+
Short philtrum	+	-	-	+	+	-	+	+	+	+	-	-
Prominent/ pointed chin	+	+	+	+	+	+	+	+	+	+	+	+
Typical ear configuration	+	+	+	-	+	+	+	+	+	+	+	+
ZEB2 variant [NM_014795.4]	c.2908 C>T	c.575 dupA	c.930 C>A	c.1337_ 1340delCTTT	c.2254 dupA	c.2083 C>T	c.2585 T>A	c.2266 delC	c.1027 C>T	c.310 C>T	c.540 delT	c.2335 delA
Exon	exon 9	exon 5	exon 8	exon 8	exon 8	exon 8	exon 8	exon 8	exon 8	exon 3	exon 5	exon 8
Amino acid change (NP_055610.1)	p.(Gln 970*)	p.(Asn192 Lysfs*7)	p.(Tyr 310*)	p.(Pro-446Hisfs*7)	p.(Thr752 Asnfs*4)	p.(Arg 695*)	p.(Leu 862*)	p.(Pro756 Leufs*5)	p.(Arg 343*)	p.(Gln 104*)	p.(Glu181 Argfs*31)	p.(Arg779 Glyfs*8)
Genomic position [hg38] (NC_000002.12)	g.144396 571G>A	g.14440 4852 A>AT	g.144400 257G>T	g.144399847 delAAAG	g.1443989 32G>GT	g.144399 104G>A	g.144398 602A>T	g.144398 920delG	g.144400 160G>A	g.144429 790G>A	g.144404 888delA	g.144398 852delT
Novelty	VCV00037 3064.3 (Clin-Var)	novel	VCV00016 7857.4 (ClinVar)	novel	VCV0026 87506.1 (ClinVar)	PMID: 33510600	novel	novel	PMID: 31376723	PMID: 19006215	VCV00268 7499.1 (ClinVar)	novel

SD: Standard deviation scores, NA: not applicable, OFC: occipitofrontal circumference, yrs: years, mos: months, ACC: agenesis of the corpus callosum, DCC: dysgenesis of the corpus callosum, CH: callosal hypoplasia, CSP: cavum septum pellucidum, HSCR: Hirschprung disease, PPS: peripheral pulmonary stenosis, blt: bilateral, ASD: atrial septal defect, VSD: ventricular septal defect, PDA: patent ductus arteriosus, PLSVC: persistent left superior vena cava, PHT: pulmonary hypertension, PFO: patent foramen ovale, L: left, R: right, CAL: café au lait, p: percentile

age at unsupported sitting was 13.6 months (range 5-25 months). When patients who were over 18 months of age were considered, five out of eleven could walk unsupported at a mean age of 34.8 months (range 18-48 months). Of the five patients who could walk, two had a wide-based, ataxic-like gait. The remaining patients who could not walk were younger, with the age at the last examination varying between 19 and 42 months. Language deficit was particularly pronounced: patients had absent or severely restricted speech, except a 10-year 3-month-old patient who could build short sentences. No patient achieved bowel or bladder control.

All patients except one underwent brain MRI. The most common CNS anomaly was callosal abnormalities (n=5/11). Additional structural brain abnormalities were cavum septum pellucidum, benign tonsillar ectopia, and decreased volume of the left hippocampus with signal alterations in the parietooccipital white matter.

Four patients experienced seizures (n=4/12), with onset between 6 and 30 months, which were successfully controlled with anti-seizure medicine (ASM). In patient 10, an electroencephalogram (EEG) performed after seizure presentation showed mild slowing of background activity with frequent generalised bursts, suggestive of epileptic encephalopathy. His seizures were severe and frequently necessitated hospitalisation in the beginning, but he responded well to ASMs and showed amelioration with time. Sleep EEG demonstrated abnormal brain activity including diffuse spike and slow wave discharges in three of seven patients without seizures.

Behavioural features included repetitive oral-motor behaviour (n=3/12), self-injurious behaviour (n=2/12), happy demeanour (n=2/12), hyperorality (n=1/12), stereotypic hand movements (n=1/12), paroxysmal laughter (n=1/12), and poor eye contact (n=1/12).

Gastrointestinal anomalies
Gastrointestinal issues were present in the form of biopsy-proven Hirschsprung disease in four patients (n=4/12) and chronic constipation without an observable defect in ganglion function in three patients (n=3/12). One individual had swallowing difficulties due to neurological deficits.

Cardiac anomalies
Eight patients showed cardiac abnormalities in the form of peripheral pulmonary stenosis (n=2/12), atrial septal defect (n=2/12), ventricular septal defect (n=2/12), patent ductus arteriosus (n=2/12), bicuspid aorta (n=2/12), persistent left superior vena cava (n=1/12), and pulmonary hypertension (n=1/12).

Genitourinary abnormalities
The genitourinary abnormalities observed in eight patients included hypospadias (n=6/8), cryptorchidism (n=4/8), transient pelviectasis (n=3/12), shawl scrotum (n=1/8), hydrocele (n=1/8), and chordee (n=1/8).

Ophthalmological anomalies
Ocular abnormalities were observed in six patients as microphthalmia (n=2/12), retinal atrophy (n=2/12), microcornea (n=1/12), aniridia (n=1/12), congenital cataracts (n=1/12), peripapillary atrophy (PPA) (n=1/12), strabismus (n=1/12), iris coloboma (n=1/12), nasolacrimal duct obstruction (n=1/12), and astigmatism with myopia (n=1/12).

Immunological problems
Immunological findings were observed in the form of transient hypogammaglobulinemia of infancy (n=1/12), recurrent otitis media (n=2/12), and upper respiratory infections (n=1/12) in three patients.

Additional findings
Additional findings comprised precocious puberty (n=1/12), hypersalivation (n=1/12), occipital fibroma (n=1/12), torticollis (n=1/12), hypopigmented macules (n=3/12), café au lait spot (n=1/12), genu valgum (n=1/12), accessory nipple (n=1/12), nail hypoplasia (n=1/12), position abnormalities of the toes (1/12), pes valgus (n=1/12), choanal stenosis (n=1/12), and delayed teeth eruption (n=1/12).

***ZEB2* variants**
We identified 12 heterozygous truncating variants in *ZEB2*. These include six nonsense variants and six small indels leading to the disruption of the reading frame. Five variants were previously unreported in the literature. Eight variants were located within exon 8, two in exon 5, and one each in exons 3 and 9. Parental studies were only pursued for Patient 9 and Patient 12 by Sanger sequencing of the relevant exon and did not reveal any pathogenic variants. The molecular results are detailed in Table 1.

DISCUSSION

Here we describe a further 12 patients with a definitive clinical and molecular diagnosis of MOWS due to five novel and seven previously reported pathogenic *ZEB2* alterations.

Because all patients of the cohort were recruited through a gestalt diagnosis approach, they all shared the characteristic facial appearance comprising an elongated face with medially flared and broad eyebrows and a long chin (Figure 1). Characteristic ear shape with uplifted lobules, hypertelorism, deeply-set eyes, wide nasal root, and low-hanging columella were other consistent features observed in more than 80% of the cohort. These findings are consistent with previous, larger cohorts that elaborated the facial findings of the MOWS phenotypic spectrum (15). The facial characteristics of MOWS have been shown to temporally evolve from childhood to adulthood

(15). In infancy, the face is square shaped with a broad nasal bridge. As affected individuals get older, the face tends to elongate as the jaw becomes more prominent, eyebrows become heavier, the nasal tip lengthens and becomes depressed, and the columella overhangs the philtrum. Uplifted earlobes remain observable at all ages. Since our cohort included only three patients over the age of five, with the oldest patient being ten years of age, we were unable to evaluate the temporal evolution of the facial phenotype.

The mean gestational age at delivery of the cohort was similar to that of the general population. Except for one patient with a birth weight of -2.02 SD, all patients had a birth weight appropriate for gestational age with no significant differences between males and females. Length and OFC at birth were also within normal ranges. In the postnatal period, two patients developed short stature, and 10 out of 12 were microcephalic according to the national growth curves. However, when growth charts specifically designed for MOWS were applied, height, weight, and OFC measurements were generally within the normal range, except for one patient whose OFC was above the 95th percentile (29). In general, our growth findings are consistent with the existing notion that children with MOWS typically exhibit normal birth length and weight with a slightly smaller OFC compared to age peers and experience delayed postnatal growth, particularly in OFC, after the age of one (29).

Seizures have been reported in 78.5% of individuals with MOWS, with 25.9% exhibiting resistance to ASMs (4). Four patients in this study experienced seizures, and none were refractory to treatment with ASMs. The lower frequency of epilepsy observed in the cohort may be attributed to the small sample size and the relatively young age of the patients, limiting our ability to draw definitive conclusions. Of note, Patient 9, who presented with infantile spasms at six months of age, displayed features reminiscent of Aicardi syndrome, including dysgenetic corpus callosum and retinal anomalies. However, upon further evaluation, we concluded that these manifestations represented a severe presentation within the phenotypic spectrum of MOWS.

Callosal defects were the most prevalent form of CNS involvement in the cohort, consistent with previous reports (30). Additional anomalies included cavum septum pellucidum, benign tonsillar ectopia, decreased volume of the left hippocampus, and signal alterations in the parieto-occipital white matter in individual cases. A comprehensive study on neuroimaging findings in MOWS revealed that hippocampal abnormalities are the second most common type of CNS involvement, occurring in 79.6% of cases, followed by reduced white matter thickness in 40.7% and localised signal alterations in 22.2% (30). The low frequency of these specific brain findings in our cohort may be attributed to the lack of targeted imaging protocols because most MRI scans were performed before the definite diagnosis of MOWS and did not focus on its characteristic abnormalities.

Global developmental delay and/or cognitive impairment was a universal finding in this study, and speech was particularly affected. A range of behavioural phenotypes observed in the cohort was found to be consistent with the literature data, including oral behaviours, a happy demeanour/laughter for no apparent reason, and stereotypic hand movements (4, 31). Two patients exhibited self-injurious behaviour, which may partially be attributed to underreaction to pain, another behavioural characteristic of MOWS.

Various rare ophthalmologic findings were observed in the cohort. These include aniridia and peripapiller atrophy (PPA), which have been reported only once, and the hitherto undescribed iridodialysis and nasolacrimal duct obstruction (32, 33). Although microphthalmia is generally considered to be very rare in MOWS, two patients in our study exhibited microphthalmia (4). A similar cohort of 15 Chinese patients also had two cases of microphthalmia, while three additional cases were reported in a cohort of 28 (6, 34). Furthermore, one patient in our study had PPA, which was also reported in a previously published case of MOWS that lacked molecular confirmation and detailed clinical description (33). Collectively, these observations suggest that the frequency of microphthalmia may be higher than previously anticipated and that aniridia and PPA could be rare ocular anomalies associated with MOWS.

Patient 12 presented with a constellation of clinical features that extended beyond the core gestalt of MOWS, suggesting an overlap with CHARGE syndrome. These features included choanal stenosis, iris coloboma, congenital heart defects (patent ductus arteriosus, patent foramen ovale, bicuspid aorta), developmental delay, hypospadias, cryptorchidism, and renal pelvis dilation. To the best of our knowledge, patient 12 represents the third documented patient with a CHARGE-mimicking MOWS phenotype (14). Notably, this patient also exhibited nasolacrimal duct obstruction and a shawl scrotum, findings previously undescribed in either disorder.

Four patients exhibited skin pigmentation defects, including patchy hypopigmentation, café-au-lait spots, and scrotal hyperpigmentation during infancy. Although depigmentation has previously been associated with MOWS, it is considered rare among affected individuals (10). The high frequency observed in this study suggests that skin involvement, particularly hypopigmented patches, may be an overlooked finding that is more common in MOWS than previously thought.

All patients reported here were found to harbour nonsense or frameshift variants, the two most common intragenic alterations underlying MOWS. A milder clinical phenotype has been associated with missense variants in MOWS; however, no such cases were identified in this cohort to allow further exploration of this genotype-phenotype correlation (14, 25, 27). Eight of the 12 variants identified in our study were located in exon 8, consistent with previous reports. Parental segregation studies were not performed, as almost all reported MOWS cases to date are due to *de novo* mutations with nearly complete penetrance, except for very rare instances of low-level somatic or presumed germline mosaicism (5-8).

Our study has some limitations. The relatively small sample size, due to the rarity of MOWS, may limit the generalizability of our findings. Additionally, molecular analyses were limited to *ZEB2* sequencing, and further genetic studies in patients with atypical findings could have provided additional insights into other genetic contributors or modifiers.

CONCLUSION

This study increases the number of patients with *ZEB2* mutations in the literature. Our deep phenotyping data enabled us to identify previously uncertain clinical associations and novel or potentially underrepresented features, including several ocular anomalies (colobomas, PPA, microphthalmia), choanal atresia, skin pigmentation defects, and shawl scrotum, most of which likely constitute an expansion of the MOWS phenotype. Further cohorts may provide additional insights into MOWS by revealing new specific phenotypes associated with the syndrome and expand the *ZEB2* molecular landscape, allowing for more precise genotype-phenotype correlations.

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EVALUATION OF GROWTH IN LONG-TERM FOLLOW-UP IN INDIVIDUALS WITH PHENYLKETONURIA

FENİLKETONÜRİLİ BİREYLERDE UZUN SÜRELİ TAKİPTE BÜYÜMENİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: The aim of our study was to evaluate the anthropometric parameters of patients with Phenylketonuria (PKU) receiving special nutritional therapy and how these parameters changed over time, and to evaluate anthropometric measurements in the early years of life in terms of predicting the development of overweight/obesity later in life.

Material and Methods: In this single-centre, long-term, observational, and descriptive study, 102 individuals with phenylketonuria who were diagnosed in the first two months of life and initiated nutritional treatment were included.

Results: The ratios of overweight and obesity were found to be lower in our patient group compared with the general population in both evaluations. The weight-for-age (p=0.000), height-for-age (p=0.000), weight-for-height (p=0.000), and body mass index (p=0.052) of the patients decreased compared with the evaluation at the baseline of the follow-up period. Forty-six patients had a change in their anthropometric evaluations.

Conclusion: Monitoring anthropometric measurements and body composition changes in all patient groups receiving specialised nutritional therapies is critical in determining and monitoring treatment efficacy. The reason for the low ratios of obesity and overweight in our patient group compared with the healthy population is thought to be the close monitoring of nutritional therapies and anthropometric parameters of the patients in the follow-up.

Keywords: Phenylketonuria, nutrition therapy, body mass index, obesity, overweight

ÖZET

Amaç: Çalışmamızın amacı özel beslenme tedavisi alan Fenilketonüri (PKU) hastalarının antropometrik indekslerini ve bu indekslerin zaman içerisinde nasıl değiştiğini incelemek, hayatın erken yıllarındaki antropometrik ölçümleri hayatın ilerleyen dönemlerinde aşırı tartılılık/obezite gelişimi öngörüsü açısından değerlendirmekti.

Gereç ve Yöntem: Tek merkezli, uzun dönemli, gözlemsel ve tanımlayıcı çalışmaya, yaşamın ilk iki ayında tanı alarak beslenme tedavisine başlanmış 102 fenilketonürili birey dâhil edilmiştir.

Bulgular: Hasta grubumuzda yapılan her iki değerlendirmede de aşırı tartılılık ve obezite oranları genel topluma kıyasla düşük bulunmuştur. Hastaların boya göre tartı (p=0.000), yaşa göre tartı (p=0.000), yaşa göre boy (p=0.000) ve beden kitle indekslerinde (p=0.052) izlem sürecinin başındaki değerlendirmeye kıyasla azalma tespit edilmiştir. Kırk altı hastanın antropometrik değerlendirmelerinde değişiklik gözlenmiştir.

Sonuç: Özel beslenme tedavileri alan tüm hasta gruplarında antropometrik ölçümlerin ve vücut kompozisyonu değişikliklerinin izlemi tedavi etkinliğinin belirlenmesi ve izleminde kritik öneme sahiptir. Hasta grubumuzda obezite ve aşırı tartılılık oranlarının sağlıklı topluma kıyasla düşük olmasının nedeninin izlemde hastaların beslenme tedavilerinin ve antropometrik parametrelerinin yakından izlenmesi olduğu düşünülmüştür.

Anahtar Kelimeler: Fenilketonüri, beslenme tedavisi, vücut kütle indeksi, obezite, aşırı tartı

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INTRODUCTION

Phenylketonuria (PKU; OMIM 261600) is an inherited metabolic disorder characterised by a deficiency of the enzyme phenylalanine hydroxylase, which converts the amino acid phenylalanine (Phe) to tyrosine. The specialised nutritional therapy involved in the main treatment of the disease has three pillars; (a) regulation of natural protein intake according to individual Phe tolerance, (b) administration of a synthetic protein constructed from Phe-free amino acids and (c) use of low-protein specialised nutritional products (1). Phe-free amino acid mixtures comprise essential and non-essential amino acids other than Phe, carbohydrates and fats as energy sources, in addition to vitamins, minerals and trace elements. The protein content of low-protein foods is reduced, and the proportion of energy derived from carbohydrates and fat in these foods is increased compared to that derived from natural foods. Specialised nutritional therapies are used to achieve this, using diets in which the energy provided by carbohydrates and fats is higher than that in a healthy diet (1). There are concerns that these specialised nutritional therapies may lead to an increased prevalence of overweight and obesity in patients with PKU in the long term (2).

Since 1975, the global prevalence of overweight has almost tripled (3). Abdominal obesity is associated with dyslipidemia, hypertension, insulin resistance, and inflammation (4). In the long-term, it has been suggested that non-communicable diseases such as non-insulin-dependent diabetes mellitus, musculoskeletal disorders, lung diseases, and cancer may be linked to these changes in body composition (5, 6).

The aim of our study was to investigate the anthropometric indices of weight-for-age (WFA), height-for-age (HFA), weight-for-height (WFH) and body mass index (BMI) of PKU patients receiving special nutritional therapy and how these indices changed over time, and to evaluate anthropometric measurements in the early years of life in terms of predicting the development of overweight/obesity later in life.

MATERIAL AND METHODS

This single-centre, long-term, observational, and descriptive study was conducted between October 2023 and June 2024 in the Department of Paediatric Nutrition and Metabolism, Istanbul University, Istanbul Faculty of Medicine. The cohort consisted of PKU patients who were born at term, diagnosed in the first 2 months of life and receiving nutritional treatment. Patients with a history of small for gestational age (SGA) and other conditions that could affect normal physical development were excluded from the study. The İstanbul Faculty of Medicine Clinical Research Ethics Committee approved the study (Date: 22.09.2023, No: 19). This study was conducted in accordance with the ethical standards of the responsible institutional committee on human experimentation and with the Helsinki Declaration. Informed consent was obtained from patients or their legal guardians after the procedure had been fully explained.

Patients with Phe levels >1200 µmol/L at initial diagnosis or Phe tolerance <20 mg/dl at follow-up were classified as classic PKU (cPCU), whereas patients with Phe levels 600-1200 µmol/L at initial diagnosis or Phe tolerance >20 mg/dl at follow-up were classified as having mild PKU (mPKU). All patients received nutritional therapy consisting of a low-Phe diet and a Phe-free L-amino acid mixture since diagnosis.

Data obtained from the volunteers included age, sex, diagnostic classification, anthropometric indices of WFH, WFA, HFA, and BMI during the first 5 years of life and 9 years after the initial assessment. The first anthropometric measurements were obtained from the patients' medical records, and the second anthropometric measurements were taken using standardised techniques, without shoes and in light clothing. It was planned to measure body weight and height from the anthropometric measurements and to calculate the anthropometric indices of WFA, HFA, WFH and BMI from these data. BMI was calculated as weight (kg)/height^2 (m^2). For participants over 18 years of age, BMI <18.5 kg/m^2 was considered as underweight, 18.5-24.9 kg/m^2 as normal, 25-29.9 kg/m^2 as overweight, and >30 kg/m^2 as obese (7). Between the ages of 5 and 18 years, a BMI z-score below -2 was considered underweight, between -2 and 1 was considered normal, between 1 and 2 was considered overweight, and above 2 was considered obese (8). For the first 5 years of life, BMI was considered underweight if it was below the 5th percentile, normal if it was between the 5th and 85th percentiles, overweight if it was between the 85th and 95th percentiles and obese if it was above the 95th percentile (9).

The mean, standard deviation, median, minimum, maximum, frequency and ratio values were used in the descriptive statistics of the data. We evaluated the distribution by the Kolmogorov–Smirnov test. Paired samples t-test was used in the analysis of quantitative-dependent data. The Wilcoxon test was used to analyse qualitative-dependent data. The SPSS 28.0 (IBM SPSS Corp., Armonk, NY, USA) program was used in the analyses.

RESULTS

In the patient group, 54 of 102 participants were female (52.9%) and 48 were male (47.1%). The mean age of the patients was 15.3±3.0 (median: 14.8; range: 10.2-22.3) years. Eighty patients (78.4%) were followed up with a diagnosis of classical PKU and 22 patients (21.6%) with mild PKU. The mean follow-up period of patients was 14.9±3.9 (median: 14.5; range: 10.1-22.0) years.

The data related to WFH, WFA, HFA, and BMI of the overall patient group at the beginning and end of the follow-up period are presented in Table 1. At the end of the follow-up period, a statistically significant reduction was found in the mean values of WFH, HFA, and WFA z-score. In the first assessment, there were no underweight patients according to BMI, while the proportion of normal, overweight, and obese patients were 65%, 18.6%, and 17.6%, respectively. In the second assessment, the proportions of underweight, normal, overweight, and obese patients according to BMI were 2.9%, 59.8%, 28.4%, and 8.8%, respectively. The difference between the rates of underweight, normal weight, overweight and obese patients was not statistically significant (p=0.140). The anthropometric data of 10 patients initially classified as normal weight exhibited characteristics compatible with overweight at the subsequent evaluation. The anthropometric data of the two patients initially classified as normal weight were found to be compatible with obesity at the subsequent evaluation. The anthropometric data of two patients who were initially classified as overweight at the first evaluation were found to be compatible with the criteria for obesity at the second evaluation. Twenty-three patients had decreased BMI z-scores during the second evaluation; Nine patients initially classified as obese exhibited an overweight status at the subsequent evaluation. Four patients initially classified as obese were subsequently determined to have a normal weight at the second evaluation. Seven patients initially classified as overweight were subsequently found to have a normal weight at the subsequent evaluation. Three patients initially classified as normal weight were subsequently identified as underweight at the second evaluation.

The anthropometric parameters of the patients according to BMI percentiles in the first five years of age according to gender and anthropometric parameters according to BMI z-scores 9 years after the first evaluation are presented in Figure 1. There was a statistically significant decrease in the mean WFH, WFA, HFA, and BMI z-scores in the girls during the follow-up period. In boys, a statistically significant reduction was found in the mean WFH, WFA, and HFA z-scores during the follow-up period. Although there was a decrease in the mean BMI z-score, statistical significance was not found (Table 2).

Anthropometric evaluations of the patients according to BMI percentiles in the first five years of age according to disease classification and anthropometric evaluations according to BMI z-scores 9 years after the first evaluation are presented in Figure 2. No statistically significant change was found in the mean WFH, WFA, HFA, and BMI z-scores of mPKU patients. However, the z-scores of all anthropometric indices (WFH, WFA, HFA, and BMI) decreased. A statistically significant decrease was found in the mean WFH, WFA, HFA, and BMI z-scores of cPKU patients during the follow-up period (Table 3).

DISCUSSION

Nutritional therapies are the primary treatment option for many inherited metabolic diseases, including PKU. It has long been hypothesised that the Phe-restricted diet followed by patients with PKU may lead to abdominal obesity and metabolic syndrome, which are chronic non-communicable diseases associated with nutrition in the long term (10, 11). The determination and monitoring of body composition changes in PKU plays a fundamental role in nutritional assessment and evaluation of the efficacy of nutritional therapy (12).

