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İstanbul Tıp Fakültesi Dergisi

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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Book section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR,

editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a single author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or technical report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts accepted for publication, not published yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub ahead of print articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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RESEARCH / ARAŞTIRMA
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PREDICTORS OF INTRALUMINAL RECURRENCE AT THE ANASTOMOSIS SITE AFTER CURATIVE RESECTION FOR COLON CANCER*

REZEKTABL KOLON KANSERİNDE ANASTOMOZ HATTI NÜKSÜNE ETKİ EDEN PREDİKTİF FAKTÖRLER

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ABSTRACT

Objective: The increase in the number of patients recovering from colon cancer after primary resection inevitably increases the number of patients with local recurrence. This study was conducted to investigate the predictors of intraluminal recurrence at the anastomosis site in patients who underwent curative resection for colon cancer.

Material and Method: This study included 160 patients who underwent curative resection for colon cancer and had completed follow-up colonoscopy and surveillance for at least two years at our tertiary referral hospital. Patients with intraluminal recurrence were compared with those without locally recurrent disease. Patient data, including demographics, tumor characteristics, surgery type, and reconstruction technique, were reviewed.

Result: The median age of the study group was 61 years, and 60% were men. A total of 25 (15.6%) patients had only intraluminal recurrence at the anastomosis site. The median time to intraluminal recurrence was 21.3 months (range, 3–71 months). Univariate analysis revealed the histopathological type, histological grade, T stage, number of metastatic lymph nodes, tumor margins, presence of tumor budding, perineural invasion, and anastomosis type as risk factors for intraluminal recurrence. Multivariate analysis revealed handsewn anastomoses (odds ratio [OR]: 45.532; 95% confidence interval (CI): 5.278–392.778), T stage (OR: 3.593; 95% CI: 1.378–9.371), and the presence of

ÖZET

Amaç: Rezeksiyon sonrası kolon kanserinden (KK) iyileşen hastaların sayısındaki artış, lokal nüks olabilecek hastaların sayısını da artırmaktadır. Bu çalışmada, onkolojik prensiplere uygun yapılan cerrahi sonrası, cerrahi sınırlar negatif olmasına rağmen anastomoz hattında nükslerin gelişebilmesi nedeniyle, nüksün ortaya çıkmasına etki eden risk faktörlerini araştırmayı amaçladık.

Gereç ve Yöntem: Bu çalışmaya, kolon kanseri için küratif rezeksiyon uygulanmış ve en az iki yıl boyunca takip kolonoskopisi yapılan 160 hasta dahil edildi. İntraluminal nüksü olan hastalar, lokal nüks olmayan hastalarla karşılaştırılarak; hastaların demografik bilgileri, tümör özellikleri, cerrahi tipi ve rekonstrüksiyon tekniği gibi veriler incelendi.

Bulgular: Çalışma grubunun medyan yaşı 61 olup, %60'ı erkek hasta idi. Toplamda 25 (%15,6) hastada sadece anastomoz bölgesinde nüks görüldü. Anastomoz hattı nüksünün gelişimindeki medyan süre 21,3 ay (aralık, 3–71 ay) olarak hesaplandı. Tek değişkenli analiz, intraluminal nüks için risk faktörleri olarak histopatolojik tip, histolojik derece, T evresi, metastatik lenf nodu sayısı, tümör kenarları, tümör tomurcuklanması varlığı, perinöral invazyon ve anastomoz tipini ortaya koydu. Çok değişkenli analiz, el ile yapılan anastomozlar (odds oranı [OR]: 45.532; %95 güven aralığı (GA): 5.278–392.778), T evresi (OR: 3.593; %95 GA: 1.378–9.371) ve tümör tomurcuklanmasının varlığı (OR: 3.912; %95 GA: 1.306–11.715) olarak bağımsız risk faktörlerini ortaya

* This study was presented as an oral presentation at the 19th National Congress of Colon and Rectal Surgery, 16-20 May 2023 Antalya/Türkiye and published in the conference abstract book.

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tumor budding (OR: 3.912; 95% CI: 1.306–11.715) as independent risk factors. Adjuvant chemotherapy did not affect the rate of intraluminal recurrence.

Conclusion: This study suggests a relationship between tumor biology and intraluminal recurrence, and the T stage and tumor budding were the predictors.

Keywords: Colon cancer, intraluminal recurrence, risk factors

koydu. Adjuvan kemoterapinin, anastomoz hattı nüks oranını etkilemediği görüldü.

Sonuç: Anastomoz hattı nüksüne etki eden prediktif faktörler arasında, tümörün histopatolojik özellikleri ve T evresi ön plana çıkmaktadır.