In the cohort of 102 patients enrolled in our study, there was a reduction in the mean z-score of WFA, HFA, WFH and BMI after nine years of follow-up. However, the mean z-scores were within the healthy reference range. According to the data obtained at the baseline and at the end

Table 1: Anthropometric data at the baseline and end of the follow-up period in all patient groups, anthropometric evaluation according to BMI

	WFH		WFA		HFA		BMI z-score		BMI evaluation			
	mean±SD	median	mean±SD	median	mean±SD	median	mean±SD	median	Underweight (n-%)	Normal (n-%)	Overweight (n-%)	Obese (n-%)
Baseline evaluation	0.8±1.1	0.8 (-1.8 - 4.4)	0.6±1.0	0.6 (-2.1-3.6)	0.1±1.0	0.1 (-2.0-2.2)	0.8±1.0	0.8 (-1.5-4.5)	0	65 (63.8%)	19 (18.6%)	18 (17.6%)
Second evaluation	0.2±1.2	0.3 (-2.6 - 3.1)	-0.1±1.4	-0.2 (-3.7-3.4)	-0.4±1.0	-0.5 (-3.6-2.6)	0.5±1.2	0.7 (-2.8-2.5)	3 (2.9%)	61 (59.8%)	29 (28.5%)	9 (8.8%)
p	0.000[ps]		0.000[ps]		0.000[ps]		0.052[ps]			0.140[w]		

BMI: Body mass index, HFA: Height-for-age, SD: standard deviation, WFH: Weight-for-height, WFA: Weight-for-age, [ps]: paired samples t-test, [w]: wilcoxon test

Table 2: Anthropometric data at the baseline and at the end of the follow-up period according to gender groups, anthropometric evaluation according to BMI

		WFH		WFA		HFA		BMI z-score		BMI evaluation			
		mean±SD	median	mean±SD	median	mean±SD	median	mean±SD	median	Underweight (n-%)	Normal (n-%)	Overweight (n-%)	Obese (n-%)
Females	Baseline evaluation	0.8±1.0	0.8 (-1.7-3.2)	0.6±0.9	0.6 (-1.8-2.8)	0.0±0.7	0.0 (-2.0-1.1)	0.8±1.0	0.8 (-1.5-3.2)	0	35 (64.8%)	9 (16.7%)	10 (18.5%)
	Second evaluation	0.4±1.4	0.5 (-2.6-3.1)	-0.1±1.6	-0.1 (-3.7-3.4)	-0.5±0.9	-0.7 (-2.2 - 1.4)	0.5±1.2	0.6 (-2.1-2.5)	2 (3.7%)	32 (59.3%)	12 (22.2%)	8 (14.8%)
	p	**0.022**[ps]		**0.001**[ps]		**0.000**[ps]		**0.000**[ps]			0.591[w]		
Males	Baseline evaluation	0.8±1.1	0.8 (-1.8-4.4)	0.6±1.1	0.7 (-2.1-3.6)	0.1±0.9	0.2 (-1.8-2.2)	0.8±1.1	0.8 (-1.4-4.5)	0	30 (62.5%)	10 (20.8%)	8 (16.8%)
	Second evaluation	0.1±1.0	0.1 (-2.0-2.2)	0.2±0.9	-0.7 (-2.2-1.4)	0.2±1.0	-0.3 (-3.5-2.6)	0.5±1.0	0.7 (-2.8-2.1)	1 (2.1%)	29 (60.4%)	17 (35.4%)	1 (2.1%)
	p	**0.001**[ps]		**0.000**[ps]		**0.025**[ps]		0.392[ps]			0.124[w]		

BMI: Body mass index, HFA: Height-for-age, SD: standard deviation, WFH: Weight-for-height, WFA: Weight-for-age, [ps]: paired samples t-test, [w]: wilcoxon test

Table 3: Anthropometric data at the baseline and at the end of the follow-up period according to disease classification and anthropometric evaluation according to BMI

		WFH		WFA		HFA		BMI z-score		BMI evaluation			
		mean±SD	median	mean±SD	median	mean±SD	median	mean±SD	median	Underweight (n-%)	Normal (n-%)	Overweight (n-%)	Obese (n-%)
mPKU	Baseline evaluation	0.6±0.9	0.5 (-1.7-2.2)	0.4±0.9	0.6 (-2.1-1.8)	0.2±0.9	0.2 (-1.3-2.2)	0.6±0.9	0.6 (-1.5-2.1)	0	16 (72.8%)	4 (18.2%)	2 (9%)
	Second evaluation	0.2±1.1	0.3 (-1.2-2.5)	0.0±1.1	0.0 (-1.5-2.7)	-0.1±1.0	-0.3 (-1.4-2.6)	0.5±0.9	0.6 (-0.9-2.5)	0	16 (72.8%)	4 (18.2%)	2 (9%)
	p	0.200[ps]		0.075[ps]		0.131[ps]		0.748[ps]			1.000[w]		
cPKU	Baseline evaluation	0.8±1.1	0.9 (-1.9-4.4)	0.6±1.0	0.6 (-1.8-3.6)	0.0±0.8	0.0 (-2.0-2.1)	0.9±1.1	0.9 (-1.4-4.5)	0	49 (61.3%)	15 (18.8%)	16 (19.9%)
	Second evaluation	0.2±1.3	0.3 (-2.6-3.1)	-0.1±1.4	-0.3 (-3.7-3.4)	-0.5±1.0	-0.5 (-3.6-1.8)	0.5±1.2	0.7 (-2.8-2.5)	3 (3.8%)	45 (56.3%)	25 (31.3%)	7 (9.6%)
	p	**0.000**[ps]		**0.000**[ps]		**0.000**[ps]		**0.001**[ps]			0.108[w]		

BMI: Body mass index, HFA: Height-for-age, SD: standard deviation, WFH: Weight-for-height, WFA: Weight-for-age, [ps]: paired samples t-test, [w]: wilcoxon test

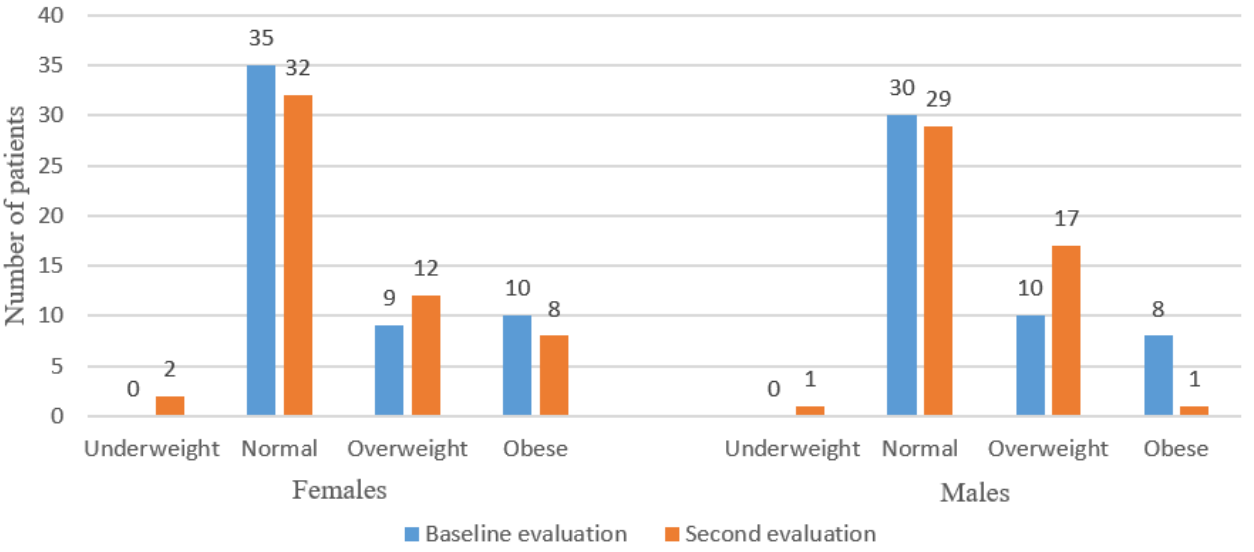

Figure 1: Anthropometric assessments of female and male patients at baseline and second evaluation

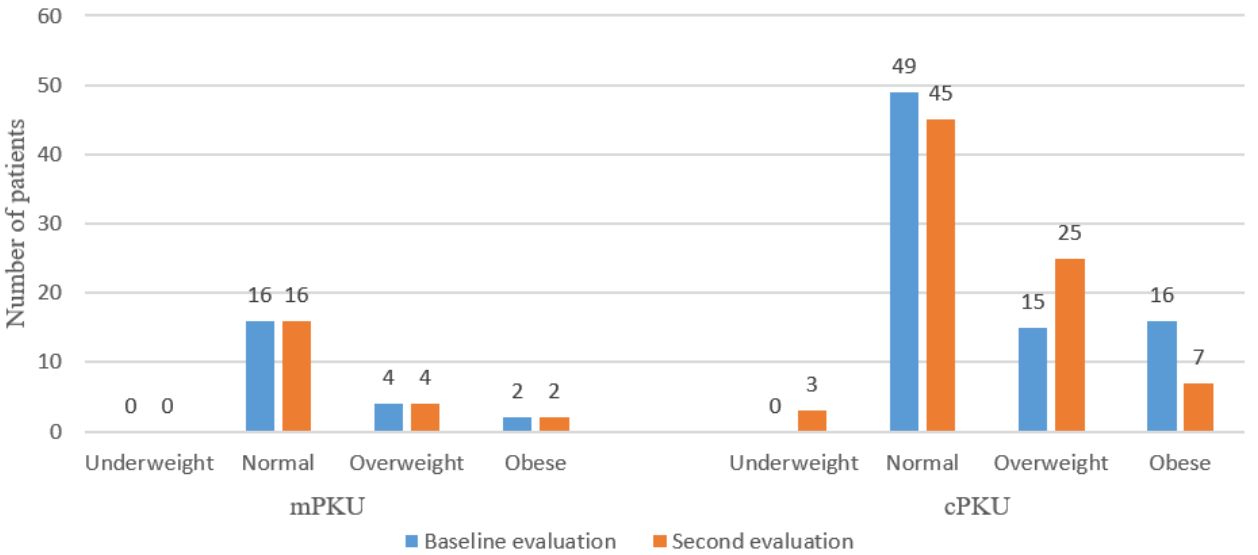

Figure 2: Anthropometric assessments of mPKU and cPKU patients at baseline and second evaluation
cPKU: clasical phenylketonuria, mPKU: mild phenylketonuria

of the follow-up period, the rate of obesity in the PKU patient group was below the Turkish average. According to the data of the Turkish Statistical Institute, the rate of obesity among individuals aged 15 years and older in our country was 19.9% and the overweight rate was 33.7% in 2014, while the rate of obesity among individuals aged 15 years and older was 20.2% and the overweight rate was 35.7% in 2022 (13, 14). Some studies have found no difference in BMI, overweight and obesity prevalence between patients with PKU and healthy individuals (15, 16). A meta-analysis showed no significant association between PKU and overweight (1). However, there are many studies showing that people with PKU have a higher BMI than the healthy population (10, 11, 17), especially in females (2, 18). Some studies have shown that the BMI and fat mass of female patients with PKU are higher than those of the control groups (19, 20). In our patient group, the obesity and overweight rates were found to be lower in both males and females than in the general population at both assessment periods. The reason for this result may be that the patients have acquired eating habits to achieve a more favourable body composition because of the special nutritional therapy. When the patients were grouped by sex, there was a decrease in anthropometric

measurements at the last assessment in both groups, and the z-score averages were within the healthy reference range at both assessments.

Grouping the patients according to disease classification, no difference was found in the WFH, WFA, HFA, and BMI of mPKU patients at the beginning and end of the follow-up period. In patients diagnosed with cPKU, a decrease was found in the mean WFH, WFA, HFA, and BMI z-scoreS at the last evaluation. The rate of obesity and overweight is below the Turkish mean rates (13, 14). In both the mPKU and cPKU groups, the mean and median values of WFH, WFA, HFA, and BMI z-scores during the first and second assessments were within the reference range considered normal. It has been reported that patients with cPKU have a significantly higher BMI than healthy controls (1). One reason for this is that more calories may be given to patients with cPKU to prevent catabolism, which causes higher blood Phe levels (1). This can lead to overweight development (1).

Studies assessing the prevalence of overweight and obesity in patients with PKU have reported different results (21-23). One of the reasons for these different results is that the studies were conducted with patient groups living in cultures with different dietary habits. Different treatment and follow-up principles of different centres may have caused such a result. Another reason is that changes in body composition that may occur during puberty may not be considered in the patient groups in the studies. In addition, caution should be taken when interpreting the results because of the different criteria used to classify overweight and obese individuals in the studies.

A comparison of the BMI z-scores of the patients revealed a shift in the distribution of BMI categories between the first and second assessments. A total of 14 patients were reclassified into a higher BMI category at the second assessment. A total of 23 patients were reclassified as belonging to a lower BMI group at the second assessment. This once again demonstrated the necessity of close follow-up of patients in terms of the results of special nutritional therapies.

The limitations of our study are that it did not include physical activity levels, dietary habits, Phe levels, which determine the metabolic control of the disease, and other parameters that provide information about body composition.

CONCLUSION

Monitoring anthropometric measurements and changes in body composition in all groups of patients receiving specific nutritional therapies is essential to determine and monitor treatment efficacy. In both evaluations of our patient group, the rates of overweight and obesity were found to be low compared with the general population. This can be explained by the fact that researchers closely monitored patients by assessing their nutritional treatment and changes in body composition at each outpatient clinic visit. Multicenter studies with more advanced methodologies are needed to properly address the issues and guide clinical practice.

Ethics Committee Approval: Ethics committee approval was received for this study from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.09.2023, No: 19).

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

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EFFICACY OF SGLT-2 INHIBITORS IN THE TREATMENT OF TYPE 2 DIABETES: SINGLE CENTRE EXPERIENCE

TİP 2 DİYABET TEDAVİSİNDE SGLT-2 İNHİBİTÖRLERİNİN ETKİNLİĞİ: TEK MERKEZ DENEYİMİ

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ABSTRACT

Objective: Sodium-glucose transporter-2 (SGLT-2) inhibitors lower blood glucose levels by reducing renal glucose reabsorption without affecting insulin secretion. The aim of our study was to evaluate the effect of SGLT-2 inhibitor treatment on glycemic control and the possible superiority of the drugs by comparing clinical parameters and laboratory findings in Type 2 diabetes mellitus (T2DM) patients.

Material and Methods: Two hundred and nineteen T2DM patients who received SGLT-2 inhibitor therapy [empagliflozin (EMPA) (n=146) or dapagliflozin (DAPA) (n=73)/10 mg] were enrolled retrospectively. The patients' demographic characteristics, detailed medical history, comorbidities, physical examination findings, complications, weight and systolic-diastolic blood pressure follow-up, laboratory findings (at baseline, 3rd, and 12th month), and overall follow-up outcomes were evaluated.

Result: The mean values of HbA1c and fasting blood glucose (FBG) decreased significantly compared with the baseline values after the treatment. The mean body weight and uric acid values were significantly reduced in the 3rd month of the treatment. Similarly, the values of the liver function tests decreased substantially after treatment.

Conclusion: The beneficial effects of SGLT-2 inhibitors on glycemic control and liver functions in patients with T2DM have been demonstrated. In addition, there was no major difference in terms of clinical parameters, laboratory findings, and drug safety in patients between EMPA and DAPA.

Keywords: Type 2 diabetes mellitus, sodium-glucose cotransporters, empagliflozin, dapagliflozin

ÖZET

Amaç: Sodyum-glukoz taşıyıcı-2 (SGLT-2) inhibitörleri, insülin sekresyonunu etkilemeden renal glukoz reabsorpsiyonunu azaltarak kan glukozunu düşürür. Çalışmamızın amacı, Tip 2 diabetes mellituslu (T2DM) hastalarda SGLT-2 inhibitör tedavisinin glisemik kontrol üzerindeki etkisini ve ilaçların olası üstünlüklerini klinik parametreler ve laboratuvar bulguları ile karşılaştırarak değerlendirmektir.

Gereç ve Yöntem: SGLT-2 inhibitörü tedavisi [empagliflozin (EMPA) (n=146) veya dapagliflozin (DAPA) (n=73)/10 mg] alan 219 T2DM hastalarının verileri geriye dönük olarak kaydedildi. Demografik özellikler, detaylı tıbbi öykü, komorbiditeler, komplikasyonlar, vücut ağırlığı ve kan basıncı takibi, laboratuvar bulguları (başlangıçta, 3. ve 12. aylarda) incelendi.

Bulgular: Bu çalışmamızda SGLT-2 tedavisinden sonra ortalama HbA1c ve açlık plazma glukoz değerleri başlangıç değerlerine göre anlamlı oranda azaldı. Ortalama vücut ağırlığı ve ürik asit değerleri tedavinin 3. ayında anlamlı oranda azaldı. Benzer şekilde karaciğer fonksiyon testleri değerlerinde de tedavi sonrası iyileşmeler görüldü.

Sonuç: SGLT-2 inhibitörlerinin T2DM hastalarında glisemik kontrol ve karaciğer fonksiyonu üzerindeki olumlu etkileri açıkça gösterilmiştir. Ayrıca klinik parametreler, laboratuvar bulguları ve ilaç güvenliği açısından EMPA ve DAPA arasında önemli bir fark saptanmamıştır.

Anahtar Kelimeler: Tip 2 diabetes mellitüs, sodyum glukoz kotransporterlar, empagliflozin, dapagliflozin

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive metabolic multi-systemic disease characterised by insulin resistance, insulin deficiency, and hyperglycaemia. T2DM carries a high risk of morbidity and mortality frequently related to renal failure, cardiovascular diseases (CVD), and micro/macrovascular complications (1, 2).

The primary target for treating T2DM is to achieve optimal glycemic control starting with lifestyle changes like diet and exercise as the first-line treatment. Oral antidiabetics (OADs) are used in the second-line treatment of T2DM (3). Conventional OADs act either by directly increasing the insulin secretion from pancreatic β-cells or by indirectly suppressing tissue insulin resistance, glucose production, and absorption in the liver and intestine, respectively (4). In recent years, studies have focused on new antidiabetic treatment modalities whose functions are independent of insulin secretion.

Sodium-glucose transporter-2 (SGLT-2) receptors expressed in the proximal renal tubules are responsible for glucose reabsorption from the glomerular filtrate independent of insulin. Furthermore, SGLT-2 receptor expression is reported to be increased in T2DM and is one of the mechanisms responsible for severe hyperglycaemia in T2DM (5). SGLT-2 inhibitors increase glycosuria by inhibiting the renal tubular reabsorption of glucose and sodium; therefore, they lower blood glucose levels without directly affecting insulin secretion or its sensitivity (5, 6). Unlike several OADs, SGLT-2 inhibitors have been frequently reported to provide glycemic control without causing side effects such as hypoglycaemia or weight gain (7). Moreover, SGLT-2 inhibitors are reported to be associated with effective blood pressure control, favourable cardiovascular risk profile, and reduced risk of cardiovascular death in the literature (8, 9).

In this study, we aimed to evaluate the effect of SGLT-2 inhibitor treatments (dapagliflozin [DAPA] and empagliflozin [EMPA]) on glycemic control and clinical and laboratory parameters in our tertiary referral centre.

MATERIALS AND METHODS

Sample

This study was conducted between January 1st 2018 to November 30th 2019 with the approval of the İstanbul Faculty of Medicine, Clinical Research Ethics Committee (Date: 06.12.2019, No; 20). Two hundred and nineteen patients with the diagnosis of T2DM aged over 18 years who received SGLT-2 inhibitor therapy (EMPA 10 mg or DAPA 10 mg) were evaluated retrospectively. The exclusion criteria were defined as; age <18 years, Type 1 Diabetes Mellitus, pregnancy, end-stage renal failure or patients on dialysis, or advanced stage (Child B or C) liver failure. All patients received appropriate antidiabetics in addition to SGLT-2 inhibitors as an add-on therapy, and prior treatment continued unless a severe side effect. Chronic renal failure (CRF) was defined as an estimated glomerular filtration rate (e-GFR)<60 ml/min/1.73 m^2 and microalbuminuria was defined as urinary albumin-to-creatinine ratio >20 mg/g (10). Patients' demographic characteristics, detailed medical history, presence of comorbidities, physical examination findings, complications of diabetes, data of weight, and systolic-diastolic blood pressure follow-up, laboratory findings (at baseline-3rd month-12th month), antidiabetic drugs other than SGLT-2 inhibitor used by patients and follow-up outcomes at 12th-month treatment were evaluated using the recorded medical data of patients.

Laboratory

Complete blood count analysis was performed from the patients' venous blood samples. Haematological parameters were analysed using a haematology analyser (CellDyne 3700, Abbott, Abbott Park, IL, USA). Biochemical analyses were performed from the serum samples by using an electro-chemiluminescence immunoassay analyser (Beckman Coulter Unicel DXI 800, Brea, CA, USA). The analysis of serum hormone levels was performed via an immunodiagnostic system (Siemens, Advia Centaur xp, Germany). HbA1c level analysis was performed in Beckman Coulter Au480 model automated HbA1c analyser using the turbidimetric immunoinhibiting method.

Statistical analysis

In our study, the 21.0 version (IBM, Armonk, NY, USA) of the SPSS (Statistical Package for the Social Sciences) programme was used for the statistical analysis of data. In descriptive statistics, discrete and continuous numerical variables were expressed as mean, ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables were expressed as the number of cases and (%). In the univariable analysis, cross-table statistics were used to compare categorical variables (Chi-Square, Fisher exact test) abnormally distributed parametric data were compared with Student's t-test and Paired t-test; non-parametric data that did not meet the normal distribution were compared with Mann–Whitney U and Kruskal–Wallis tests. P<0.05 value was considered statistically significant.

RESULTS

In this study, 219 T2DM patients were included, including 89 females (40.6%) and 130 males (59.4%). The mean age was 59.2±8.6 years (range; 21-79); there was no statistically significant difference between male (59.7±8.4 years) and female (58.5±8.8 years) patients in terms of mean age (p=0.4). The mean diabetes duration was 15.9±7.6 years. While the most common comorbidity was hypertension (70.8%), coronary heart disease was seen in

42.5%, CRF in 6.4% (no patient had stage 4 or 5 chronic kidney disease), microalbuminuria in 36.5%, congestive heart failure (CHF) (1.4%) and peripheral artery disease (1.4%). While the most used oral antidiabetic drug was metformin (94%), basal insulin was used in 38.8%, and the basal-bolus regimen in 26%. Additionally, 62.6% of the patients received angiotensin-converting enzyme (ACE) inhibitor/Angiotensin receptor blockers (ARB), and 10.5% of the patients used diuretics (loop and thiazides) (Table 1). Of the study patients, 66.7% (n=146) were treated with EMPA and 33.3% (n=73) with DAPA. Among background OAD treatment, only sulfonylurea treatment was lower in EMPA-receiving patients (22% vs 37%; p=0.02, Odds ratio [OR]:5.6), and basal insulin treatment tended to be higher in EMPA-receiving patients than in those receiving DAPA (43.2% vs 30.1%; p=0.06). Other background medications did not differ between the two treatment groups (p=0.8 for metformin, p=0.6 for thiazolidinediones, p=1 for GLP-1 agonists, p=0.7 for basal-bolus regimen).

The mean values of HbA1c and fasting blood glucose (FBG) were significantly reduced compared with the baseline values in all patients after treatment (Table 2 and Figure 1). Although the microalbuminuria levels decreased numerically, they did not reach statistical significance. Similarly, the mean values of body weight and uric acid levels decreased significantly during 3rd month of treatment. In addition, it was determined that the mean values of liver function tests (ALT, GGT, AST; at the end of the 12th month; ALP at the end of the 3rd month) reduced significantly after the treatment. Moreover, the mean serum HDL value increased significantly at the end of the 12th month of treatment. On the other hand, although a slight decline was observed in GFR values at the beginning, there was no statistically significant difference in e-GFR and systolic and diastolic blood pressure values during the follow-up period (Table 2).

Table 1: Clinical features of the study participants

Variables	Results
Age, years, mean±SD (range)	59.2±8.6 (21-79)
Gender, female, n (%)	89 (40.6)
Duration of diabetes, years, mean±SD	15.9±7.6
Smoking history (ever), n (%)	111/216 (41.3)
Hypertension, n (%)	155 (70.8)
Hyperlipidaemia, n (%)	142 (64.8)
Chronic kidney failure, n (%)	14 (6.4)
Heart failure, n (%)	3 (1.4)
Microalbuminuria, n (%)	61/167 (36.5)
Coronary heart disease, n (%)	93 (42.5)
Cerebrovascular accident, n (%)	7 (3.2)
Peripheral artery disease, n (%)	3 (1.4)
Diabetic foot, n (%)	9 (4.1)
Treatment history, n (%)	
Metformin	206 (94)
Sulphonylureas	59 (27)
Meglitinids	48 (22)
DPP-4 inhibitors	63 (28.8)
Tiazolidinediones	7 (3.2)
GLP-1 analogues	6 (2.7)
Basal insulin	85 (38.8)
Basal-bolus regimen	57 (26)
Statins	142 (64.8)
Fenofibrates	21 (9.6)
ACE-I/ARB	137 (62.6)
Diuretics	23 (10.5)

SD: Standard deviation, DPP-4: Dipeptidyl peptidase-4, GLP-1: Glucagon-like peptide-1, ACE-I: Angiotensin-converting enzyme inhibitors, ARB: Angiotensin receptor blockers

While the pre-treatment hypoglycaemia rate was 15.5% in all cases; it was determined to be 15.4% in 3rd month and 14.5% in the 12th month of treatment (p>0.05 for each). Besides, SGLT inhibitor treatment was discontinued in 14 patients (6.4%). All patients who discontinued the treatment had diabetes for more than 7 years, most of them were female (57.1%) and EMPA users (85.7%). Discontinuation of treatment was due to hypoglycaemia in four patients, urogenital infection in six patients, impaired renal function in one patient, and other reasons in three patients. The treatment was discontinued in 10 patients (4.6%) at 3rd month. due to genital infection in one patient, hypovolemia/dehydration in two patients, and due to other reasons in seven patients.

In our study, in patients who received EMPA therapy; mean values of HbA1c, FBG, and microalbuminuria decreased significantly at the 3rd and 12th month compared with the baseline values. The mean serum uric acid value was also reduced in the 3rd month compared with the baseline. Additionally, liver function tests (ALP, ALT, GGT) decreased significantly at the end of the 3rd month (Table 3). In the group receiving DAPA, it was determined that the mean HbA1c value during the entire follow-up period and FBG, microalbuminuria at the 3rd month decreased significantly compared to the baseline values. In addition, it was observed that there was a significant reduction in body weight during the follow-up period. The values of the liver function tests were also decreased significantly (Table 4). However, there was no statistically significant difference between the hypoglycaemia rates (DAPA 16.7% vs. EMPA 11.0%) at the end of the 3rd month in both groups (p=0.166). No statistically significant difference was found when comparing the effects of EMPA vs. DAPA on the clinical parameters during the follow-up period.

Table 2: Clinical characteristics and laboratory values of all patients at baseline and 3rd and 12th month of treatment

Variables (mean±SD)	Baseline	3rd month	12th month	p-value[1]	p-value[2]	p-value[3]
Body weight (kg)	83.4±14.5	83.1±14.2	83.92±14.5	**0.001**	**0.052**	**0.008**
HbA1c (%)	9.24±1.6	8.010±1.5	7.64±1.4	**<0.001**	**<0.001**	**<0.001**
Fasting plasma glucose (mg/dL)	195.0±72.9	162.4±54.3	172.8±68.2	**<0.001**	0.833	**0.022**
Urea (mg/dL)	33.59±10.6	35.41±10.4	38.44±14.2	0.096	**0.046**	**0.001**
Creatinine (mg/dL)	0.86±0.2	1.14±3.5	0.89±0.2	0.504	0.665	0.063
Uric acid (mg/dL)	5.04±2.0	4.82±1.1	5.11±1.2	**0.001**	0.490	0.845
Sodium (mmol/L)	139.9±2.4	140.2±2.3	140.6±2.4	0.744	0.582	0.259
ALP (U/L)	80.02±27.1	74.39±24.1	78.59±24.3	**0.002**	0.275	0.609
AST (U/L)	20.51±7.9	19.66±6.7	19.00±6.1	0.118	0.601	**0.039**
ALT (U/L)	25.01±13.4	22.75±11.9	23.05±11.9	**<0.001**	0.762	**0.005**
GGT (IU/L)	25.90±17.0	23.87±20.1	23.67±11.7	**<0.001**	0.256	**0.015**
Triglyceride (mg/dL), median (IQR)	157 (111)	152 (109)	181.6 (154)	**0.8**	0.7	0.9
HDL-C (mg/dL)	44.07±12.3	44.64±12.1	44.67±12.1	0.4	0.6	0.6
LDL-C (mg/dL)	115.0±50.5	108.4±32.8	106.4±29.9	0.535	0.549	0.629
Total-C (mg/dL)	189.1±43.1	185.2±38.5	181.0±36.6	0.598	0.622	0.918
Microalbumin/creatinine (mg/g), Median (IQR)	11.4 (37)	10 (29)	4.4 (18)	0.1	0.9	0.3
e-GFR (ml/min)	87.6±18	86.06±16.6	84.1±20	0.6	0.06	0.04
Systolic BP (mmHg)	130.7±19.4	133.7±17.5	137.0±19.6	0.290	0.797	0.944
Diastolic BP (mmHg)	78.65±10.5	81.78±9.3	80.60±10.6	0.089	0.138	0.573
Red Blood Cell (10^6/uL)	4.86±0.48	5.03±0.4	5.07±0.5	**<0.001**	0.929	**<0.001**
Haemoglobin (g/L)	13.72±1.4	14.05±1.5	14.30±1.5	**<0.001**	0.067	**<0.001**
Haematocrit (%)	41.08±3.9	42.45±4.2	43.21±4.3	**<0.001**	**0.042**	**<0.001**

[1]: Baseline 3rd month [2]: 3rd month-12th month [3]: Baseline-12th month
Bold values are statistically significant
SD: Standard deviation, IQR: Interquartile range, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, Total-C: Total cholesterol, e-GFR: Estimated glomerular filtration rate, BP: Blood pressure

DISCUSSION

SGLT-2 inhibitors have additional beneficial effects other than their glucose-lowering effects. SGLT2 inhibitor therapy also provides weight loss due to the burning of fatty acids induced by glycosuria and blood pressure control due to osmotic diuresis (11). SGLT-2 inhibitor therapy has been associated significantly with a decrease in systolic (3-6 mmHg) and diastolic (1-2 mmHg) blood pressure in patients with T2DM (12). Berhan A. and Barker A. reported that there was a significant reduction in HbA1c and FBG levels with SGLT-2 inhibitors compared to placebo in their studies (13). However, we did not observe a significant change in blood pressure with the SGLT-2 inhibitor, possibly due to the measurement difference. In addition, due to the retrospective characteristics of our study, the blood pressure values may have been recorded incompletely, which may be the cause of the inconsistency of the blood pressure results in contrast to the literature. In a meta-analysis in which the outcomes of 27 published studies were reviewed and involving a total of 7363 T2DM patients; Toyama et al. reported that SGLT-2 inhibitors were highly effective and reliable agents in lowering HbA1c level (0.29%; 95% CI, −0.39 to 0.19). Additionally, the researchers noted that these drugs effectively reduced FBG, systolic-diastolic blood pressure, and body weight (14). In another study, Liakos et al. reported a significant reduction in body weight and blood pressure values by EMPA treatment (15). Similarly, in our study, it was determined that the mean values of HbA1c and FBG decreased significantly in comparison to the baseline values in all patients after treatment. In addition, the mean body weight values were also significantly reduced in the 3rd month of treatment compared with the baseline values. It was also observed that there was a significant reduction in body weight during the follow-up period in patients treated with DAPA. However, there were no statistically significant differences in the systolic and diastolic blood pressure values after DAPA-EMPA treatment during the follow-up period. In our consideration, the findings that were inconsistent with the published data about the body weight observed in the 12th month of treatment may be due to the higher frequency of insulin treatment with EMPA.

The elevated uric acid concentration has been closely associated with cardiovascular disease, hypertension, and

Table 3: Clinical and biochemical parameters in patients treated with empagliflozin

Variables (mean±SD)	Baseline	3rd month	12th month	p-value[1]	p-value[2]	p-value[3]
Body weight (kg)	83.24±13.9	83.77±14.2	85.25±13.3	**<0.001**	0.185	0.065
HbA1c (%)	9.29±1.6	8.10±1.6	7.68±1.4	**<0.001**	**0.002**	**<0.001**
Fasting Plasma Glucose (mg/dL)	199.1±80.2	160.8±57.8	168.3±70.3	**<0.001**	0.775	**0.010**
Urea (mg/dL)	33.64±10.7	34.69±9.7	40.49±16.6	**0.045**	0.167	**0.001**
Creatinine (mg/dL)	0.88±0.2	0.89±0.2	0.95±0.2	0.554	0.466	**0.019**
Uric acid (mg/dL)	5.19±2.3	4.85±1.1	5.28±1.3	**0.015**	0.239	0.485
Sodium (mmol/L)	140.1±2.4	140.3±2.3	140.6±2.3	0.4	0.3	0.3
ALP (U/L)	82.4±26.3	73.6±21.8	76.9±22.5	0.001	0.3	0.5
AST (U/L)	25.16±14.4	22.86±11.0	24.89±13.3	**0.007**	0.585	0.130
ALT (U/L)	26.45±18.5	24.34±21.8	24.56±12.3	**<0.001**	0.351	0.095
GGT (IU/L)	26.5±18.6	24.2±21	24.6±12	0.6	0.6	0.08
Triglyceride (mg/dL), median (IQR)	147 (98)	140 (98)	168 (154)	0.853	0.419	0.734
HDL-C (mg/dL)	44.69±12.4	45.32±11.6	45.26±11.6	0.335	0.112	**0.031**
LDL-C (mg/dL)	106.0±374.8	33.69±92.2	72.86±153.4	**0.016**	0.433	**0.006**
Total-C (mg/dL)	186.8±44.7	183.2±37.5	177.0±33.3	0.608	0.681	0.820
Microalbumin/creatinine (mg/g), Median (IQR)	11 (40)	9. 8 (25)	5.3 (15)	0.1	0.6	0.1
e-GFR (ml/min)	86.3±19	84.3±17	80.3±21	0.4	0.15	0.049
Systolic BP (mmHg)	131.2±18.8	133.8±16.2	134.0±18.3	0.2	0.5	0.5
Diastolic BP (mmHg)	78.5±10.3	82.5±9.3	78.7±11	**0.005**	0.14	**0.35**
Red Blood Cell (10^6/uL)	4.9±0.5	5.04±0.5	5.1±0.5	**<0.001**	0.001	0.9
Haemoglobin (g/L)	13.75±1.3	14.07±1.4	14.29±1.4	**<0.001**	0.943	**0.037**
Haematocrit (%)	41.20±3.6	42.55±3.9	43.29±4.0	**<0.001**	0.933	**0.011**

[1]: Baseline 3rd month, [2]: 3rd month-12th month, [3]: Baseline-12th month
Bold values are statistically significant
SD: Standard deviation, IQR: Interquartile range, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, Total-C: Total cholesterol, e-GFR: Estimated glomerular filtration rate, BP: Blood pressure

CRF. It has been demonstrated that after SGLT-2 inhibitor therapy, due to the increase in uric acid excretion, uric acid levels, and renal functions improved, which reduces the risk of cardiovascular disease (16). In a meta-analysis in which 62 studies were reviewed and 34,941 T2DM patients were included, Zhao et al. reported that SGLT-2 inhibitors significantly reduced serum uric acid levels (17). In our study, the mean uric acid value was significantly decreased at the 3rd month of treatment compared with the baseline values in all patients.

It has been reported that post-treatment improvement of hepatic dysfunction with SGLT-2 inhibitors was achieved possibly due to the improvement in hyperglycaemia and insulin resistance independent of reduction in body weight. In a study consisting of 115 T2DM patients treated with DAPA (n=69) and EMPA (n=46), Lee et al. reported that ALT levels statistically decreased in all cases [40.3±28.0 vs. 29.0±14.1 U/L (p<0.001)] at the end of 6th month of SGLT-2 inhibitor treatment (18). Similarly, Gunhan et al. documented a significant reduction in ALT and AST levels (p=0.001 and 0.007, respectively) in 119 T2DM patients receiving DAPA (41.2%) and EMPA (58.8%) after 6 months of treatment (19). In accordance with these data, we detected that the post-treatment liver function tests' values (ALT, GGT, AST; at the end of the 12th month; ALP at the end of the 3rd month) significantly decreased in our study.

In diabetic patients, SGLT-2 inhibition is considered to reduce albuminuria by improving glomerular filtration in the early stages of diabetic nephropathy (20). However, Liu et al. concluded that SGLT-2 inhibitors had no significant effect on e-GFR levels in their meta-analysis (21). In another meta-analysis, Xu et al. noted that SGLT-2 inhibitor therapy was not significantly associated with e-GFR change in 22,843 T2DM cases (22). Although a slight decline was observed with SGLT-2 inhibitor treatment, no statistically significant difference was found in the mean values of creatinine, glomerular filtration rate, and sodium after SGLT-2 inhibitor therapy in our study.

Table 4: Clinical and biochemical parameters in patients treated with dapagliflozin

Variables (mean±SD)	Baseline	3rd month	12th month	p-value[1]	p-value[2]	p-value[3]
Body weight (kg)	83.75±15.4	81.71±14.1	81.80±16.2	**0.012**	**0.034**	**0.021**
HbA1c (%)	9.13±1.4	7.82±1.1	7.57±1.5	**<0.001**	**0.036**	**<0.001**
Fasting Plasma Glucose (mg/dL)	186.9±55.2	165.6±47.0	179.0±66.3	**0.007**	0.466	0.764
Urea (mg/dL)	33.47±10.5	36.83±11.6	35.71±9.9	0.818	0.158	0.176
Creatinine (mg/dL)	0.82±0.1	1.61±6.0	0.81±0.2	0.803	0.750	0.820
Uric acid (mg/dL)	4.72±1.2	4.76±1.1	4.87±1.2	**0.029**	0.575	0.262
Sodium (mmol/L)	140±2.4	140.3±2.4	140.6±2.7	0.8	1	1
ALP (U/L)	76.3±27.4	76±28.3	81.1±27.1	0.4	0.2	0.8
AST (U/L)	24.69±11.2	22.52±13.6	20.45±9.2	**0.002**	0.720	**0.008**
ALT (U/L)	24.79±13.4	22.94±16.3	22.43±11.0	**0.020**	0.513	0.088
GGT (IU/L)	24.8±13.5	22.95±16.3	22.4±11	0.6	0.5	0.1
Triglyceride (mg/dL), median (IQR)	190.5 (130)	174.5 (148)	206 (160)	0.220	0.935	0.205
HDL-C (mg/dL)	42.83±12.3	43.31±13.2	43.87±13.0	0.520	0.389	0.106
LDL-C (mg/dL)	116.6±35	110±36	110.5±36	0.16	0.9	0.3
Total-C (mg/dL)	193.9±39.3	189.2±40.4	186.3±40.8	0.165	0.765	0.896
Microalbumin/creatinine (mg/g), median (IQR)	12.9 (36)	11.6 (40)	3.45 (17)	0.8	0.7	1
e-GFR (ml/min)	90.29±16.3	89.49±15.3	91.78±25.8	0.468	0.964	0.639
Systolic BP (mmHg)	129.7±20.4	133.6±20.1	139.7±21.1	0.610	0.832	0.608
Diastolic BP (mmHg)	78.93±11.0	80.50±9.5	82.38±10.2	0.685	0.476	0.959
Red Blood Cell (10^6/uL)	4.82±0.5	5.02±0.5	5.06±0.5	**<0.001**	0.194	**0.003**
Haemoglobin (g/L)	13.67±1.5	14.02±1.6	14.30±1.7	**<0.001**	**0.009**	**<0.001**
Haematocrit (%)	40.85±4.3	42.27±4.6	43.11±4.7	**<0.001**	**0.011**	**<0.001**

[1]: Baseline 3rd month, [2]: 3rd month-12th month, [3]: Baseline-12th month
Bold values are statistically significant
SD: Standard deviation, IQR: Interquartile range, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, HDL-C: high-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, Total-C: Total cholesterol, e-GFR: Estimated glomerular filtration rate, BP: Blood pressure

The inhibition of glucose and sodium reabsorption in the proximal renal tubules by SGLT-2 inhibitors triggers osmotic/natriuretic diuresis and reduction of plasma, interstitial, and extravascular volume. The reduction of plasma volume increases haematocrit (HCT) and haemoglobin (HGB) levels (23). In addition, recently, SGLT2 inhibitors have been associated with elevations in serum erythropoietin secretion, which results in increased haemoglobin and haematocrit values (24). In a study conducted in 808 patients with the diagnosis of T2DM, Aberle et al. reported significantly increased HCT and RBC values at the 56th week of DAPA treatment (25). In accordance with published data, it has been observed that RBC, HGB, and HCT levels significantly increased after SGLT-2 inhibitor therapy in our study.

Microalbuminuria is strongly associated with cardiovascular and progressive kidney diseases (20). In a meta-analysis including 7363 T2DM cases, Toyama et al. highlighted that SGLT-2 inhibitors effectively reduced the microalbuminuria values (14). Similarly, in another study consisting of 119 patients treated with DAPA (41.2%) and EMPA

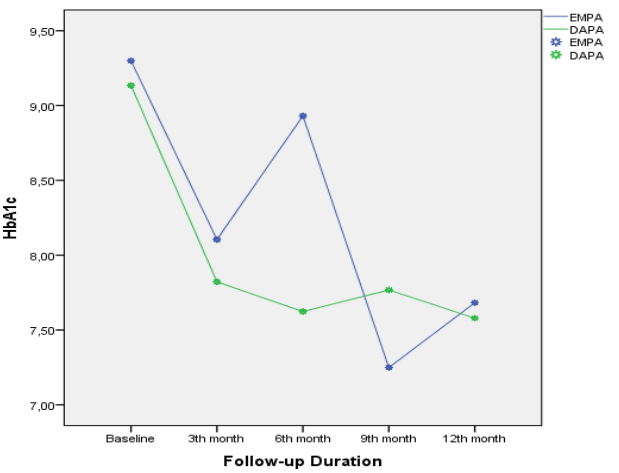

Figure 1: Comparison of change in HbA1c levels during the treatment in patients treated with EMPA and DAPA (p<0.001)
EMPA: Empagliflozin, DAPA: Dapagliflozin

(58.8%), Gunhan et al. concluded that SGLT-2 inhibitors had a beneficial effect in reducing microalbuminuria (189. In addition, Dekkers et al. reported that the microalbuminuria values decreased by 43.9% with DAPA treatment (26). Although microalbuminuria levels decreased numerically with SGLT-2 inhibitor treatment, they did not reach the statistical significance possible due to the small sample number in our study (Type 2 error).

An important clinical advantage of SGLT-2 inhibitors over other antidiabetic drugs is that they are not associated with the risk of hypoglycaemia (27). In a study including 350 T2DM patients, Ku et al. stated that there was no significant difference in the post-treatment Hypoglycemia rates between DAPA and EMPA (28). In our study, the hypoglycaemia rates did not show significant differences between patients receiving DAPA and EMPA treatments (16.7% vs. 11.0%, respectively) at the end of the 3rd month.

Our study has some limitations. First, the retrospective design of the study was the main limitation. Having missing data was an important limitation. On the other hand, this is one of the largest real-world data in terms of the efficacy and safety of SGLT-2 inhibitor treatment in Turkey.

CONCLUSION

In conclusion, the beneficial effects of SGLT-2 inhibitors (EMPA and DAPA) on glycemic control and liver functions in patients with T2DM were demonstrated in this study. Moreover, the protective effects of both agents on cardiovascular and renal diseases have been highlighted in association with a decrease in serum uric acid concentration and microalbuminuria. In addition, there was no major superiority of one of the two SGLT-2 inhibitors (EMPA, DAPA) over the other in terms of clinical parameters, laboratory findings, and patient drug safety.

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RESEARCH / ARAŞTIRMA
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AN EVALUATION OF THE AST/ALT RATIO IN PATIENTS WITH MYCOSIS FUNGOIDES AND ITS ASSOCIATION WITH THE SEVERITY OF CUTANEOUS INVOLVEMENT

MİKOZİS FUNGOİDES HASTALARINDA AST/ALT ORANININ DEĞERLENDİRİLMESİ VE KUTANÖZ TUTULUMUN ŞİDDETİ İLE İLİŞKİSİ

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ABSTRACT

Objective: Mycosis fungoides (MF) is the most common T-cell skin lymphoma, and a simple and applicable parameter is needed to monitor the prognosis of the disease. We investigated the ratio of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in patients with MF.

Material and Methods: The research involved a retrospective, cross-sectional study. The records of MF patients were analysed. AST and ALT levels were recorded and the AST/ALT ratio was calculated and compared with the control group.

Results: Eighty-five MF patients and 85 healthy controls were included in the study. Males accounted for 56.5% (n=48) of MF patients and 57.6 % (n=49) of healthy group, with no significant difference (p>0.05) between them. AST levels were notably elevated in the MF group compared with the healthy group (p<0.001). Furthermore, there was a significant difference in the AST/ALT ratio between the two groups (p=0.005). The AST/ALT ratio cut-off value was 1.067. The AST value in the patients with abnormal lymphadenopathy (LAP) on ultrasonography (USG) was higher than in those with reactive LAP. The AST values of the patients with LAP with fluorodeoxyglucose (FDG) involvement on positron emission tomography (PET)/computed tomography (CT) were significantly higher than those of the patients without abnormal LAPs (p<0.05). The AST/ALT ratio was weakly positively correlated with the disease stage (p=0.018).

Conclusion: AST values and AST/ALT ratios of patients with MF were significantly higher than those of the control group. The

ÖZET

Amaç: Mikozis fungoides (MF), derinin en sık görülen T hücreli lenfoması olup hastalığın prognozunu takip etmede kullanışlı, basit parametrelere ihtiyaç duyulmaktadır. Çalışmamızda son zamanlarda birçok malignitede prognostik faktör olarak araştırılan aspartat aminotransaminaz (AST) ve alanin aminotransaminaz (ALT) oranının bir deri lenfoması olan MF'de değerlendirilmesini amaçladık.

Gereç ve Yöntem: Çalışmamız retrospektif kesitsel bir çalışmadır. Çalışmada MF tanısı alan hastaların dosya taraması yapıldı. AST, ALT değerleri kaydedilerek AST/ALT oranı hesaplanıp, kontrol grubu ile istatistiksel olarak karşılaştırıldı.

Bulgular: Çalışmaya 85 MF hastası ve 85 sağlıklı kontrol olmak üzere toplam 170 kişi dahil edildi. Buna göre, MF grubunun %56,5'i (n=48) ve sağlıklı kontrol grubunun %57.6 (n=49)'sı erkek idi ve gruplar arası anlamlı farklılık bulunmuyordu (p>0,05). MF grubunun AST değeri sağlıklı gruba göre daha yüksekti ve istatistiksel anlamlı farklılık saptandı (p<0,001). AST/ALT oranı her iki grup arasında istatistiksel olarak anlamlı farklılık saptandı (p=0,005). AST/ALT oranı cut-off değeri ise 1,067 bulunmuştur. USG'de anormal LAP saptanan hastalarda AST değeri reaktif LAP olanlara göre daha yüksek bulundu. PET CT'de ise, FDG tutulumu olan LAP'ları saptanan hastaların AST değeri, anormal LAP'ları olmayan hastalardan anlamlı yüksek olarak saptandı (p<0,05). AST/ALT oranı ise hastalık evresi ile zayıf pozitif korele bulundu (p=0,018).

Sonuç: Çalışmamızın sonucunda, MF'li hastaların AST değeri ve AST/ALT oranı sağlıklı kontrol grubuna göre istatistiksel olarak

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AST/ALT ratio can be used as an independent factor in monitoring the prognosis of patients with MF.

Keywords: Mycosis fungoides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST/ALT ratio, prognostic factor

anlamlı yüksek bulunmuştur. MF hastalarının prognozunun takibinde AST/ALT oranı bağımsız bir faktör olarak kullanılabilir.

Anahtar Kelimeler: Mikozis fungoides, aspartat aminotransferaz (AST), alanin aminoaminotransferaz (ALT), AST/ALT oranı, prognostik faktör

INTRODUCTION

Mycosis fungoides (MF) is the most common primary T-cell skin lymphoma. Its aetiology is uncertain although the emphasis is on genetic and environmental factors. The disease may progress with only cutaneous lesions. However, in patients with advanced disease, visceral involvement may occur. Skin lesions include patches, indurated plaques and tumours. Lesions of different morphologies may co-exist, and these lesions may also transform into each other. They tend to affect covered body areas (1). Survival in the early stages of the disease is 10-35 years, while around 25% of cases progress to the advanced stage with an average survival of 1-4 years (2). The tumour (T), lymph node (N), metastasis (M) and blood (B) (TNMB) system is the most important prognostic factor for MF. The early stage is defined as IA-IIA and the advanced stage as IIB-IV (3). Several independent prognostic factors in MF have been described in addition to TNMB staging. Increased lactate dehydrogenase levels, increased β2-microglobulin, eosinophilia, large cell transformation, older age, male sex and folliculotropic type MF have all been recognised as poor prognostic criteria (4). Aspartate aminotransferase (AST) is an enzyme that can be present in the cell, cytoplasm, and mitochondria. In addition to the liver, it can be found in several organs including the muscle, skeletal muscle, blood cells such as erythrocytes, the pancreas, and the brain. Alanine aminotransferase (ALT) is a cytoplasmic enzyme and is more specific to the liver. A more marked increase in ALT occurs in the event of liver damage (5). Aminotransferases like AST and ALT participate in cellular metabolism in healthy cells, and they also play a significant role in cancer cells and their turnover. ALT is crucial for the glucose-alanine cycle, whereas AST is necessary for aerobic glycolysis through adenine dinucleotide translocation within the mitochondria. These metabolic processes are particularly vital for cancer cells because of their heightened metabolic activity (6). The average reported AST/ALT ratio, also known as the "De Ritis ratio", in the cytoplasm of a normal hepatocyte cell is 0.6 (7). Recent research shows that the ratio of AST to ALT serves as an independent prognostic factor in many different types of cancer (8). The purpose of the present study was to investigate the relationship between the ratio of AST to ALT and the disease stage and extent of the affected body surface area in patients with MF. Our scan of the literature revealed no previous studies examining the "De Ritis ratio" in MF patients with T-cell lymphoma of the skin, and this study is thus the first on the subject.

MATERIAL AND METHODS

Study design

The research was conducted as a retrospective, single-centre, cross-sectional study.

Ethical approval

The study has ethical approval from the Atatürk University, Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (Date: 29.03.2024, No: 192).

Setting

The study was conducted in a tertiary university hospital in a region in the east of Türkiye with approximately 4.5 million inhabitants, with a large patient population. The MF patient group participated from the chronic dermatological disease's clinic, while the control group consisted of individuals presenting to our hospital's occupation health and safety clinic for routine checks.

Participants

One hundred and seventy patients were included in the study. These consisted of 85 patients aged 18-85 presenting to our hospital's chronic dermatological diseases clinic between January 2018 and February 2024 with histopathologically confirmed MF. The healthy control group consisted of 85 individuals with demographic characteristics similar to those of the patient group and with no dermatological or systemic diseases. The data for healthy controls were obtained retrospectively from individuals presenting to our hospital's health and safety clinic for routine check-ups. Official permission for the use of these individuals' data was received from the chief physician's office. The patients and controls' demographic data and the patient group's duration of disease and clinical findings were recorded with retrospective screening of the files. Patients with a history of using dietary supplements or herbal products that may affect liver enzymes were not included in the study. The patient groups with existing symptoms as of the date of admission and their AST and ALT results for that time were screened and recorded. The AST/ALT ratio was calculated as the proportion of AST and ALT values. The patients' abdominal ultrasonography (USG) and positron emission tomography (PET)/computed tomography (CT) results were also recorded. The distribution and extent of the lesions and the stage of the disease-based lymph node involvement were determined. Lymph nodes whose USG findings revealed a loss of oval shape, larger than 1.5 cm in size, with

an indistinct hilus, a thick cortex, and hyperechoic necrotic areas were abnormal in structure. Our hospital's laboratory values were used for AST and ALT test result reference values. Accordingly, 0-40 U/L were evaluated as normal for AST and 0-50 U/L for ALT. The rule of nines was applied to calculate the body surface area. Accordingly, each leg was constituting 18% of the body area, each arm 9%, the front and rear trunk 19% each, and the head 9% (9). Patients without histologically confirmed diagnoses of MF, those aged under 18 or over 85 years, with other diseases affecting the liver, with histories of alcohol use, or with previously known malignancies were excluded from the study. Since the study was conducted retrospectively and it was designed as an archive scan of all patients' files, it was not necessary and not possible to obtain informed consent from the patients.

Statistical analysis

The study data were analysed using SPSS version 27 software (IBM SPSS Corp., Armonk, NY, USA). Categorical data are presented as frequency and percentage and numeric data as mean, standard deviation, median, and interquartile range values. The Kolmogorov–Smirnov test was applied to assess the normality of the distribution of the continuous variables. The Mann–Whitney U test was used in the analysis of two independent non-normally distributed groups. Tukey's HSD test was employed in the post hoc analysis of variables with assumed equal variances. Spearman's correlation analysis was applied to non-normally distributed continuous variables. The area under the curve (AUC) was calculated using ROC analysis, and the cut-off values were determined using the Youden index. Categorical variables were analysed using Pearson's chi-square test. P values less than 0.05 were deemed statistically significant.

RESULTS

One hundred and seventy individuals were included in the study, 85 patients with MF and 85 healthy controls. A comparison of the participants' demographic characteristics and blood test values is shown in Table 1. Males represented 56.5% (n=48) of the MF group and 57.6% (n=49) of the healthy group, and the difference between the groups was not statistically significant ($p>0.05$). The median age was significantly higher, at 50 Interquartile Range (IQR) =31 in the MF group than in the healthy control group at 43 (IQR= 2) ($p<0.05$). The median AST value in the MF group was significantly higher than that in the healthy group [24.00 (IQR=15.00) vs. 18.50 (IQR=6.00), respectively] ($p<0.001$). However, there was no significant difference between the two groups' ALT values ($p>0.05$). The ratio of AST to ALT in the MF group, 1.2095 (IQR=0.56) was significantly higher than that in the healthy control group, at 1.0629 (IQR=0.54) ($p=0.005$).

AST and the ratio of AST to ALT ROC curves between the MF and healthy groups are shown in Figure 1. In the ROC analysis, the AUC value for AST was 0.729 (95 % CI: 0.653-0.806), and that for the AST/ALT ratio was 0.623 (95 %CI: 0.540-0.707) (Table 2). The cut-off values were 20.25 (with 75.3% sensitivity and 64.7% specificity) for AST and 1.067 (with 70.6% sensitivity and 51.8% specificity) for the AST/ALT ratio.

A comparison of the MF patients' AST, ALT, and AST/ALT ratio values in terms of their demographic characteristics and morphological and clinical disease findings is shown in Table 3. ALT was significantly higher in males and the AST/ALT ratio in women. AST values were higher in patients with abnormal LAP detected at USG than those with reactive LAP. At post-hoc analysis, AST values were similarly significantly higher in the presence of LAP and in the presence and absence of hepatosteatosis, in other words, in individuals with abnormal LAP, compared to those with reactive LAP. The ADT values of patients with LAP and FDG involvement at PET CT were significantly higher than those of the patients without abnormal LAP ($p<0.05$).

Analysis between continuous variables revealed a weak positive correlation between AT values and age, disease stage, the region of the body, and LAP circumference. ALT values exhibited no correlation with any parameters, whereas the AST/ALT ratio was weakly positively correlated with the disease stage (Table 4).

DISCUSSION

Mycosis fungoides (MF), the most common T-cell skin lymphoma, is capable of affecting the haematological system and the visceral organs in the advanced stage (1). More easily applied and inexpensive methods than expensive and time-consuming tests have recently become highly attractive for estimating the prognosis of several diseases. Several biomarkers have recently been investigated for monitoring the prognosis in malignant diseases. One such biomarker is the AST/ALT ratio. On that basis, we set out to add to the existing literature by investigating the AST/ALT ratios of patients with MF and a healthy control group and its relationship with the stage of the disease and spread of cutaneous involvement. The AST/ALT ratio is also known as the "De Ritis ratio", the concept first being employed in a study of the aetiology of hepatitis (10). ALT is more specific to the liver, while AST is an enzyme involved in aerobic glycolysis and capable of being synthesised in several types of tissue. AST may therefore rise more markedly than ALT in cases of widespread tissue damage and high tumour cell turnover. This makes the AST/ALT ratio a potential biomarker in malignancies (6). Aerobic glycolysis and pyruvate production are highly important for the increased metabolism of tumour cells. Increased nucleotide biosynthesis and the synthesis of non-essential amino acids in tumour cells contributes to the proliferation of cancer cells. Tumour cells should increase the synthesis of

Table 1: A comparison of the two study groups

				MF group	Healthy group	P
Sex	Female		n	37	36	**0.877***
	%		43.5%	42.4%		
	Male		n	48	49	
	%		56.5%	57.6%		
Age	Mean			50.79	42.84	**0.007†**
	95% Confidence Interval for mean	Lower bound		46.92	42.66	
		Upper bound		54.66	43.01	
	Median			50.00	43.00	
	Std. deviation			17.93	0.80	
	Interquartile range			31.00	2.00	
AST	Mean			27.37	19.91	**<0.001†**
	95% Confidence Interval for mean	Lower bound		24.89	18.69	
		Upper bound		29.86	21.13	
	Median			24.00	18.50	
	Std. deviation			11.52	5.65	
	Interquartile range			15.00	6.00	
ALT	Mean			22.84	19.95	**0.131†**
	95% Confidence Interval for mean	Lower bound		20.44	18.01	
		Upper bound		25.25	21.89	
	Median			21.00	18.40	
	Std. deviation			11.14	8.98	
	Interquartile range			16.10	9.70	
AST/ALT ratio	Mean			1.3468	1.1336	**0.005†**
	95% Confidence Interval for mean	Lower bound		1.2188	1.0330	
		Upper bound		1.4748	1.2341	
	Median			1.2095	1.0629	
	Std. deviation			0.5934	0.4660	
	Interquartile range			0.56	0.54	

*: Pearson Chi–square test, †: Mann–Whitney U test, MF: Mycosis Fungoides, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.
Note: Bold p values indicate statistical significance between the groups.

Table 2: Area under the curve (AUC) values for AST and AST/ALT ratio levels were determined in the ROC curve analysis

Test result variable(s)	Area	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
AST	0.729	0.039	0.000	0.653	0.806
AST/ALT ratio	0.623	0.043	0.004	0.540	0.707

[a]: Under the non-parametric assumption, [b]: Null hypothesis: true area = 0.5, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

the non-essential amino acid glutamine to ensure this. In contrast, ALT catabolizes pyruvate and glutamine to α-ketoglutarate in order to reduce glutamine. One in vitro experiment showed lower ALT levels in invasive cancer cells than in non-invasive cancer cells. Cancer cells can cause this by increasing ALT consumption (11). In

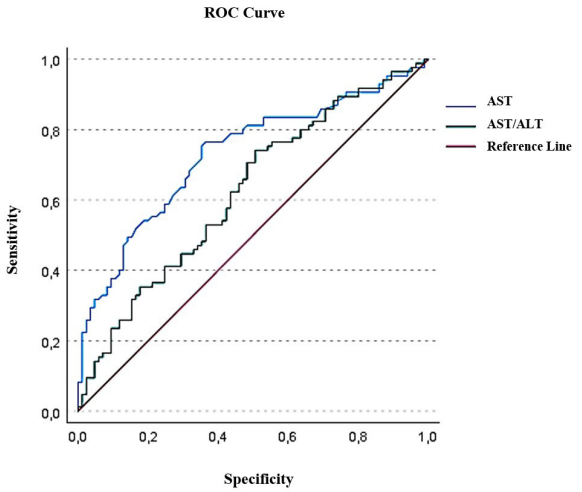

Figure 1: ROC curve analysis of AST and AST/ALT ratio between the study groups
AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 3: Statistical comparisons of AST, ALT, and AST/ALT ratio values according to various morphologic features in the MF group

	AST	ALT	AST/ALT ratio
Sex	0.062*	**0.003***	**0.007***
Smoking	0.270†	0.741†	0.122†
Lesion type	0.377†	0.440†	0.681†
Subjective finding	0.343†	0.490†	0.840†
Symptom	0.372†	0.461†	0.561†
LAP present	0.052*	0.119*	0.972*
LAP morphology	**0.011†**	0.079†	0.926†
LAP location	0.491†	0.607†	0.528†
USG findings	**0.024†**	0.070†	0.716†
PET findings	**0.039***	0.073*	0.549*

*: Mann–Whitney U test, †: Kruskal–Wallis test, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LAP: Lymphadenopathy, PET: Positron Emission Tomography, USG: Ultrasonography.
Note: Bold p values indicate statistical significance between the groups.

Table 4: Correlation analysis between AST, ALT and AST/ALT ratio values and demographic variables in the MF group

		AST	ALT	AST/ALT Ratio
Age	r	0.216*	0.092	0.061
	p	**0.047**	0.404	0.579
Disease Duration (months)	r	-0.032	-0.021	-0.033
	p	0.775	0.848	0.766
Disease Stage	r	0.388**	0.100	0.256*
	p	**<0.001**	0.363	0.018
Body Surface Area Involved	r	0.244*	0.146	0.074
	p	**0.025**	0.181	0.500
LAP Number	r	0.184	0.134	0.037
	p	0.093	0.221	0.734
LAP Circumference	r	0.359**	0.184	0.147
	p	**0.001**	0.092	0.180

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LAP: Lymphadenopathy
Note: Bold p values indicate statistical significance between the groups.

support of that finding, this study revealed no significant difference in ALT levels between the patients with MF and the healthy controls. In the previous literature, the AST/ALT ratio has been shown to be higher in several malignancies, such as renal cell carcinoma, bladder cancer, cholangiocarcinoma, pancreatic carcinoma, and breast carcinoma, compared to control groups, and be capable of use as a prognostic marker in patients with malignancies (12-16). However, we encountered no previous study investigating the AST/ALT ratio in haematological malignancies. This study is thus the first involving MF, a condition capable of causing dermatological, haematological, and visceral involvement. Significant differences in AST and AST/ALT ratio values were determined between the MF patients and the control group. The AST/ALT ratio was 1.2095 in the MF group and 1.0629 in the healthy control group. At the same time, a positive correlation was found between the extent of the skin surface and AST values in the MF patients. This may indicate that widespread cutaneous involvement may have raised AST values by increasing the tumour burden. Monitoring of liver enzymes may be particularly important in MF patients with diffuse skin involvement. Although several studies have reported that the AST/ALT ratio may be useful for determining prognosis in malignancies, a weak positive correlation was observed in the present study between the disease stage of MF patients and the AST/ALT ratio. The AST/ALT ratio may be a prognostic factor that becomes more important in aggressive malignancies with much faster metabolic activity. The statistically weak correlation between MF stage and AST/ALT ratio found in this study may be explained by the fact that MF is a slowly-progressive T-cell lymphoma. It can be posited that the lack of a significant correlation between the AST/ALT ratio and the disease stage may be attributable to the predominance of early stage MF patients within the study sample, potentially masking the potential relationship between these variables. In our opinion, the AST/

ALT ratio may be a more useful prognostic factor in the follow-up of patients with progressive MF. Therefore, we believe that our findings need to be supported by further prospective studies involving greater participation. MF is more common at the ages of 55-60 and in males (17). In agreement with the previous literature, the median age of the MF group in the present research was 50 (IQR=31) years, and 56.5% (n=48) of the MF patients were males. The ratio of AST to ALT showed a significant increase in female MF patients compared to males. Although a previous study reported that hepatic enzyme levels may vary by gender for genetic reasons, this is still unclear. There is a need for further research into this issue. (18).

ROC analysis between the MF patients and the healthy control group revealed AUC values of 0.729 for AST and 0.623 for the AST/ALT ratio. The cut-off values were 20.25 for AST (75.3% sensitivity and 64.7% specificity) and 1.067 for the AST/ALT ratio (70.6% sensitivity and 51.8% specificity). The patients' USG and PET results were examined, but no visceral organ involvement was observed in any case in the MF group. This may derive from the patients included in the study being in the early stage of MF. Hepatosteatosis and LAP were evaluated on the basis of USG and PET results, and the relationship between these findings and the AST/ALT ratio in the patient group was investigated. AST values were higher in the patients with abnormal LAP determined by both USG and PET compared with those with reactive LAP. However, no statistically significant association was observed with the AST/ALT ratio. We therefore think that hepatic enzymes should be measured during the disease in patients with MF with abnormal LAP detected both clinically and using tests such as USG and PET.

There are many limitations to this study. The first involves its retrospective nature. Another limitation is that some patients were evaluated while in receipt of treatment. A third limitation is that the single centre-nature of the research represents an obstacle to the generalisation of the results.

However, there are also some strengths to this study. The most powerful of these is that it is the first to evaluate the AST/ALT ratio in patients with MF. The relatively high numbers of both MF patients and healthy controls taking part constitutes another strength.

CONCLUSIONS

In conclusion, both AST and AST/ALT ratio values were higher in the MF group than in the healthy control group. Although the AST/ALT ratio exhibited a weak positive correlation with the stage of MF, we believe that this ratio, and particularly AST, can be used as a potential biomarker for monitoring the prognosis of the disease. To confirm and support the relationship between MF and the AST/ALT ratio, further multicenter prospective studies with larger numbers of participants are needed. We think that if this relationship is supported by further studies, it may contribute to the identification of patients who require close monitoring and to deciding whether to apply additional and more aggressive treatments in MF, a malignant disease.

Ethics Committee Approval: Ethics committee approval was received for this study from the Atatürk University, Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (Date: 29.03.2024, No: 192).

Informed Consent: Since the study was conducted retrospectively and it was designed as an archive scan of all patients' files, it was not necessary and not possible to obtain informed consent from the patients.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.P., M.B.; Data Acquisition- E.P., M.B.; Data Analysis/Interpretation – E.P., M.B.; Drafting Manuscript- E.P., M.B.; Critical Revision of Manuscript- E.P., M.B.; Final Approval and Accountability- E.P., M.B.; Technical or Material Support- E.P., M.B.; Supervision- E.P., M.B.

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EFFECT OF S-ADENOSYLMETHIONINE ON HEPATIC AND METABOLIC DISORDERS IN GUINEA PIGS WITH NON-ALCOHOLIC STEATOHEPATITIS

NON-ALKOLİK STEATOHEPATİT OLUŞTURULAN KOBAYLARDA S-ADENOZİLMETIYONİNİN KARACİĞER VE METABOLİK BOZUKLUKLAR ÜZERİNE ETKİSİ

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ABSTRACT

Objective: S-adenosylmethionine (SAM) has antioxidant and anti-inflammatory actions and hepatoprotective potential. In this study, the therapeutic effectiveness of SAM was investigated in high-fat/cholesterol diet (HFCD)-induced non-alcoholic steatohepatitis (NASH).

Material and Methods: In this study, guinea pigs were fed a HFCD for ten weeks to induce NASH. SAM (50 mg/kg, i.p.) was administered to the animals during the last four weeks of the 10-week HFCD regimen. Hepatic damage markers, lipid levels (total cholesterol and triglyceride), inflammatory cytokines (tumour necrosis-α and interleukin-6) levels, and insulin resistance (HOMA-IR) were determined in the serum. Moreover, hepatic lipids, SAM and cytochrome p450-2E1 (CYP2E1) levels, prooxidant parameters (reactive oxygen species, lipid peroxides and protein carbonyls) and antioxidant parameters (glutathione levels and antioxidant activity) together with fibrosis indicators (α-smooth muscle actin and transforming growth factor-β1 protein expressions and hydroxyproline levels) were investigated in the liver. Steatosis, inflammation, and fibrosis scores were also detected histopathologically.

Result: SAM treatment diminished the increase in hepatic damage markers, inflammatory cytokine levels, and HOMA-IR levels

ÖZET

Amaç: S-adenozilmetiyonin (SAM), antioksidan ve anti-enflamatuar etkilere ve hepatoprotektif potansiyele sahiptir. Bu çalışmada, yüksek yağ/kolesterollü diyet (YYKD) ile indüklenen non-alkolik steatohepatit (NASH) üzerinde SAM'ın terapötik etkinliği araştırılmıştır.

Gereç ve Yöntem: Bu çalışmada, NASH oluşturmak için kobaylara 10 hafta boyunca YYKD verildi. Hayvanlara, 10 haftalık YYKD uygulamasının son dört haftasında SAM (50 mg/kg, i.p) uygulandı. Serumda hepatik hasar belirteçleri, lipitler (total kolesterol ve trigliserit), inflamatuar sitokin (tümör nekroz faktörü-α ve interlökin-6) düzeyleri ve insülin direnci (HOMA-IR) ölçüldü. Ayrıca, karaciğerde hepatik lipitler, SAM ve sitokrom p450-2E1 (CYP2E1) düzeyleri, prooksidan parametreler (reaktif oksijen türleri, lipid peroksidleri ve protein karbonil) ve antioksidan parametreler (glutatyon düzeyleri ve antioksidan aktivite) ile birlikte fibrotik parametreler (α-düz kas aktin ve transforme edici büyüme faktör-β1 protein ekspresyonları ve hidroksiprolin düzeyleri) belirlendi. Steatozis, inflamasyon ve fibrozis skorları da histopatolojik olarak tespit edildi.

Bulgular: SAM tedavisi, YYKD ile indüklenen NASH'lı kobayların serumunda hepatik hasar belirteçleri, enflamatuar sitokinler düzeyleri ve HOMA-IR düzeylerinde azalmaya neden oldu. Ayrıca,

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in the serum of guinea pigs with HFCD-induced NASH. Elevated levels of hepatic triglyceride and CYP2E1 and fibrosis indicators were also detected to decrease due to SAM treatment. This treatment reduced the decrease in SAM levels, disturbance in the prooxidant and antioxidant balance, and diminished the increases in steatosis, inflammation, and fibrosis scores in the liver of guinea pigs fed the HFCD diet.

Conclusion: These results indicate that SAM may be effective in HFCD-induced NASH as a therapeutic agent by decreasing lipogenesis, oxidative stress, inflammation, and fibrosis.

Keywords: S-adenosylmethionine, nonalcoholic steatohepatitis, high fat/cholesterol diet, oxidative stress, inflammatory cytokines, guinea pigs

trigliserit ve CYP2E1 düzeyleri ile fibroz belirteçlerindeki yüksek seviyelerin de SAM tedavisine bağlı olarak azaldığı tespit edildi. Bu tedavi, YYKD diyeti ile beslenen kobayların karaciğerinde SAM düzeylerindeki azalmayı, prooksidan ve antioksidan dengesindeki bozukluğu iyileştirdi, steatozis, inflamasyon ve fibrozis skorlarındaki artışları azalttı.

Sonuç: Bu sonuçlar, SAM'ın lipojenez, oksidatif stres, enflamasyon ve fibrozisi azaltarak YYKD ile indüklenen NASH'ta terapotik bir ajan olarak etkili olabileceğini göstermektedir.

Anahtar Kelimeler: S-adenozilmetyonin, non-alkolik steatohepatit, yüksek yağlı/kolesterollü diyet, oksidatif stres, inflamatuvar sitokinler, kobay

INTRODUCTION

S-adenosylmethionine (SAM) is the main donor of the methyl group in the organism. It has direct antioxidant activity by scavenging reactive oxygen species (ROS) and is a precursor of glutathione (GSH), a major antioxidant in the cells. It inhibits the formation of proinflammatory molecules [tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6)], inhibits cytochrome P450-2E1 (CYP2E1) enzyme activity, and prevents mitochondrial dysfunction (1). Therefore, SAM is accepted as a hepatoprotective agent (1, 2). Indeed, SAM treatment has been found to have a protective potential against acetaminophen-(3), ischaemia-reperfusion, alcohol and cholestasis induced liver injuries and experimental alcohol plus carbon tetrachloride induced-fibrosis by inhibiting hepatic stellate cell activation (4-7).

The liver plays an important role in maintaining SAM homeostasis by regulating its synthesis and degradation. Approximately 85% of methylation reactions in the body are carried out by the liver. SAM is responsible for methylating various molecules, including phospholipids. Thus, phosphatidylcholine (PC) is formed by the methylation of phosphoethanolamine (PE). Since a low PC/PE ratio diminishes the secretion of very low density lipoproteins (VLDL) from the liver, the decrease in SAM levels impairs the export of VLDL from the liver and thus triglyceride accumulated in the liver. Furthermore, a low PC/PE ratio elevates membrane permeability and the sensitisation of liver to endotoxin-induced proinflammatory cytokines (8, 9).

Non-alcoholic fatty liver disease (NAFLD) is the most important cause of chronic liver disease. NAFLD encompasses nonalcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NASH is the severe form of NAFLD and is characterised by steatosis, inflammation, and progressive fibrosis. According to the two-hit hypothesis, steatosis is the first hit and enhances the hepatic susceptibility to subsequent secondary hits such as oxidative stress, endotoxemia, and inflammatory cytokines, which contribute to the development of NASH and other advanced pathologies such as fibrosis/cirrhosis and hepatocellular carcinoma (8, 10). However, the pathophysiology of NASH and effective pharmacological treatment tools are not yet fully clarified (11). Low hepatic SAM levels have also been suggested to play a role in NASH development by serving as a second hit (8, 9, 12), and SAM treatment may be useful in the prevention of liver damage in NAFLD/NASH (13-16). However, there are few experimental and clinical studies on NASH, and the clinical benefit of SAM remains controversial (2, 8, 17).

In our previous study, it was determined by histopathological and metabolic markers that high fat/cholesterol diet (HFCD) feeding on for six weeks caused NASH in guinea pigs (18). In addition, when SAM was administered simultaneously with HFCD in this process, it was determined that SAM reduced the NASH formation process and this effect was achieved by reducing steatosis, inflammation, fibrosis and oxidative stress and had a preventive potential. Our aim in the current study was to investigate the therapeutic effect of SAM on NASH. For this purpose, in our experimental groups, which we planned independently from our previous study, we extended the feeding period with HFCD to 10 weeks and evaluated the therapeutic effect by giving SAM together in the last four weeks. Because steatosis, inflammation, fibrosis and oxidative stress play a fundamental role in the formation and progression of NASH, our investigations were performed within the framework of these parameters.

MATERIALS AND METHODS

Chemicals

The chemical, S-adenosyl-L methionine disulphate tosylate (SAM), was donated by Pure Encapsulations, Inc. (Sudbury, MA, USA). Cholesterol was purchased from Alfa Easer (Kandel, Germany), and other chemicals were obtained from Sigma-Aldrich (Darmstadt, Germany).

Animals and the experimental design

Dankin Hartley guinea pigs, weighing 600-650 g, were obtained from Aziz Sancar Institute of Experimental Medicine, İstanbul University. The animals were housed in a light- and temperature-controlled room on a 12 h:12 h light:dark cycles in stainless steel cages (two or three

per cage). The experimental procedures used in this study were approved by the İstanbul University Animal Care and Use Ethics Committee (Date: 02.03.2108, No: 2018/18).

Animals were divided into four experimental groups (each n=6) as follows: a) Control group: Animals were fed a normal guinea pig diet for 10 weeks and injected with 0.9% NaCl as a vehicle in the last four weeks. b) Control SAM group: They received a normal diet for 10 weeks and injected with SAM (50 mg/kg; five days per week; i.p.; freshly dissolved in 0.9%NaCl) in the last four weeks. c) HFCD-10w group: Guinea pigs were fed an HFCD diet (81% standard guinea pig chow diet, 1% cholesterol, 8% yolk powder and 10% beef tallow) for 10 weeks. d) SAM+HFCD-10w group: Guinea pigs were fed HFCD for 10 weeks as described above and were injected with SAM (50 mg/kg; freshly dissolved in 0.9% NaCl solution; five days per week; i.p) in the last 4 weeks.

The dose and duration of SAM used in our study are based on previous studies (15, 18, 19). Diets were prepared by the Barbaros Denizeri Company (Gebze) and kept at 4°C. There were no restrictions on water and food for animals, and food and drinking water intake were periodically monitored.

Samples

At the end of the experimental period, the guinea pigs were fasted overnight. They were then anaesthetised with ketamine (40 mg/kg, i.p., Pfizer, USA) and xylazine HCl (5 mg/kg, i.p., Bioveta, Czech Republic). Blood samples were collected via cardiac puncture into dry tubes and, then centrifuged at 1500xg for 10 min to separate the sera. The liver tissues from the animals were homogenised in ice-cold phosphate-buffered saline (PBS; 0.01M, pH: 7.4), then they were centrifuged at 600xg for 10 min at 4°C, and the supernatants were used for biochemical analyses in the liver. Both serum and liver tissue were stored at -80°C until analysed.

Hepatic damage markers in the serum

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in an autoanalyzer (Cobas Integra 800, Roche Diagnostics, Germany) to evaluate hepatic damage in the serum using enzymatic methods.

Determination of the glucose and insulin levels in the serum

Total cholesterol (TC), triglyceride (TG), and fasting glucose levels were determined using an autoanalyser (Cobas Integra 800, Roche Diagnostics, Germany). Serum insulin levels were estimated using guinea pig insulin ELISA kits (#KTE120010, Abbkine, Wuhan, China) in accordance with the manufacturer's instructions. Glucose and insulin levels were used to calculate the homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR), an index of insulin resistance, which is defined as follows: fasting insulin levels (pmol/L) x fasting glucose levels (mmol/L)/135 (20).

Determination of TNF-α and IL-6 levels in the serum

TNF-α (#KTE120004, Abbkine, China) and IL-6 (#KTE120003, Abbkine, China) levels were measured using ELISA kits according to the manufacturers' instructions. Results are expressed in nanograms per L.

Determination of lipids in the liver

Hepatic TC (#87356, Biolabo Biochemistry and Coagulation, France) and TG (#87319, Biolabo Biochemistry and Coagulation, France) levels were assayed using commercial colorimetric kits in lipid extracts obtained from the tissues. Results were expressed as µmol per g liver.

Determination of SAM, hydroxyproline (Hyp), and CYP2E1 levels in the liver

Hepatic SAM (#201-01-1072, Sunred Bio, Shanghai, China), Hyp (#E0148Gp, Bioassay Technology Laboratory, Shanghai, China) and CYP2E1 (#KTE120024, Abbkine, China) levels were measured in liver homogenates using ELISA kits according to the manufacturers' instructions.

Assessment of oxidative stress parameters in the liver

The level of reactive oxygen species (ROS) in the liver homogenates was assayed using a fluorescent compound (2',7'-dichlorodihydrofluorescein diacetate) that is sensitive to oxidation (21). Fluorescence intensities were detected at Ex 485/Em 538 using a Fluoroskan Ascent microplate fluorometer from Thermo Scientific Inc., USA. Results were given as relative fluorescence units per mg protein.

Hepatic lipid peroxidation was assessed by determining the levels of thiobarbituric acid reactive substances (TBARS) and diene conjugate (DC) levels. TBARS was determined using the spectrophotometric method developed by Buege and Aust (22). The liver homogenate and Buege-Aust reagent (consisting of 26 mM thiobarbituric acid and 0.92 M trichloroacetic acid in 0.25 M HCl), the mixture was heated in boiling water for 15 min. Following cooling and centrifugation at 1000g, the absorbances of the resulting supernatants were read at 532 nm. The results were computed using a molar extinction coefficient of $1.56 \times 10^{-5} M^{-1} cm^{-1}$. Results of TBARS were expressed in pmol per mg protein. For this assay, the levels of DC in the hepatic lipid extracts were also measured spectrophotometrically at 233 nm. For this assay, tissue lipids were extracted with a chloroform/methanol (2:1, v:v) mixture. The extracted lipids were evaporated and dissolved in cyclohexane. The absorbances were read at 233 nm, and the results were computed using a molar extinction coefficient of $2.52 \times 10^{4} M^{-1} cm^{-1}$ (22). Results of DC were expressed in nmol per mg protein.

The level of oxidative protein damage in the liver homogenates was assessed by measuring the protein carbonyl (PC) groups using a method developed by Reznick and Packer (23). This involved calculating the absorbance of the protein hydrazone formed by reacting the protein carbonyls with 2,4-dinitrophenylhydrazine, and the absorbances were read at 360 nm. The results were then calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1}. Results were expressed in nmol per mg protein.

Glutathione (GSH) levels were measured spectrophotometrically using 5,5-dithiobis (2-nitrobenzoic acid) as an indicator, following the method described by Beutler et al. (24). The ferric reducing antioxidant power (FRAP) assay was used for the spectrophotometric determination of the antioxidant power of the liver homogenates, based on the method outlined by Benzie and Strain (25). In this assay, the ferric-tripyridyltriazine complex was reduced to the ferrous form, which is blue coloured and the absorbances were monitored at 593 nm. Results were expressed in nmol per mg protein.

Protein levels in the liver homogenates were determined using the bicinchoninic acid assay, with serum albumin serving as the standard (26).

Histopathological analysis

Liver tissues were fixed in a 10% formalin buffer solution for 24 h before embedding in paraffin. After that, 5 µm slides were obtained from each paraffin block, and all paraffin was removed from the slides and stained with haematoxylin and eosin (H&E) for histological examinations. Reticulin staining was also performed to show reticulin fibres of fibrotic areas. Steatosis, liver damage, and fibrosis scores were calculated according to the protocol proposed by Goodman, which was previously reported by us (20, 27).

Steatosis was scored as 0 = <5% (none), 1 = 5%–33% (mild), 2 = 34%–66% (moderate), 3 = ≥67% (severe). Fibrosis was classified using Ishak's staging (27). 0 = no fibrosis, 1 = fibrous expansion of some portal areas, with or without short fibrous septa, 2 = fibrous expansion of most portal areas, with or without short fibrous septa, 3 = fibrous expansion of most portal areas with occasional portal to portal bridging, 4 = fibrous expansion of portal areas with marked bridging, 5 = marked bridging with occasional nodules, 6 = cirrhosis, probable or definite.

Liver damage parameters were scored as follows: 0 = no visible cell damage, 1 = focal damage on <25% of the tissue (mild), 2 = focal damage on between 26 and 50% of the tissue (moderate), 3 = extensive lesions in >51% of the tissue (severe), 4 = global lesion (global) (27).

Protein expressions of α-smooth muscle actin (α-SMA) and transforming growth factor-β1 (TGF-β1)

The immunochemistry analysis was used to measure the expressions of α-SMA and TGF-β1 in the liver, as described previously (18, 20). Briefly, liver sections were incubated with α-SMA (dilution 1:100, #ABP52852, rabbit polyclonal, Abbkine, Wuhan, China) and TGF-β-1 (dilution 1:100, #APB52598, rabbit polyclonal, Abbkine, Wuhan, China) as primary antibodies for 1 h at room temperature. Negative control sections treated with phosphate-buffered antibodies were confirmed to be unstained. In addition, positive control studies were conducted in sections of healthy human liver. The secondary antibody reacted with the sections for 25 min. AEC (ScyTek Laboratories, Inc.205 South 600 West Logan, UT 84321, USA) chromogen was used to visualise the reaction, and the sections were then washed in distilled water. The presence or absence of brown staining was considered indicative of a positive or negative result for each antibody, respectively. The sections were evaluated under the light microscope and the score was made up of 0-5% positive cells as (-), 5- 30% positive cells as (+), 30-60% positive cells as (++) and 60% and over positive cells as (+++). Digital photographs were assessed using the Olympus AnalySIS five image analysis programme.

Statistical analysis

Results are presented as mean±standard deviation (SD). The normality of the results was tested using the Kolmogorov-Smirnov test. Parametric data was analysed using a one-way ANOVA test with post-hoc Tukey's test, while non-parametric data was compared using the Kruskal-Wallis test with post-hoc Mann Whitney-U test. A P-value <0.05 was considered statistically significant. The analyses were performed using SPSS for Windows, version 21.0 (IBM SPSS Corp., Armonk, NY, USA).

RESULTS

SAM treatment did not alter the increases in liver weight and liver index values in the HFCD group

Body weight did not alter in the HFCD and SAM+HFCD groups compared with the controls. However, the liver weight and liver index values remained unchanged in the HFCD group due to SAM treatment (Table 1).

SAM treatment decreased the high levels of ALT and AST activities in the serum of the HFHC group

Serum ALT and AST activities were significantly elevated in guinea pigs fed HFCD. SAM treatment decreased these enzyme activities in the HFCD group (Table 1).

SAM treatment reduced the high levels of TC and HOMA-IR in the serum of the HFCD group

In the HFCD group, the serum TC increased, but not the TG levels. Additionally, serum glucose and insulin levels and HOMA-IR values also shown an increase. However, among these parameters, the serum TC and glucose levels, as well as the HOMA-IR values, diminished due to SAM treatment in the HFCD group (Table 2).

Table 1: The effect of SAM treatment on body weight, liver weight and liver index values and ALT and AST activities in guinea pigs fed on HFCD (mean±SD; n=6, each)

	Control	SAM	HFCD	SAM+HFCD
Body weight (g)	746.7±40.8	730.0±28.3	689.0±98.1	715.4±37.2
Liver weight (g)	34.8±2.86	34.3±2.71	69.8±8.11[a]	68.5±12.3[a]
Liver index* (%)	4.68±0.54	4.71±0.39	10.2±0.54[a]	9.51±1.28[a]
ALT (U/L)	51.2±6.27	53.0±6.16	127.1±22.4[a]	65.8±7.01[a,b]
AST (U/L)	95.5±15.0	99.0±16.7	757.5±68.9[a]	379.5±71.9[a,b]

[a]: p<0.05 as compared to control, [b]: p<0.05 as compared to HFCD group, *Liver weight x100/body weight, SAM: S-adenosylmethionine, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HFCD: High-fat/cholesterol diet.

Table 2: The effect of SAM treatment on serum TC, TG, glucose, insulin, HOMA-IR levels, as well as TNF-α and IL-6 levels in guinea pigs fed on HFCD (mean±SD; n=6, each)

	Control	SAM	HFCD	SAM+HFCD
TC (mmol/L)	1.55±0.47	1.53±0.30	8.93±1.16[a]	5.78±0.75[a,b]
TG (mmol/L)	0.83±0.14	0.80±0.11	0.90±0.12	0.79±0.08
Glucose (mmol/L)	8.24±1.38	8.14±1.37	11.0±1.01[a]	8.72±0.78[b]
Insulin (pmol/L)	27.6±3.04	25.1±2.26	29.6±1.02[a]	27.8±0.86
HOMA-IR	1.61±0.38	1.53±0.36	2.34±0.09[a]	1.80±0.16[b]
TNF-α (ng/L)	19.0±2.74	17.5±2.09	27.4±2.34[a]	20.3±2.11[b]
IL-6 (ng/L)	23.9±2.19	22.3±2.32	32.6±5.03[a]	22.3±2.33[b]

[a]: p<0.05 as compared to control, [b]: p<0.05 as compared to HFCD group, SAM: S-adenosylmethionine, TC: Total cholesterol, TG: Triglyceride, HOMA-IR: Homeostasis model assessment for insulin resistance, TNF-α: Tumour necrosis factor-alpha, IL-6: Interleukin-6, HFCD: High-fat/cholesterol diet.

SAM treatment reduced the high levels of TNF-α and IL-6 in the serum of the HFCD group

Both TNF-α and IL-6 levels were detected to increase in the serum of the HFCD group. The SAM treatment diminished the levels of these cytokines in the HFCD group (Table 2).

Changes in the liver histology

The control and SAM-control groups showed normal hepatic architecture. The HFCD group exhibited severe macrovesicular steatosis, along with fibrous bands and mild chronic infiltration between the central and portal veins. Sinusoids could not be observed. Increased reticulin fibres, which form bundles in fibrous bands, were detected by reticulin staining. Less steatosis was observed in guinea pigs with HFCD due to SAM treatment. Furthermore, the fibrous bands were thinner and had a shorter course. The reticulin stain demonstrated that reticular fibres reduced, and the bundles became thinner and had a shorter course due to SAM treatment (Figure 1).

The reticulin stain revealed an increase in reticulin fibres forming bundles in the fibrous bands. In guinea pigs with HFCD, less steatosis was observed due to SAM treatment. Additionally, the fibrous bands were thinner and had a shorter course. The reticulin stain demonstrated a reduction in reticular fibres, with the bundles becoming thinner and having a shorter course due to SAM treatment (Figure 1).

According to the histopathological scorings, SAM treatment significantly diminished the increased hepatic steatosis, inflammation, and fibrosis scores in the HFCD group (Figure 2).

SAM treatment increased hepatic SAM levels and decreased lipid, Hyp, and CYP2E1 levels in the HFCD group

The hepatic SAM levels significantly decreased in the HFCD group compared to the controls. However, TC and TG as well as Hyp and CYP2E1 levels increased significantly in the liver of the HFCD group. SAM treatment caused increases in SAM levels and decreased hepatic TC, TG, Hyp and CYP2E1 levels in the HFCD group (Figure 3).

SAM treatment reduced hepatic oxidative stress parameters in the HFCD group

The levels of oxidant parameters [ROS, lipid peroxides (TBARS and DC) and protein oxidation products (PC)] significantly increased, while the levels of antioxidant

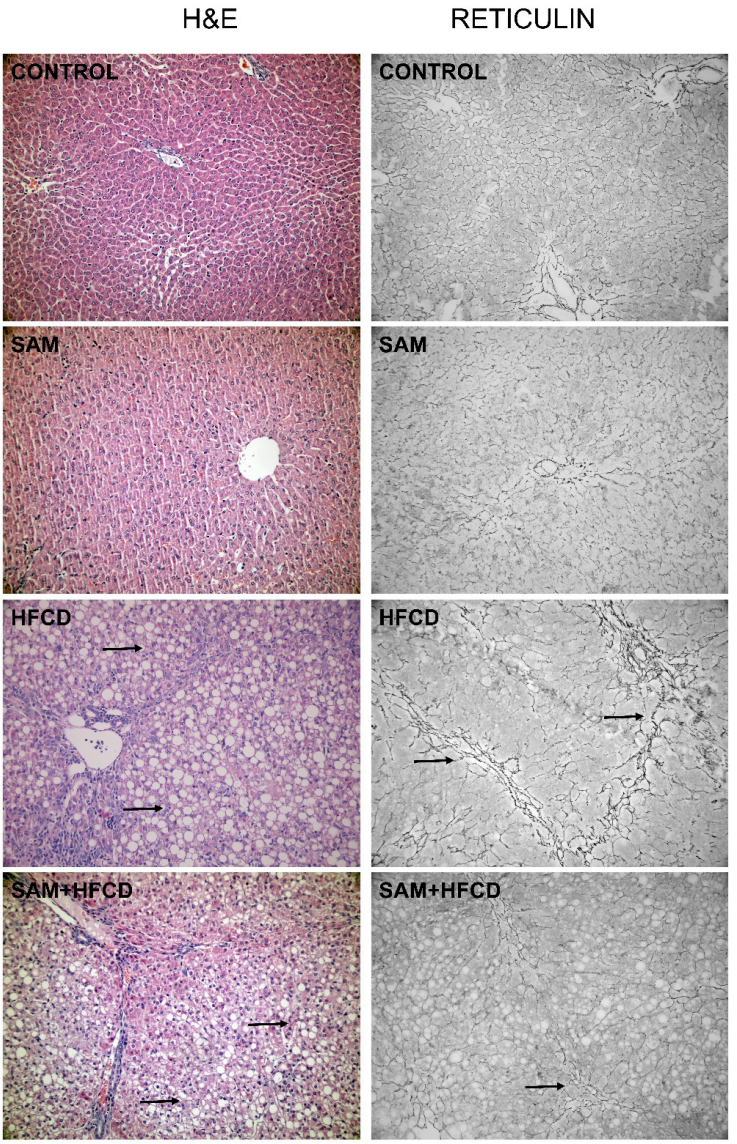

Figure 1: The effect of S-adenosylmethionine treatment on the hepatic and histopathology of guinea pigs fed a high-fat/cholesterol diet. (Haematoxylin and eosin x200 and Reticulin x200). Arrows in haematoxylin and eosin staining of the liver (first column) indicate macrovesicular steatosis, arrows in reticulin staining (second column) also show reticular fibres. Groups: Control; SAM; HFCD; SAM + HFCD. H&E: Haematoxylin and eosin, HFCD: High-fat/cholesterol diet, SAM: S-adenosylmethionine

parameters (GSH and FRAP levels) decreased in the HFCD group. SAM treatment diminished oxidant parameters and increased antioxidant parameters in the HFCD group (Figure 4).

Changes in the hepatic α-SMA and TGF-β1 protein expressions

Significant increases in α-SMA and TGF-β1 protein expressions were observed in guinea pigs fed the HFCD. However, these expressions decreased in the HFCD group due to SAM treatment (Figure 5).

DISCUSSION

Diets rich in fat, fructose or cholesterol or their combinations and the methionine-choline deficiency (MCD) diet are dietary experimental models used to understand the pathogenesis of NAFLD/NASH and to test treatment possibilities (28). The development of fibrosis was the primary feature that distinguished NASH from NAFLD. It has been suggested that fibrosis development is probably influenced not only by dietary cholesterol but also by the interaction between dietary

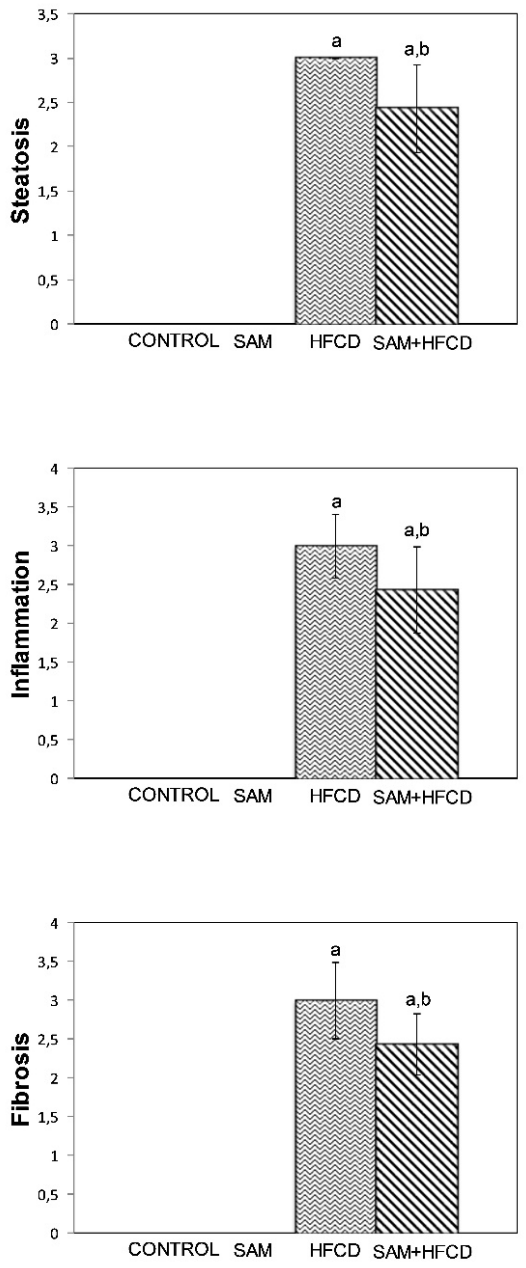

Figure 2: The effect of S-adenosylmethionine treatment on hepatic steatosis, inflammation, and fibrosis scores in guinea pigs fed a high-fat/high-cholesterol diet. No steatosis, inflammation and fibrosis were seen in the control and S-adenosylmethionine groups. The high-fat/cholesterol diet group exhibited severe macrovesicular steatosis, along with fibrous bands and mild chronic infiltration between the central and portal veins. The high-fat/high-cholesterol diet + S-adenosylmethionine groups showed decreases in steatosis inflammation and fibrosis scores (Mean±SD; n=6; each). Groups: Control; SAM; HFCD; SAM + HFCD. HFCD: high-fat/cholesterol diet. SAM: S-adenosylmethionine. [a]: $p<0.05$ as compared to control, [b]: $p<0.05$ as compared to HFCD group

cholesterol and dietary fat (29,30). Therefore, HFCD is recognised as a suitable dietary experimental model for inducing hepatic steatosis and inflammation and NASH in mice and rats (29, 30). Unlike rats and mice, guinea pigs and humans share an LDL-dominant lipoprotein profile and show a high degree of similarity with humans in terms of hepatic lipid metabolism, inflammation, and fibrogenesis (32). Guinea pigs are susceptible to HFCD-induced NASH and are used as a suitable model to study the pathogenesis of the disease (31, 33).

In guinea pigs fed on HFCD for 10 weeks, the increases were detected in ALT and AST activities, indicating liver damage, TC and inflammatory cytokine (TNF-α and IL-6) levels as well as HOMA-IR values in their serum of guinea pigs. Hepatic TC, TG and Hyp levels, profibrotic α-SMA and TGF-β1 protein expressions were increased. Hepatic histopathological observations also showed significant increases in steatosis, inflammation, and fibrosis scores in this group. All these results demonstrated the occurrence of NASH due to HFCD feeding, which is characterised by increased steatosis, inflammation, fibrosis and hepatocyte necrosis, consistent with previous reports (29-31). HFCD feeding also caused an increase in ROS levels in the liver of guinea pigs. As is known, ROS produced by CYP2E1 plays an important role in triggering oxidative stress in NASH (1, 34). In this study, increased ROS levels were associated with higher formation of lipid peroxides (such as TBARS, DC) and protein oxidation products (such as PC). The decrease in the antioxidant parameters (GSH and FRAP) reflects the deficiency in the antioxidant power. The results of the study indicate that a pro-oxidant state is present in the livers of guinea pigs with NASH, as previously reported (35-37).

In chronic liver diseases, SAM levels decreased due to increased use as an antioxidant and/or a decrease in its synthesis (8, 17, 38). Since methionine adenosyltransferase 1 (MAT1), the key enzyme in SAM synthesis, is an enzyme sensitive to oxidation, it may be inhibited under conditions where oxidative stress is induced (17, 38). In MAT1-deficient mice, steatosis was detected to progress towards NASH, and SAM treatment reduced hepatic damage, ALT and AST activities, and TG levels in these mice (9). Therefore, it has been suggested that SAM homeostasis may have an active role in the pathogenesis of NASH and that SAM supplementation can be used as a therapeutic agent in NAFLD/NASH (8, 12).

Some investigators have tested whether SAM treatment is effective in experimental dietary models of NAFLD/NASH. SAM treatment was able to ameliorate fatty acid-induced lipid accumulation and oxidative stress through promoting β-oxidation in hepatocyte cultures (39). Similarly, the administration of SAM decreased fatty liver and oxidative

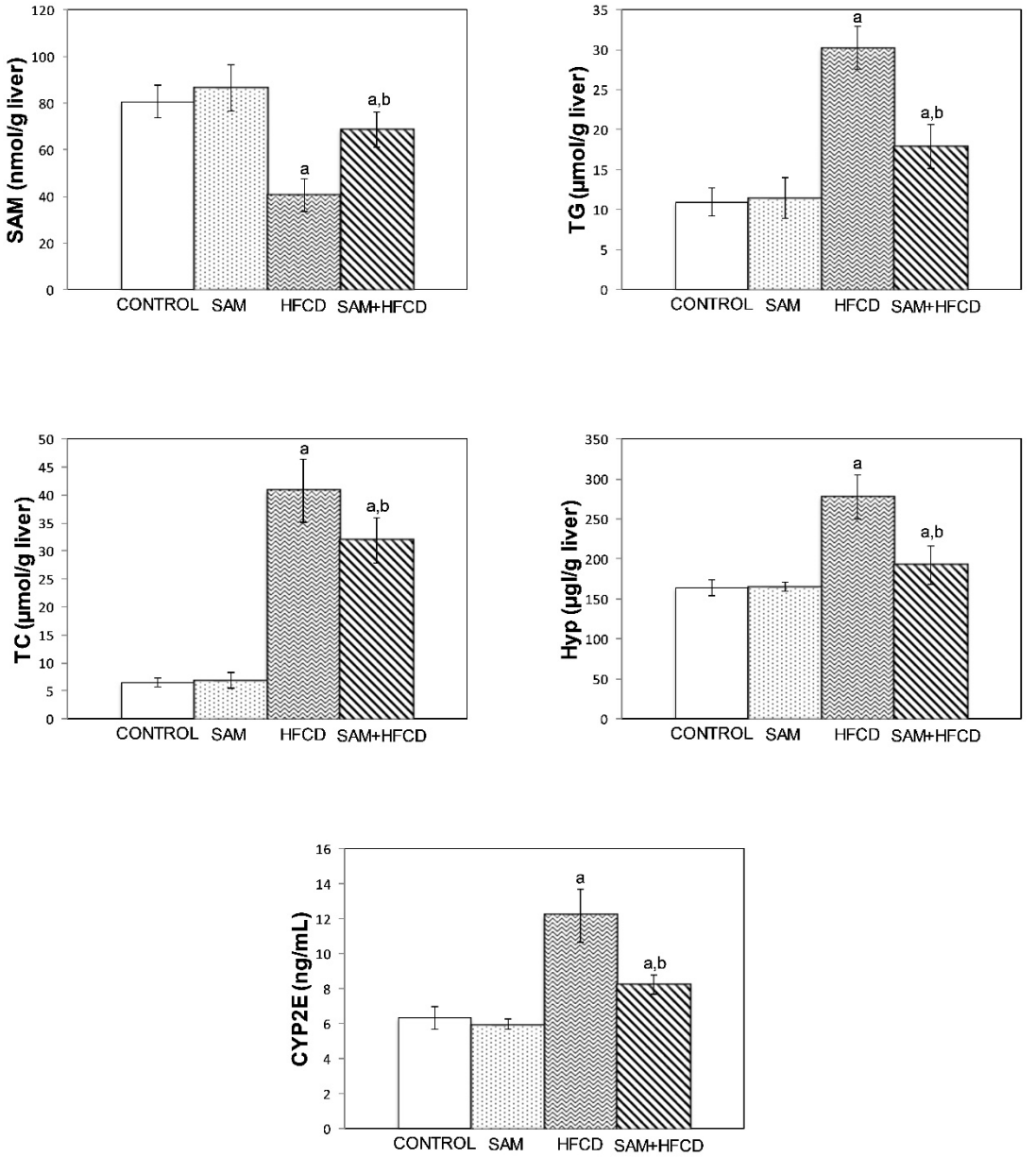

Figure 3: The effect of S-adenosylmethionine treatment S-adenosylmethionine, hepatic total cholesterol, triglyceride, hydroxyproline (Hyp), and cytochrome P450-2E1 (CYP2E1) levels in guinea pigs fed on a high-fat/cholesterol diet (HFCD). (Mean±SD; n=6; each). CYP2E1: cytochrome P450-2E1, HFCD: high-fat/cholesterol diet, S-adenosylmethionine, Hyp: hydroxyproline, total cholesterol: TC, triglyceride: TG. [a]: $p<0.05$ as compared to the control, [b]: $p<0.05$ as compared to the HFCD group

stress in the liver of rats fed a high fructose diet induced NAFLD (13). Moreover, in mice fed an MCD diet, SAM administration reduced liver damage by increasing hepatic SAM and GSH levels and by downregulating the expression of inflammatory and fibrogenic cytokines (16). On the other hand, angiotensin II (Ang II), as the main component of the renin-angiotensin system (RAS), is known to influence lipid metabolism and insulin sensitivity via its receptor, Ang II type 1 receptor (AT1R), thereby contributing to NAFLD progression (40). SAM was reported to prevent intrahepatic RAS activation by upregulating the expression of the AT1R-associated protein (ATRAP), an inhibitor of AT1R. Therefore, it has been suggested that SAM may be useful as a therapeutic agent in the prevention of NAFLD/NASH through this mechanism (15). Indeed, it has been reported that both hepatic SAM levels and ATRAP protein expression are decreased in patients with NAFLD. Similar results were also found in HFCD-fed rats, andwhen SAM

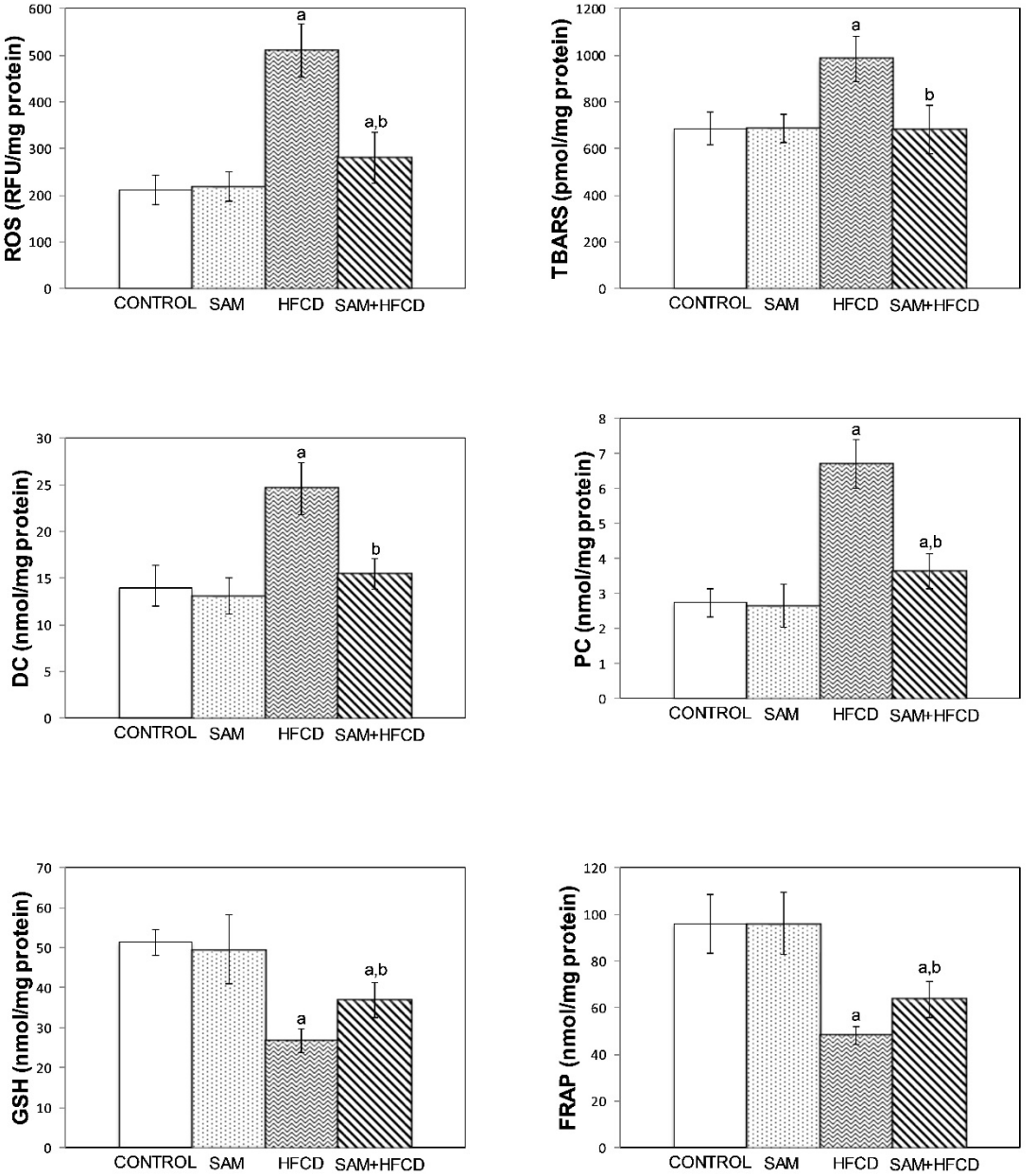

Figure 4: Hepatic reactive oxygen species, thiobarbituric acid reactive substances, diene conjugate and protein carbonyl, glutathione and ferric reducing antioxidant power levels in guinea pigs fed on a high-fat/cholesterol diet. (Mean±SD; n=6 each). DC: diene conjugate, FRAP: ferric reducing antioxidant power, GSH: glutathione, HFCD: high-fat/cholesterol diet, PC: protein carbonyl, ROS: reactive oxygen species, TBARS: thiobarbituric acid reactive substances. [a]: $p<0.05$ compared to control, [b]: $p<0.05$ compared to HFCD group

was administered to these rats, the decreased ATRAP expression was restored and steatosis alleviated (14). In our previous study, SAM was administered to guinea pigs together with HFCD for six weeks and SAM was found to have a preventive effect on NASH (18). In contrast, SAM treatment alone was not effective, but its combination with dilinoleoylphosphatidylcholine (DLPC) prevented CYP2E1 activation, TG accumulation, oxidative stress and fibrotic changes in the liver of rats fed on a high-fat diet (41). However, in these experimental studies, SAM was administered along with different dietary models of NAFLD/NASH in the same period.

In the current study, guinea pigs were given HFCD for 10 weeks and SAM treatment was administered in the last 4 weeks of this treatment. Thus, we investigated whether SAM may show a therapeutic potential in pre-existing

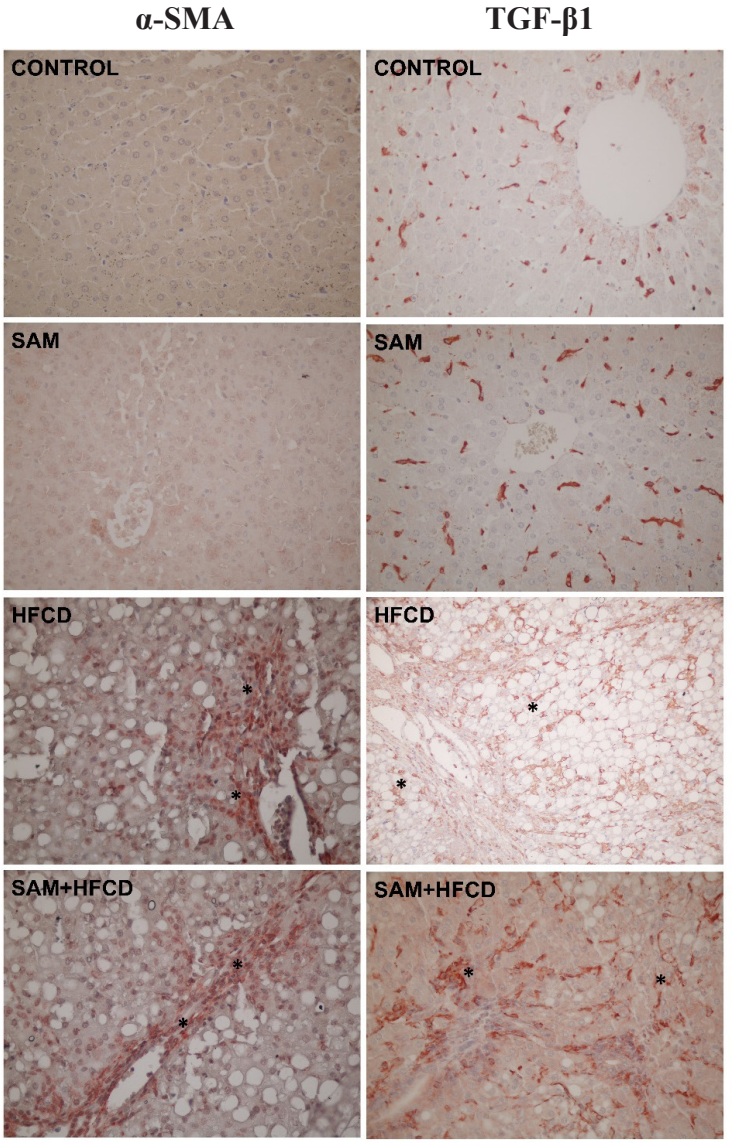

Figure 5: The effect of S-adenosylmethionine treatment on hepatic α-smooth muscle actin and transforming growth factor-β1 protein expression of guinea pigs fed a high-fat/cholesteroldiet (x400). Groups: Control; SAM; HFCD; SAM + HFCD. While no abnormal staining was observed in the control and SAM groups, positive staining was observed in the HFCD and SAM+HFCD groups (asterix). Positive staining was detected in the cells within the fibrous bridging areas between the central vein and the portal region with α-SMA, as well as in the immature mesenchymal cells with the TGF-β1. α-SMA: α-smooth muscle actin HFCD: high fat/cholesterol diet, S-adenosylmethionine, TGF-β1: transforming growth factor- β1

NASH. It was observed that SAM treatment decreased ALT and AST activities, TC, TNF-α and IL-6 levels in serum along with insulin resistance in guinea pigs with NASH. In addition, SAM treatment decreased hepatic TC, TG and Hyp levels and α-SMA and TGF-β1 protein expressions; histopathological improvements were observed with a decrease in steatosis, inflammation and fibrosis scores. Furthermore, a decrease in CYP2E1 levels and improvement in the pro-oxidant-anti-oxidant balance were detected, indicating that SAM effectively reduces oxidative stress in HFCD-induced NASH.

In conclusion, SAM seems to alleviate hepatic injury and metabolic disorders in HFCD-induced NASH by reducing lipogenesis, oxidative stress, inflammation, and fibrosis. In addition to the preventive effect of SAM on NASH observed in our previous study (18), this study demonstrated that SAM may also have therapeutic efficacy.

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REVIEW / DERLEME
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BREAST MILK IN ALL ASPECTS

HER YÖNÜYLE ANNE SÜTÜ

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ABSTRACT

Exclusive breastfeeding for the first six months and continuing to breastfeed until at least 2 years of age is recommended by the Ministries of Health of countries worldwide. Breastfeeding contributes to health not only through its nutritional but also immunological properties. Breast milk has unique ingredients that ensure healthy growth. It is rich in bioactive substances such as growth factors; cytokines, microRNAs, human milk oligosaccharides, and cells. Epigenetic studies have identified differences in gene expression between breastfed and formula-fed infants. DNA methylation (DNAm) has been suggested as a mechanism underlying the long-term health effects of breastfeeding. Breastfeeding promotes proper maxillofacial growth and development by stimulating intense oral muscular activity, promoting correct lip closure, mandibular function, and tongue positioning on the palate. In economics, merit good is generally accepted as good (product) that societies should consume regardless of individuals' wishes or demands. The most typical examples of merit products are education and health. From an economic perspective, it can be concluded that breast milk is an economically merit product, and the states should allocate resources by using their public power to increase breastfeeding. Pharmacists and nurses play important and crucial roles in supporting and assisting mothers during the breastfeeding period. Nurses should always play an active role in initiating and maintaining breastfeeding at every stage of life. In this review, breast milk is discussed in every aspect.

Keywords: Breastfeeding, health, economics, immunology, epigenetic

ÖZET

İlk altı ay sadece anne sütü ile beslenmeyi ve en az iki yaşına kadar emzirmeye devam etmeyi dünya çapında ülkelerin sağlık bakanlıkları önermektedir. Emzirme sadece besleyici değil aynı zamanda immünolojik özellikleriyle de sağlığa katkıda bulunur. Anne sütü, sağlıklı büyümeyi sağlayan benzersiz bileşenlere sahiptir. Büyüme faktörleri, sitokinler, mikroRNA'lar, anne sütü oligosakkaritleri ve hücreler gibi biyoaktif maddeler açısından zengindir. Epigenetik çalışmalar, anne sütü ile beslenen ve mama ile beslenen bebekler arasında gen ifadesinde farklılıklar tespit etmiştir. DNA metilasyonu (DNAm), emzirmenin uzun vadeli sağlık etkilerinin altında yatan bir mekanizma olarak öne sürülmüştür. Emzirme, yoğun oral kas aktivitesini uyararak, doğru dudak kapanmasını, mandibular fonksiyonu ve dilin damak üzerinde konumlanmasını teşvik ederek uygun maksillofasiyal büyüme ve gelişmeyi destekler. Ekonomide "erdemli mal" genellikle bireylerin istek ve taleplerinden bağımsız olarak toplumların tüketmesi gereken mal (ürün) olarak kabul edilir. Erdemli ürünlerin en tipik örnekleri eğitim ve sağlıktır. Belirtilen iktisadi bakış açısı ile anne sütünün ekonomik olarak erdemli bir ürün olduğu ve devletlerin emzirmeyi artırmak için kamu gücünü kullanarak kaynak ayırması gerektiği sonucuna varılabilir. Eczacılar ve hemşireler, emzirme döneminde annelere destek ve yardımcı olma konusunda önemli ve hayati bir role sahiptir. Hemşireler emzirmenin başlatılması ve sürdürülmesinde yaşamın her aşamasında aktif rol oynamalıdırlar. Bu derlemede anne sütü her yönü ile ele alınarak tartışılmıştır.

Anahtar Kelimeler: Emzirme, sağlık, ekonomi, immünoloji, epigenetik

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INTRODUCTION

Academicians from six different institutions of Istanbul University (Faculty of Dentistry, Faculty of Economics, Faculty of Medicine, Faculty of Nursing, Faculty of Pharmacy, and Institute of Child Health) came together as speakers for the symposium "Breast Milk with All Aspects" on December 27, 2023 at the Doctorate Conference Saloon of the historical main building of Istanbul University. The aim of the symposium held under the theme of Child Care, Health and Development from Tradition to Future in the 100th Anniversary of our Republic was to discuss different aspects of breast milk from the perspective of different disciplines; to share the accumulated knowledge created by the institutions of Istanbul University in this field, to carry out joint studies, to carry out multidisciplinary projects, to conduct researches, to examine the results of researches that have been carried out or are being carried out in order to bring breastfeeding to the levels targeted in the United Nations sustainable development goals together with different disciplines. This article aims to convey the main themes of the symposium with the multidisciplinary approach and to explain the importance of breast milk in all aspects.

Role of breast milk in strengthening immunity

Exclusive breastfeeding for the first six months and continuing to breastfeed until at least 2 years of age is recommended by the Ministries of Health of countries worldwide. It has been reported that with this proposal, over 800,000 child deaths and at least 20,000 maternal deaths will be prevented, and an economic gain of 300 million dollars will be achieved (1). The 2025 target of the Global Nutrition Goal is to increase the rate of exclusive breastfeeding within the first six months to at least 50%. The central part of the Sustainable Development Goals (SDG) for 2030 is breastfeeding. Breastfeeding is at the centre of the first, eighth, and tenth goals of the SDGs, which aim to end poverty and economic growth worldwide; the twelfth goal aims to have the least ecological footprint; the fourth goal aims to achieve global learning goals; and the second and third goals aim to prevent hunger and ensure healthy individuals (2).

Breast milk has unique ingredients that ensure healthy growth. Breastfeeding contributes to health not only through its nutritional but also immunological properties. This contribution begins with the mother's first milk after birth. The first milk, called colostrum, is a source of nutrients and has a high immunoglobulin (Ig) content (3). Although all Ig subtypes are present in colostrum, the largest component is secretory IgA (sIgA). SIgA, which is found in breast milk, particularly in colostrum, has a protective effect against infections. In a study conducted by Juncker et al. in the Netherlands, IgA antibodies specific to SARS-CoV-2 were detected in breast milk, and it was reported that this high antibody level caused passive immunity in many breastfed babies and protected them against COVID-19 infection (4). Studies have shown that breast milk protects against infections not only because of its sIgA content but also because of its IgG and IgM antibody contents (5-7). Because of these ingredients, promoting and protecting breastfeeding in extraordinary situations such as pandemics is of great importance for the health of the child, mother, and society (8).

The lysozyme content of breast milk is another factor that contributes to the immunological and immunomodulatory effects of breastfeeding. In addition to causing bacterial lysis, lysozyme provides a synergistic effect with Ig and lactoferrin. Lactoferrin in breast milk also has a broad antimicrobial spectrum and has been considered to prevent excessive immune response by blocking inflammatory cytokines (3, 7, 9). Mothers infected with the Hepatitis B virus will not pose a risk of virus transmission to their babies through breastfeeding because of the lactoferrin content of their breast milk, according to the findings of an in vitro study (10).

Another immunomodulator found in breast milk is human alpha-lactalbumin, which is lethal to tumour cells (HAMLET). HAMLET is a protein-lipid complex consisting of alpha-lactalbumin and oleic acid that is lethal to tumour cells. In a meta-analysis examining the relationship between breastfeeding and childhood cancers, breastfeeding was found to reduce the risk of leukaemia by 0.77 for both breastfeeding at any time and for long breastfeeding durations, emphasising the protective effect of milk (11).

Breast milk is rich in bioactive substances such as growth factors, which are important in organogenesis; cytokines with immunomodulatory effects; microRNAs effective in epigenetic regulation; human milk oligosaccharides with antipathogenic, immunomodulatory, anti-inflammatory, and prebiotic effects; and cells such as stem cells, lymphocytes, natural killer cells, and neutrophils. Factor content is another component of breast milk that contributes to immunity (12, 13).

Epigenetic perspective on breast milk

The term 'epigenetics' is used to describe the changes in the expression of genetic information encoded in DNA without any change in the structure or sequence of DNA, with the suffix 'epi' meaning 'above' in Latin, meaning 'genetics above genes'. Epigenetics are natural control mechanisms that affect gene expression (14). Considering that the genome is like a computer; Genetics (Genome) is the hardware (functional activity) of the computer. Epigenetics (Epigenome) is a software that tells the computer when to work, how to work, and how long to work. Epigenetics is like an on/off switch that regulates the operation of genes. Epigenetic switches and labels

turn off or on the expression of certain genes. The main epigenetic mechanisms include DNA methylation, histone modification, noncoding RNAs, such as microRNAs, and RNA-associated silencing. Recent epigenetic studies have identified differences in gene expression between breastfed and formula-fed infants. DNA methylation (DNAm) has been suggested as a mechanism underlying the long-term health effects of breastfeeding (15).

DNA methylation (DNAm), the most extensively studied epigenetic mechanism associated with early life nutrition, involving the addition or removal of a methyl group to cytosine-guanine dinucleotides (CpGs), has been suggested as a key factor in the long-term health effects of breastfeeding. In the study examining the relationship between breastfeeding and DNA methylation in the peripheral blood cells of 37 children aged 9 months to 4 years, the Epigenome-Wide Association Study (EWAS) method was used (16). As a result, significant differences between breastfeeding duration and methylation level were detected for 2635 genes. According to the functional analysis, these genes were predominantly involved in the control of cell signaling systems and, most importantly, in the development and function of the immune and central nervous systems (CNS). In a case-control study of asthma in 200 children, the duration of breastfeeding (none, less than 3 months, more than 3 months) was associated with different patterns of whole-genome methylation (17). Leptin (LEP) is a hormone important in growth, insulin sensitivity, and appetite control. LEP promoter methylation was examined in relation to breastfeeding duration in toddlers, and LEP promoter methylation in white blood cells was lower and serum leptin levels were higher in children who were breastfed for at least 1 to 3 months than in children who were never breastfed (18). Recent studies have shown that breastfeeding is negatively associated with the promoter methylation of LEP, CDKN2A (gene involved in tumour suppression), and SLC2A4 (gene encoding an insulin-related glucose transporter) and positively correlated with Nyp (gene encoding an orexigenic neuropeptide). In addition, breastfeeding duration modulates the epigenetic effects of global methylation patterns and genetic variants (19).

In breast milk, some noncoding RNAs that play a role in epigenetic mechanisms have been identified, but their function is less well known. MicroRNAs (miRNAs) surrounded by membranous microvesicles called exosomes play a crucial role in horizontal miRNA transfer. Long non-coding RNAs and miRNAs in milk exosomes, along with breast milk stem cells, survive digestion, enter the bloodstream, and cross the blood-brain barrier. Some of these non-coding RNAs may regulate genes involved in brain development and function, whereas nestin-positive stem cells can differentiate into neural cells and potentially serve as epigenetic regulators in the brain (20). A study conducted on 18 pregnant women found a strikingly different miRNA composition in breast milk obtained during colostrum and mid-lactation. Seven miRNAs (miR-148a-3p, 22-3p, 26a-5p, 21-5p, 7b-5p, 7g-5p, and 24-3p) were found to be common in breast milk, nipple aspirate, serum, plasma, and breast tissue (21). The study involving breast milk and colostrum reported different miRNA compositions and, interestingly, no common miRNAs were identified. This finding supports the production of milk specific to infants' needs (22).

The epigenetic effects of breastfeeding are still in their early stages. When the study results are considered in terms of clinical significance, variations in laboratory analyses are high, and their reproducibility is low. Therefore, more trials are needed to provide evidence-based information.

Effect of breastfeeding on oral health

The numerous benefits of breastfeeding for general health are well documented in the literature (23). Research in the field of oral health has specifically investigated the long-term effects of breastfeeding on both jaw development and the occurrence of dental caries (24). Breastfeeding promotes proper maxillofacial growth and development by stimulating intense oral muscular activity, promoting correct lip closure, mandibular function, and tongue positioning on the palate. This dynamic process encourages a more intensive squeeze action for milk extraction, in contrast to the more passive feeding motion associated with bottle feeding. Thus, breastfeeding potentially facilitates better occlusal development and is associated with a lower risk of malocclusion (25). According to the results of a recent systematic review, children who are breastfed for at least six months are less likely to have class II malocclusion and posterior crossbite (26).

The relationship between breastfeeding and dental caries is controversial in dentistry. A meta-analysis showed that breastfeeding during the first year of life has a potential protective effect against dental caries in children. This was attributed to the possibility that breastfeeding delays the introduction of free sugar-containing foods and the initiation of bottle feeding (27). Another factor is related to breast milk's composition, which is rich in immunomodulatory factors, thus supporting the establishment of a healthy oral microbiome in infants, potentially offering initial protection against dental caries. (27-29). However, the risk of dental caries may change as the child's oral microbiome evolves with the eruption of new teeth.

The same meta-analysis demonstrated that prolonged breastfeeding (beyond 12 months), particularly during nighttime and more frequently, was a contributing factor for an increased risk of dental caries. However, the authors indicated that it was not the act of breastfeeding

itself but rather other confounding factors, such as maternal oral health status, poor oral hygiene practices, and the introduction of other sugary foods and drinks that contribute to dental caries in breastfed children (27). Mutans streptococci, such as *Streptococcus* mutans, can be transmitted from mother to child via direct contact with the mother's saliva. Kissing, sharing utensils, or cleaning a pacifier with the mother's mouth can all transfer bacteria to the child's oral cavity. However, the cariogenicity and levels of these bacteria can vary between individuals. Several factors influence this variation, including maternal bacterial levels, caries prevalence, and oral hygiene practices. For instance, mothers with higher levels of mutans streptococci or poor oral hygiene practices are more likely to transmit these bacteria to their children, increasing the child's risk of developing dental caries (30, 31). Similarly, if a child is exposed to sugary foods and drinks frequently, it can further promote the growth and activity of cariogenic bacteria in their oral cavity, contributing to the development of dental caries. Importantly, the cariogenicity of different milks and formulas, related to their carbohydrate content, could also contribute to the observed differences in caries risk before and after 12 months of age (27).

Various studies, ranging from meta-analyses to in vitro investigations, have investigated the relationship between breastfeeding and dental caries. However, despite this extensive research, there is no clear consensus regarding the possible cariogenic potential of breastfeeding. The lack of a definitive cause-effect relationship in these studies can be attributed to various factors, including the development of diverse feeding patterns unique to each child with the introduction of solid foods after 6 months, differences in oral hygiene habits, and the socioeconomic status of the parents. Furthermore, it is not possible to consider all these confounding factors when designing a study (32, 33). Therefore, further research is necessary to elucidate the complex interaction between breastfeeding practices and the risk of dental caries comprehensively. Given the many benefits of breastfeeding for overall health, the potential association with dental caries should prompt an emphasis on improving oral hygiene practices rather than advocating the cessation of breastfeeding.

Breastfeeding in the context of economics: The public nature of breast milk as a merit product

In economics, merit goods are generally accepted as goods (products) that societies should consume regardless of individuals' wishes or demands. The most typical examples of merit goods (products) are education and health (34). In this study, the term 'product' will be used, as it was suggested and decided to be a more appropriate expression at the Symposium "Breast Milk with All Aspects.

The acceptance that merit products should be consumed without considering the wishes or demands of individuals is a result of the characteristics of these products. These products have the following characteristics:

- Merit products have positive externalities. Social costs and benefits extend beyond the private costs and benefits of consumers (individuals) and are therefore outside the prices realised through the market mechanism. With such externalities, the market mechanism fails and leads to inefficient decisions regarding resource allocation (35). For example, if more education increases productivity not only of an individual worker but also of co-workers, the individual chooses the level of education and ignores this production externality. In this context, if people demand too little education, the provision of education to society should be encouraged (34).

- Another characteristic of merit products is that people have insufficient information about their benefits. If consumers have insufficient information and consequently consume products below their optimal consumption levels, the market mechanism fails to increase social welfare (35).

These two fundamental characteristics of merit products imply that individuals may not be able to act in their own and public interests and therefore require decisions to be made on their behalf. In this context, the function of state intervention, which has more information or is in a more favourable position to make a decision, is to enable people to access and consume merit products rather than telling them what is beneficial or not beneficial (34).

Accepting the postulate that societies should consume merit products regardless of the wishes or demands of individuals makes it necessary for governments to provide these products (although they can be consumed and purchased individually through the market mechanism) and intervene to achieve the level of consumption required for society. This is because if the task of providing merit products is left to the market mechanism alone, such products will be under-consumed in society (34). In this context, for example, individual preferences regarding breastfeeding should be guided by public interventions, and the production and consumption levels of merit products should be increased.

When we evaluate the benefits of breast milk and breastfeeding as an economic product based on consumption data, the following conclusions are reached (36):

- Breast milk is a unique source of nutrients for the growth and development of children. Breast milk has benefits that extend from infancy to childhood and adulthood.

- The World Health Organisation (WHO) considers the exclusive breastfeeding of infants for the first 6 months and the continuation of breastfeeding for at least 2 years as natural nutrition. Breastfeeding also provides maternal health benefits in addition to child health.
- Breast milk has a species-specific and dynamic structure. Its content changes during breastfeeding and varies according to the age of the baby.
- Babies who cannot be fed breast milk are more likely to be hospitalised, especially due to infectious diseases, resulting in treatment and high health costs.
- Babies fed with breast milk become healthier and more successful adults. With these features, breastfeeding benefits the future of society in a sense.
- According to the results of the 2018 Demographics and Health Survey (37), breastfeeding of infants and children in Turkey is not at the desired level.

The aforementioned qualities of breast milk require it to be accepted as an economically merit product. Because: It provides benefits to the baby and mother; it has external benefits to society depending on its production and consumption; and it is consumed below the optimum level.

The acceptance of breast milk as a merit product requires the state to make public interventions to increase its consumption. These interventions are outlined as follows:

- Use of legal power: Dissemination and continuity of baby-friendly hospital practice.
- Improving information: Making it clear to people, especially mothers, the risks they face if they do not breastfeed.
- Incentive: Provide the necessary conditions to achieve optimal breastfeeding rates using various incentive mechanisms to address the difficulties faced by families (especially mothers).

In this context, it can be concluded that breast milk is an economically merit product, and the state should allocate resources by using its public power to increase breastfeeding. Otherwise, societies and individuals may make choices that they regret in the future. Furthermore, this will reduce social welfare.

Proactive role of pharmacists in breastfeeding

Pharmacists play an important role as primary healthcare providers in many healthcare settings, including community pharmacies, hospitals, and clinics. They are often the first point of contact for patients and work with physicians and other healthcare professionals to ensure optimal patient care. Pharmacists also play a crucial role in supporting and assisting mothers during the breastfeeding period (38, 39).

The key roles and responsibilities of a pharmacist during the breastfeeding period include (40, 41).

1. Help to increase breastfeeding rates in their country, in line with the WHO guidelines, based on the principle that every child has the right to the best start in life, thus helping society achieve a healthier and sustainable future.
2. Provide information and education on the importance of breastfeeding, correct positioning, proper nutrition, and/or supplementation, as appropriate.
3. Educate mothers about medication safety: During breastfeeding, interrupting treatment because of concerns about drug use can cause undesirable and permanent damage to the health of the mother and baby. In cases where the mother should take medication while breastfeeding, the ability of the mother to continue to breastfeed should be assessed on an evidence-based risk-benefit basis. Pharmacists can provide information about the transfer of medicines into breast milk, potential adverse effects, and alternative treatments, if necessary.

Approaches to reducing infant exposure to medicines include (42, 43):

- As most drugs are excreted in breast milk, the main question regarding the use of drugs during lactation is not whether the medicine is excreted in breast milk; it is whether the amount of the drug excreted in breast milk is likely to cause a serious or severe adverse effect in the infant.
- Drug selection during lactation is a multifactorial process that should be evaluated on an individual basis. Therefore, pharmacists can relieve the concerns of mothers and direct them to physicians for safer drug alternatives.
- Drugs reach peak concentration 1-2 hours after oral administration. Breastfeeding at the end of the dosing interval, expressing milk 1-2 hours after taking the medication, and excreting the milk can be used. This method may be effective in drugs with short half-lives and infrequent breastfeeding.
- Selection of drugs with known pharmacokinetic properties and toxic effects, less excretion into breast milk, and a low relative infant dose. For example, sertraline may be preferred over fluoxetine as an antidepressant in postpartum depression because it is less excreted in breast milk.

- Generally, drugs without oral absorption do not have any systemic effects in infants, even if they are passed into milk. For example, aminoglycosides, vancomycin,
- If possible, use local or generic drugs instead of systemic.
- As drugs with a long half-life may cause accumulation in infants; therefore, it is appropriate to administer a single dose just before the infant's longest sleep period.
- Drugs with active ingredients that are widely used in children and whose effects are well known should be preferred for treating mothers.
- Drugs with a high protein-binding rate, such as warfarin, should be preferred because they are less likely to pass into milk.
- Temporarily interrupt breastfeeding when medications are used temporarily: If the mother is undergoing short-term treatment after dental or surgical intervention, she can pump extra milk before the procedure.
- Breastfeeding should be stopped when taking medication known to be harmful

In conclusion, pharmacists play a crucial role in empowering and motivating mothers during lactation by providing education, guidance, medication safety, and breastfeeding support, thus contributing to the UN Sustainable Development Goals on women's and children's health. Therefore, it is crucial that pharmacists properly learn how to use evidence-based scientific sources that provide the greatest quantity of safe-rated drugs among all lactation resources while making maternal medication decisions for women in the lactation period. To enhance the contribution of community pharmacists, the primary most accessible health care provider, to preventive health practices that are more visible and effective, the cooperation between healthcare teams (physician, nurse and pharmacist) and recognition by the health authority is very critical; thus, future focus should be placed on implementing this issue through undergraduate pharmacy education and continuous vocational training.

Role of nurses in lactation counselling

All health authorities recommend that children receive exclusive breast milk for the first 6 months of life and that breastfeeding should continue at least until 2 years of age (44). However, promotion, protection, and support for breastfeeding, such as breastfeeding counselling, are needed to achieve this recommendation (45).

Breastfeeding counselling is an integral part of professional practice. Nurses play a key role in promoting breastfeeding and supporting breastfeeding mothers. One of the important roles of nurses is to implement the Baby Friendly Hospital Initiative (BFHI) in hospitals. Nurses are the first health care professionals to be consulted when there is a problem with the mother or baby during the breastfeeding process after birth (46). Nurses are effective in helping mothers have positive views about breastfeeding (47). Breastfeeding counselling given to mothers plays a great role in the continuation of breastfeeding and prevention of problems that may occur (45, 46). Nurses should adopt a broad social approach that includes both mothers and fathers to maintain breastfeeding (47).

Breastfeeding counselling should cover all stages of life for children and their mothers. Breastfeeding counselling should anticipate and address important challenges related to breastfeeding, and nurses should be involved at every stage of breastfeeding counselling. For example, every step of the hand expression technique should be explained by nurses (48). The mothers should be informed by nurses how to feed their babies. Nurses should provide breastfeeding support in their routine nursing care and recommend that mothers use community resources to provide breastfeeding support. Nurses should work with many communities to improve breastfeeding support services in the society (44, 46, 49). Therefore, during the education and training of nurses on breastfeeding counselling, attention should be paid not only to the theoretical explanation but also to the clinical practical part. For this reason, it would be useful to add the applied Breastfeeding Consultancy as an elective course to the program. To address graduate nurses' lack of knowledge about breastfeeding through post-graduation in-service training programs will support breastfeeding (50).

For successful breastfeeding, the health status of the infant and mother should be monitored (44). In some cases, breastfeeding should be stopped temporarily or permanently because of maternal and infant diseases. In such cases, a decision should be made in consultation with the treating paediatrician.

While providing support, the nurse should help the mother gain self-confidence. Starting from birth, during breastfeeding counselling, the nurse should identify the difficulties experienced and provide counselling again when necessary (44, 47).

Regardless of the reasons for applying to health institutions, mothers should be encouraged to continue to breastfeed. Within the scope of public health practices, families should be reached through home visits or telephone calls if necessary (50).

In conclusion, nurses play an important role as breastfeeding counsellors. In line with these duties, nurses should play an active role in initiating and maintaining

breastfeeding and ensure that mothers have positive views about breastfeeding.

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CASE REPORT / OLGU SUNUMU
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MEASLES-MUMPS-RUBELLA VACCINATION IN A PAEDIATRIC TRANSPLANT PATIENT USING EVEROLIMUS

EVEROLIMUS KULLANAN BİR PEDİATRİK TRANSPLANT OLGUSUNDA KIZAMIK-KABAKULAK-KIZAMIKÇIK AŞISI UYGULAMASI

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ABSTRACT

There is limited information regarding the administration of live vaccines to paediatric transplant patients who are at increased risk of infection. A 7-year-old liver transplant patient who had everolimus therapy was evaluated for Measles-Mumps-Rubella (MMR) vaccination. After checking the lymphocyte count, immunoglobulin G, and serum everolimus levels, the MMR vaccine was administered. No side effects were observed during follow-up, and the antibody levels were found to be positive after two months. This article presents a case of paediatric solid organ transplantation patient who received a measles-mumps-rubella vaccination while receiving immunosuppressive everolimus therapy.

Keywords: Child, vaccination, everolimus, solid organ transplantation

ÖZET

İnfeksiyon riskinin artmış olduğu pediatrik transplantasyon hastalarında canlı aşıların uygulanması ile ilgili bilgiler sınırlıdır. Yedi yaşında, halen evorolimus kullanmakta olan karaciğer nakli hastası Kızamık-Kabakulak-Kızamıkçık (KKK) aşısı yapılmak amacıyla değerlendirildi. Lenfosit sayısı, immünoglobulin G ve serum everolimus düzeyleri kontrol edildikten sonra KKK aşısı uygulandı. İzlemde yan etki saptanmayan olguda, iki ay sonra bakılan antikor düzeyleri pozitif bulundu. Bu yazıda immünosupresif everolimus tedavisi alırken Kızamık-Kabakulak-Kızamıkçık aşısı uygulanan bir pediatrik solid organ nakli olgusu sunulmaktadır.

Anahtar Kelimeler: Çocuk, aşılama, everolimus, solid organ nakli

INTRODUCTION

Children who receive solid organ transplants (SOTs) are at an increased risk of vaccine-preventable infections. Although the use of live attenuated vaccines in children after SOT is still controversial, there is increasing literature showing that live attenuated vaccines are safe and effective in selected cases receiving immunosuppressive therapy after transplantation (1). In this article, we present a paediatric solid organ transplantation patient who was given a measles-mumps-rubbella (MMR) vaccination while receiving immunosuppressive everolimus treatment.

CASE PRESENTATION

A 7-year-old liver transplant patient was evaluated for the Measles-Mumps-Rubella (MMR) vaccine. The patient, who underwent transplantation at 11 months of age, was given inactivated vaccines six months and varicella vaccine three years after the transplant while on tacrolimus treatment. The patient, who had no history of rejection, had been using everolimus instead of tacrolimus for two years due to recurrent angioedema and had not received any other treatment or blood products. His lymphocyte count was 1620/mm^3, IgG was 620 mg/dl, CD4/CD8 ratio was 1.68%, and the last two serum everolimus levels were 3 ng/mL

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and 2.7 ng/mL. After obtaining consent from the family, the MMR vaccine was administered to the serologically negative case, and no side effects related to the vaccine were detected. Specific IgG antibodies were measured using the chemiluminescent immunological method (CLIA) with the VIRCLIA® KIT assay after two months of vaccination. Values above the 1.0 signal cut-off ratio (S/CO) were considered positive (2). The MMR vaccine was found to be immunogenic in the patient on everolimus treatment, as the antibody titres for measles, mumps and rubella were found to be 1.5, 2.2 and 30.5 S/CO, respectively.

DISCUSSION

Children receiving SOTs are likely to have an increased risk of vaccine-preventable infections. The considerable morbidity, mortality and costs associated with these infections highlight the importance of immunisation of all transplant candidates and recipients, especially children aged two years and younger (3). During outbreaks, transplanted children are at a high risk of exposure to measles and are more likely to develop life-threatening complications such as pneumonia, encephalitis and myocarditis (4).

The recommended essential criteria for the administration of attenuated live vaccines after SOTs have been a-established/presented in Pediatric Transplantation consensus meeting (5). MMR vaccine (except combined MMRV) is considered safe in patients more than one year after liver or kidney transplantation and two months after an episode of acute rejection, who are clinically stable, can be closely monitored, and meet certain criteria for "low-level" immunosuppression as defined in the guidelines; for steroids (prednisone equivalent) <2 mg/kg/d or total cumulative <20 mg/d, for tacrolimus <8 ng/mL for two consecutive readings and for cyclosporine <100 ng/mL for two consecutive readings. Patients also need to meet the minimum immune criteria defined by absolute lymphocyte count (ALC) >1500 for children ≤6 y and >1000 cells/μL for children >6 years, CD4 >700 cells/μL for children ≤6 y and >500 cells/μL for children >6 years and normal total serum IgG levels for age (5). In our case, the MMR vaccine was administered six years after transplantation and after evaluating the IgG levels, lymphocyte count, lymphocyte profile tests, and everolimus levels.

There is no clear information about what blood level of everolimus is considered low immunosuppression. The target blood everolimus level to prevent rejection has been reported to be 3-8 ng/ml when used in combination with low-dose tacrolimus and 5-10 ng/ml when used alone (6-8). The only paper in the literature regarding the administration of live vaccines during everolimus treatment reported that the MMR vaccine was safe and immunogenic in children who had undergone liver transplantation. In this study, two consecutive serum everolimus levels <5 ng/ml were accepted as the criteria for live vaccine administration (9). In our patient who was switched from tacrolimus to everolimus because of angioedema, everolimus levels were below the minimum blood concentration levels indicated in the studies, and the level at the time of vaccine administration was the lowest in the last 3 months.

A recent study has revealed that immunisation with live attenuated vaccine showed a high seroconversion rate for those who met the clinical criteria after liver transplantation (10). In another research, the seroprotection rates for measles, rubella, mumps, and varicella were generally low in children after the first post-transplant accination and a significant number of recipients required re-vaccination. Serological evidence of seroprotection following immunisation should be routinely checked (11). In our case, seroconversion occurred after a single dose of the MMR vaccine.

To date, it has been reported that a live attenuated vaccine has been administered to 2091 people who receive immunosuppressive drugs (1). Twenty-three patients (1.1%) became infected with the virus strain used in the vaccine, which was the varicella virus in 21 patients. No serious life-threatening complications have been reported. Shinjoh et al. reported no severe side effects after live attenuated vaccination in 48 paediatric patients undergoing living donor liver transplantation. Transient swelling of the parotid glands was reported in two cases after mumps vaccination, and fever was reported in two cases 2-3 weeks after measles vaccination, but no measles symptoms were observed in the patients (10). In our case, no side effects were observed after vaccination.

Various factors such as level of immunosuppression, infrequent consultation with primary care physicians, and lack of awareness of the vaccination status among specialists lead to low vaccination rates. The new literature on live-attenuated vaccines in post-transplant paediatric patients provides more insight into the vaccines' safety and efficacy, and communication between specialists and primary care physicians plays a key role in optimising immunisation of immunocompromised patients (12).

CONCLUSION

The MMR vaccine is safe and immunogenic in the presence of "low-level" immunosuppression in paediatric patients receiving everolimus after living donor liver transplantation. The increase in the number of cases similar to our patient, who is one of the few in the literature, is important in establishing guidelines for the administration of live vaccines in transplant patients.

Informed Consent: Written informed consent was obtained from patients' parents who participated in this case

Peer Review: Externally peer-reviewed.

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CASE REPORT / OLGU SUNUMU
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A CASE REPORT OF GALLBLADDER CANCER IN A 17-YEAR-OLD PATIENT: DIAGNOSTIC CHALLENGES AND SURGICAL MANAGEMENT

ON YEDİ YAŞINDAKİ BİR HASTADA SAFRA KESESİ KANSERİ OLGUSU: TANISAL ZORLUKLAR VE CERRAHİ TEDAVİ

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ABSTRACT

Gallbladder cancer is a rare and aggressive malignancy with a poor prognosis, often diagnosed incidentally or at an advanced stage. This case study demonstrates an uncommon clinical entity of Gallbladder cancer in a 17-year-old male. The patient was presented with generalised abdominal pain and leukocytosis and mildly elevated liver enzymes. Initial imaging showed no gallstones; however, subsequent ultrasonography revealed an intraluminal hyperechoic lesion in the gallbladder. Laparoscopic cholecystectomy and biopsy confirmed well-differentiated adenocarcinoma without lymphovascular or perineural invasion. On follow-up, no complication was reported. In this case report, we highlight the importance of considering Gallbladder cancera differential diagnosis after excluding the most common aetiologies at the young age group.

Keywords: Gallbladder cancer, cancer, young age

ÖZET

Safra kesesi kanseri, genellikle tesadüfen veya ileri evrede teşhis edilen, kötü prognozlu nadir ve agresif bir malignitedir. Bu olgu sunumunda, 17 yaşında bir erkek hastada nadir görülen bir safra kesesi kanseri olgusu sunulmaktadır. Yaygın karın ağrısı, lökositoz ve karaciğer enzimlerinde hafif artış sebebiyle başvurmuştu. İlk yapılan görüntülemede safra kesesinde taş görülmedi ancak daha sonraki ultrasonografide safra kesesinde intraluminal hiperekoik bir lezyon saptandı. Laparoskopik kolesistektomi uygulanan hastada patolojik incelemede lenfovasküler veya perinöral invazyon göstermeyen iyi farklılaşmış adenokarsinom saptandı. Takipte herhangi bir komplikasyon görülmedi. Bu olgu sunumunda, genç yaş grubunda en yaygın etiyolojileri dışladıktan sonra safra kesesi kanserini ayırıcı tanı olarak düşünmenin önemini vurguluyoruz.

Anahtar Kelimeler: Safra kesesi kanseri, kanser, genç yaş

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INTRODUCTION

The gallbladder is a pear-shaped intra-abdominal cavity small organ located in the right upper quadrant (RUQ) on the undersurface of the liver and functions as a condensing storage house of bile before its release into the small intestine (1). Similar to any other organ in the body, the gallbladder may undergo malignant changes, and hence, cancer develops. Gallbladder cancer was first described in 1777 by Maxmillan de Stol (2).

Gallbladder cancer (GBC) is a relatively unusual illness with variable incidence globally. According to global cancer statistics in 2018, approximately 219,420 new cases were recorded worldwide, representing a percentage of 1.2% of all other cancer sites (3, 4). According to GLOBOCAN 2018 data, gallbladder cancer is considered the 22nd most incident type of cancer however, it is the sixth most prevalent cancer of the gastrointestinal tract and the most common cancer of the biliary tract (1, 5). The highest frequency of the disease is found among females over the age of 65 years (2).

GBC can be discovered incidentally, such as during laparotomy or histological examination. However, GBC can be clinically obvious and show evidence of local invasion, large-scale regional nodal metastasis, encasement of blood vessels, and distant metastases (6).

Generally, GBC is considered an aggressive disease among malignancies of the biliary cancers with a short median survival period (6). In that the prognosis is poor, only about a 32% five-year survival rate for lesions confined to the gallbladder mucosa and a 10% one-year survival rate for more advanced stages. In addition, in autopsy studies throughout the world, gallbladder cancer represents 80% to 95% of cancers of the biliary tree (2).

There are marked regional and ethnic variations in the incidence of gallbladder cancer. The highest mortality rates have been reported in India and Hispanics. Incidence rates are much lower in Europe and India (2).

In addition, there is a clear association between chronic cholelithiasis and gallbladder cancer worldwide. Aside from gallstones and female sex, some associated risk factors were discovered to favour the establishment of gallbladder cancer as neoplastic initiators. Unknown internal and probiotic mutagens and neoplastic promoters; including chronic inflammation and infection, were observed to contribute to the development of GB adenocarcinoma (2). For example, gallbladder cancer has been found to be linked to chronic infection with *Helicobacter bilis* and *Salmonella typhi* (7).

Surgery is the most efficient and possible curative management. Proper resection is considered to be curative for early-stage neoplasms; nevertheless, surgical intervention is not effective in many patients, as they have advanced disease due to late presentation (6). Unfortunately, patients who are found to be beyond surgical resection or have distant metastases have a poor prognosis. Open surgical resection is recommended for patients with suspected GBC. Efficient therapeutic choices for managing advanced gallbladder cancer are emerging, including adjuvant combination chemotherapy and targeted therapy. In cases of advanced disease, palliation is the mainstay of management, and endoscopic metallic stents for biliary obstruction are used to improve the quality of life (6). In this case report, we present a case of gallbladder adenocarcinoma in a 17-year-old patient.

CASE PRESENTATION

17 year old male with average weight patient who was previously healthy presented to the emergency room complaining of abdominal pain for several hours prior to presentation. The pain was vague and generalised with no other symptoms, such as vomiting, anorexia, and nausea. There were no constitutional symptoms such as headache, fever, or weight loss. There was no history of any medical diseases, such as haemolytic anaemia.

Moreover, the clinical examination showed no localised tenderness, negative Murphy's sign, rigidity, or guarding. Initial workup showed leukocytosis and mild elevation in liver enzymes, otherwise normal CBC and Biochemistry results (Hb: 14 g/dl WBC: 16.9 10^3/µL PLT:399 10^3/µL, AST: 59 U/L, ALT: 75 U/L, Total Bilirubin: 5.9 µmol/L, Creatinine: 41 µmol/L, Albumin: 42 g/L, Potassium: 4.7 mmol/L and Sodium: 139 mmol/L) and x-ray imaging did not reveal any acute surgical condition. A primary ultrasound of the abdomen and pelvis imaging was performed, showing no gallbladder stones, pericholecystic oedema with normal wall thickening. In addition, there was no hydronephrosis or free fluid collection.

The decision to admit this patient to our hospital for further evaluation was made. Thorough history shows no similar attacks of abdominal pain, changes in bowel habits, dyspepsia, or changes in the colour of urine or stool. There was also no evidence of a family history of malignant diseases, particularly gallbladder or liver cancers. Another confirmatory ultrasonography study was conducted in the presence of an intraluminal hyperechoic irregular margin lesion attached to the wall of the gallbladder fundus without colour flow. The liver was homogenous, measuring approximately 14.5 cm craniocaudally at the midclavicular line with no obvious focal lesions. The spleen was normal in size and shape with no focal lesions. Both kidneys were normal in size, position, and corticomedullary differentiation.

Subsequently, the patient was scheduled for a surgical intervention. Intraoperative laparoscopy revealed a

nodule-like lesion adjacent to the gallbladder fundus and an intraoperative gross image of the fatty liver. Moreover, a histopathological study demonstrated the presence of a gallbladder biliary-type well-differentiated adenocarcinoma measuring 2.5 cm in the greatest dimension, with no definite lymphovascular perineural invasion. In addition, the cystic duct margin was free of tumour invasion (Figure 1).

The pathological results of a true cut biopsy of the liver showed a disturbed architecture with a vague nodular pattern and septal fibrosis, with an overall picture suggesting hepatitis. The patient's postoperative recovery was uneventful. The patient was discharged. Unfortunately, the patient was lost to follow-up as he travelled to another city.

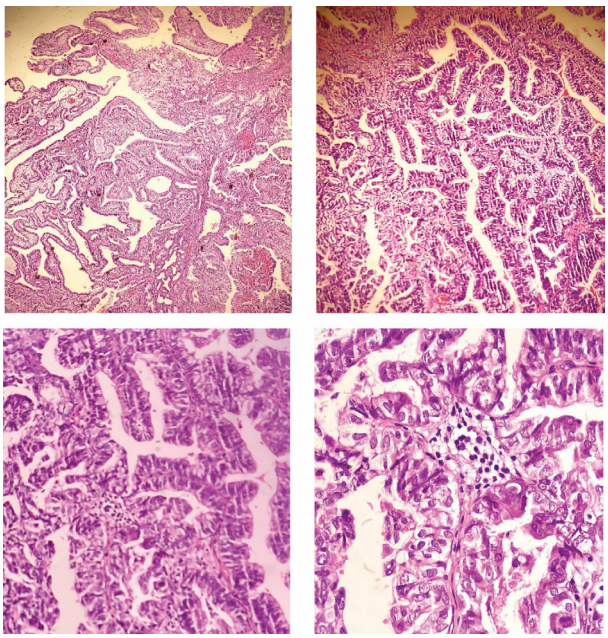

Figure 1: A well-differentiated adenocarcinoma of the gallbladder, biliary type, and 2.5 cm in the greatest dimension. Histological staging is pT2a Nx Mx

DISCUSSION

GBC is an uncommon malignancy that affects the elderly in the first place (1). Studies validate a percentage of 0.26% cumulative risk of gallbladder cancer for females and 0.25% for males up to 74 years of age (5).

Gallbladder malignancy is the most common primary cancer of the biliary tract. Adenocarcinomas arising from secretory cells are nearly the typical form of GB malignancies. Papillary adenocarcinoma is a common form of gallbladder adenocarcinoma that materialises from papillary cells and assists in promoting bile motility in the gallbladder.

It is worth mentioning that the incidence of GBC differs geographically. For example, the United States of America (USA) has a lower incidence than the rest of the world, and Eastern Europe, East Asia, and Latin America denote the highest incidence rates (5).

Gallbladder cancer presents a higher proportion of malignancy mortality than incidence. To clarify, GBC mortality accounts for 1.7% of all cancer-related deaths, although its incidence accounts for only 1.2% of all cancer diagnoses (5). This is due to the poor prognosis of gallbladder cancer, which is attributed to its late diagnosis. For instance, recent epidemiological studies in the USA have revealed that 43% of gallbladder cancers are diagnosed after metastasis to neighbouring structures. However, only 42% of cases were identified after spreading to lymph nodes or distant organs (5).

A retrospective study performed in Jordan between 2002 and 2016 revealed that the GBC rate and histological patterns among patients who underwent cholecystectomy in the northern region were 0.003. Adenocarcinoma remained the dominant type, accounting for 87% of the cases (8). Out of all targeted population, those who underwent cholecystectomies at the time of the study, only 31/11,391 (0.27%) patients had GBC confirmed by histopathology with a mean age of 68 (8). Similarly, in another study carried out in Qatar, five cases per year were diagnosed with GBC with a total of 35 cases in years, which represent the study period. In other words, GBC has an annual incidence of 0.2/100,000 people in Qatar. The median age of the patients was 54 years (3).

Malignancy of the gallbladder is tremendously rare in the young age group and children, with only two case reports available in the English literature and few in the non-English literature (7). However, a 15-year-old boy with gallbladder carcinoma was reported in a study by Muduly et al. (3). Similarly, this report presents a case of GBC in a 17-year-old boy.

Similar to all malignancies, several risk factors predispose patients to cancerous neoplastic growth. In addition to gallstones, age, genetics, obesity, chronic infection and occupational exposure to mutagens are some vital aetiological factors for harbouring gallbladder cancer (5).

Chronic inflammation often leads to gallbladder adenocarcinomas. Physiological cell signalling and cell growth are disrupted. Additionally, gallbladder cancer may be preceded by cholelithiasis in approximately 20 years. Approximately 85% of the patients with cholelithiasis develop GBC. This is assumed to be due to the chronic irritation caused by gallstones, along with the carcinogen production, i.e secondary bile acids. Consequently, sequential growth of metaplasia, hyperplasia, dysplasia, and then carcinoma may occur. Although gallstones are

ultimately linked to the aetiology of gallbladder cancer, their role remains unclear (5).

Calcification of the gallbladder is termed as a porcelain gallbladder attributed to its morphology on radiographic studies. Porcelain GB is particularly commonly observed among middle-aged females or those who are overweight. Through scientific history, porcelain gallbladder has been linked to gallbladder malignancy, with an incidence rate above 60%; however, recent studies have demonstrated a less than 6% accompanying incidence (5).

Potential cancerous growth has been observed in gallbladder polyps for several decades. The exact pathogenesis of this malignancy is still not well understood. In the literature, 23% of the polyps demonstrated malignant changes during the follow-up period. Polyps that have a length of more than 1 cm are noted to be more prone to malignancy. Currently, there are no strict guidelines regarding the follow-up schedule for small asymptomatic polyps, although some studies have concluded that polyps less than 10 mm in length have the capacity to become cancerous (5). In this case report, there were no previous predisposing factors for gallstones, and abdominal ultrasound imaging revealed that the patient had a gallbladder polyp, rather than a stone.

Genetic studies have implied that multiple genetic mutations are involved in gallbladder cancer. Most are tumour suppressor genes or common oncogenes such as c-*erb-B2, KRAS*, *P16*, and *TP53*, which are also implicated in other types of cancers. Thus, they cannot be uniquely hypothesised to be driving mutations in GBC (5). In addition, diabetes is also a risk factor for GBC. Controlling diabetes and high-density lipoprotein levels may reduce GBC levels. Similarly, obesity and being overweight are associated with a risk of GBC (6). In this case, the patient had an average weight and no previous medical diseases, such as diabetes mellitus. Also, unfortunately, genetic study is not available at our facility.

The clinical presentation is not explicit and may include abdominal pain, loss of weight, pyrexia, and jaundice, which are often observed in acute cholecystitis and other non-malignant gallbladder diseases along with some other abdominal malignancies (6). Moreover, most GBCs are still incidentally diagnosed in patients having cholecystectomy for gallstones or gallbladder polyps (8).

Furthermore, in the study carried out in Jordan, almost 70% of patients were diagnosed by chance, whereas in the study conducted by Sulieman et al. in Qatar, the intraoperative suspicion of the presence of the gallbladder was observed in 8.6% of the studied population and proven by intraoperative frozen section (3, 8). In this same study, of all GBC diagnosed cases, 40% were discovered by chance, and another 40% were confirmed by histopathology prior to surgery; in that, tissue core needle biopsy and cytological study by fine needle aspiration were conducted (3). This corresponds to what we found in our case study; in that, our patient was incidentally diagnosed with GB adenocarcinoma after laparoscopic cholecystectomy due to a GB polyp.

Therefore, it is crucial to make a diagnosis in an early setting. Ultrasonography, computed tomographic scan (CT), and magnetic resonance imaging (MRI) have improved the ability to differentiate and select the proper management. In addition, endoscopic ultrasound (EUS) has acceptable sensitivity and can differentiate non-malignant gallbladder conditions from frank gallbladder cancer (6). At initial detection, in 40-65% a mass-occupying lesion was identified in patients with GBC. A large, GB lumen filling or replacing mass with evident direct invasion of the surrounding parenchymal tissue of the liver is strongly suggestive of GBC (6). Interestingly, in the study by Sulieman et al., late presentation was observed in 11.4% of patients as they had metastatic disease on imaging (3).

In imaging, GB cancerous neoplasms may present as focal or diffused asymmetrical wall thickening. Nevertheless, these features can be related to variable benign differential diagnosis, such as acute and chronic cholecystitis, xanthogranulomatous cholecystitis, and adenomyomatosis. Moreover, diffuse hepatic or systemic diseases, such as acute hepatitis, portal hypertension, and congestive heart failure can have similar presentation and findings (6). In our case, ultrasonography showed an intraluminal hyperechoic irregular margin lesion attached to the wall of the gallbladder fundus, without colour flow.

Surgery was the only treatment option available. A small group of patients of patients are diagnosed with the initial clinical phase and can be completely cured by cholecystectomy (5, 6). Tragically, cholecystectomy alone is not curative in all stages of the disease, and a more inclusive surgical approach, consisting of gallbladder, liver, and regional lymph node resection, may be needed. Many hepatobiliary surgeons are convinced that an aggressive surgical strategy enhances the survival rate for stage II and III patients. Some scientists recommend gallbladder and adjacent liver resection including or excluding the extrahepatic bile ducts plus regional lymph nodal clearance as the most acceptable surgical approach for selected patients with GBC (6).

In the management of our patient, laparoscopic cholecystectomy was performed using intraoperative findings demonstrating a nodule-like lesion adjacent to the gallbladder fundus along with a gross picture of a fatty liver.

In terms of GBC pathological characteristics, more than 90% of gallbladder cancers a well to moderately differentiated adenocarcinomas. However, some of these are

papillary lesions that arise mainly in the gallbladder lumen. Nevin's and TNM systems are two clinical and/or pathological systems developed to determine the prognosis of gallbladder carcinoma (2).

In 1976, a new staging system was introduced by Nevin et al., in which Stage I Cancer is confined to the mucosa, in Stage II to the muscular layer, and in Stage III to the perimuscular layer. Stage IV demonstrates lymph node metastases, whereas Stage V shows hepatic or other distant metastases (2). Likewise, in the TNM classification, there are five different pathologically identified stages. Stage I tumours are limited to the mucosa or muscular layers, whereas stage II neoplasms invade the perimuscular tissue. The third stage invasion to liver than two centimetres, or regional (hepatoduodenal ligament) lymph node metastasis. Stage IV shows liver invasion greater than two centimetres (Stage IVA) or metastasis to non-regional lymph nodes and/or distant organs (Stage IVB) (2). In this case report, histopathological examination showed a well-differentiated gallbladder adenocarcinoma measuring 2.5 cm with no definite lymphovascular or perineural invasion, which is consistent with early-stage disease.

There is an argument in the literature regarding prophylactic cholecystectomy. Some studies have argued that prophylactic cholecystectomy is recommended in high-risk populations. This conclusion was based on the hypothesis that there is a strong association between long-standing gallstone disease and the development of GBC (6, 8). Nevertheless, cancer is found only in less than 1% of gallbladders resected for common reasons, such as stones or polyps; thus, there is insufficient evidence supporting the indication of prophylactic cholecystectomy for asymptomatic gallstone disease to prevent GBC (6, 8).

In conclusion, this case report highlights a rare instance of gallbladder cancer in a 17-year-old male, emphasising the need for a comprehensive diagnostic workup to aid early detection and consequently treatment. Despite the typical association of GBC with older age and certain risk factors, this case demonstrates that it can occur in previously healthy young individuals without significant predisposing conditions. Further research is warranted to explore the genetic and environmental factors that contribute to early-onset gallbladder cancer.

Informed Consent: Written consent was taken from the parents of the patient for publishing this case report.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.N.; Data Acquisition- H.M.A., J.H.J.; Drafting Manuscript- A.N., H.M.A., J.H.J., A.A.A.; Critical Revision of Manuscript- H.M.A., J.H.J.; Final Approval and Accountability- A.N., H.M.A., J.H.J., A.A.A.; Technical or Material Support- A.A.A., H.M.A.; Supervision- A.N.

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LETTER TO THE EDITOR / EDİTÖRE MEKTUP
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THE ROLE OF CLINICAL METHODS IN THE DIAGNOSIS OF LYMPHEDEMA AND THE TRANSITION TO ADVANCED IMAGING TECHNIQUES

LENFÖDEM TANISINDA KLİNİK YÖNTEMLERİN ROLÜ VE İLERİ GÖRÜNTÜLEME TEKNİKLERİNE GEÇİŞ

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Dear Editor,

I read with great interest the review titled 'Lymphedema and Peripheral Lymphoscintigraphy' by Mudun A. in Volume 87, Issue 3 of your journal dated July 19, 2024. I would like to thank the author for their valuable contribution to this important topic, which is highly relevant to physicians managing musculoskeletal disorders (1). While the review effectively highlights the significance of advanced imaging modalities, I would like to emphasise the fundamental role of clinical evaluation in the diagnosis of lymphedema from a physiatrist's perspective.

The diagnostic process begins with a detailed anamnesis, which remains indispensable. Key elements include the onset of swelling, symmetry, triggering factors such as surgery or infections, and family history. Additionally, medications and comorbidities should be thoroughly evaluated as they can provide crucial insights into the aetiology. For example, acute swelling may bring to mind conditions such as deep vein thrombosis, cellulitis, or trauma. In contrast, gradual, progressive swelling developing over weeks or months may point towards chronic venous insufficiency, lipoedema, renal disease, or heart failure (2, 3).

Clinical examination through inspection and palpation is equally essential. Features such as changes in skin colour, texture, pain, temperature, and the presence of Stemmer's sign provide important diagnostic clues. Stemmer's sign, assessed by attempting to lift the skin over the second toe or third finger, is positive when the skin cannot be pinched, showing at least stage 2 lymphedema due to fibrosis. However, a negative sign does not exclude the diagnosis (3, 4).

Volume measurement methods further enhance the clinical assessment, offering valuable information for diagnosis, treatment monitoring, and follow-up. Circumferential measurements, the most common method in daily practice, involve measuring the limb circumference at defined intervals. While practical, accuracy relies on consistent landmarks and proper technique. It is also important to consider normal physiological volume differences, such as the 8-9% variation between the dominant and non-dominant limbs (3, 4).

The water displacement method provides highly accurate limb volume measurements by collecting the displaced water when a limb is immersed. However, its use is limited due to hygiene concerns, water consumption, and challenges in accommodating larger limbs (3, 5). Modern alternatives such as perometry, which employs infrared

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sensors for automated circumference measurements, offer speed and accuracy but are hindered by high costs. Bioimpedance spectrometry, a method that measures tissue resistance to electrical currents, stands out for its non-invasive, fast, and relatively affordable nature. It is particularly useful in detecting early-stage lymphedema, making it a valuable tool for timely intervention (5).

In conclusion, clinical methods remain the cornerstone of lymphedema diagnosis, offering a cost-effective, practical, and readily available approach. These methods not only guide diagnosis but also help in distinguishing lymphedema from other causes of swelling before resorting to advanced imaging techniques such as lymphoscintigraphy. A multidisciplinary approach that integrates clinical methods with imaging is essential for accurate diagnosis and effective management.

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