Anahtar Kelimeler: Kolon kanseri, anastomoz hattı nüksü, risk faktörü

INTRODUCTION

Colon cancer (CC) is the most frequent cancer of the gastrointestinal tract and one of the major cause of cancer-related deaths worldwide, affecting women and men equally. Although recent improvements in both diagnosis and treatment have improved survival, the increasing number of CC survivors has posed a high risk for subsequent disease recurrence, either local or metastatic (1). Disease recurrence is detected in up to 30% of CC cases, with the highest expected probability found within the first two years after primary surgery (2, 3). Most cases of recurrence are hepatic or pulmonary metastases, whereas up to 13% of patients have isolated locoregional recurrence (4-7). Locoregional recurrence is mostly extramurally defined as intraabdominal, whereas intramural recurrence occurring in 12% of cases is defined as mucosal involvement (8). Although the disease burden of only intraluminal recurrence (IR) at the anastomosis site has not been well established, it is classified distinctively from locoregional recurrence or second primary CC (9, 10). Theoretically, locoregional recurrence has been believed to develop due to either exfoliation of cancer cells during primary resection or metachronous carcinogenesis (11, 12). In a recent case series, Costi et al. suggested a persistent, patient-specific alteration as the trigger of colorectal cancer IR (13). Subsequently, they performed a similar genetic analysis for microsatellite instability (MSI) and loss of heterozygosity and could not identify any potential risk factor (8).

A precise understanding of IR and predicting the intervals and patterns of recurrence determine effective treatment and follow-up strategies. The poor outcome of disease recurrence necessitates early diagnosis to perform salvage surgery. Cross-sectional imaging is the most widely used diagnostic tool for extraluminal recurrence, whereas endoscopy is primarily used for IR. Nonetheless, there is still a lack of consensus regarding the perfect timing for diagnostic workup after primary surgery. The commonly scheduled endoscopy or imaging is performed in the first year after primary surgery, which may be too late in the case of early recurrence without symptoms or signs. Therefore, identifying the risk factors for IR after primary resection may be helpful in determining high-risk patients in the postoperative follow-up. Considering these data, we conducted this study to explore the possible predictors of IR in patients with CC.

MATERIALS and METHODS

Study design and study population

This single-center, retrospective study was conducted at a general surgery outpatient clinic of a tertiary care center between January 2013 and December 2018. Patients who underwent curative-intent resection for CC were screened. We excluded patients in whom the proximal part of the tumor could not be reached by endoscopy; patients with peritoneal recurrence, pelvic recurrence, or systemic disease; and patients with less than 24 months of follow-up or in whom cancer surveillance was performed at an external center. Patients aged <30 years or those with a family history of cancer affecting at least two generations or with polyposis syndromes were also excluded to avoid the bias of hereditary cancer syndromes. We also excluded patients with synchronous primary cancer, those who underwent nonanastomotic surgery, patients who died within 30 days of surgery, those with incomplete data, and patients with primary rectal tumors. Finally, of 883 patients, we included 160 patients who met the inclusion criteria. All patients were informed about the diagnostic and therapeutic procedures, and written informed consent was obtained. The study protocol was approved by the Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethics Committee (Date: 22.02.2019, No: 04). This study was conducted according to the principles of the Declaration of Helsinki.

Assessment and data collection

Colon cancer was defined as a primary tumor developing between the cecum and rectosigmoid junction. All patients were evaluated and staged by colonoscopy and imaging techniques before surgery. Tumors were categorized anatomically according to the site of development as right-sided (cecum and ascending colon), transverse colon, descending colon, and sigmoid colon. All patients underwent primary surgery according to oncological principles and *en-bloc* resection of the invaded adjacent organs, if applicable to achieve R0 resection. Data, including primary tumor resection, patient demographics, surgery

type, pathology records, and the adjuvant therapy used, were recorded. The following clinical and pathological features were included for risk analysis: age at the time of surgery, sex of the patient, tumor location, surgery type, surgical approach, anastomosis type, histopathological type and grade, tumor diameter, disease stage according to the Tumor, Node, Metastasis (TNM) classification, total/positive/negative lymph node number, tumor margin, resection margin (infiltrative/expansive), distance of the tumor to the proximaistal and radial margin on the specimen, angiolymphatic invasion, venous invasion, peritumoral budding, perineural invasion, mesenteric tumor nodules, and receiving adjuvant chemotherapy.

Postoperative follow-up

Postoperative follow-up was performed at our institution in the outpatient setting. Patients were scheduled for physical examination, routine blood chemistry, serum carcinoembryonic antigen screening, and complete blood count every 3–6 months for the first three years and every six months in the 4th and 5th years. Colonoscopy along with thoracic, abdominal, and pelvic computed tomography was conducted annually, otherwise earlier depending on the patient's complaints. IR was diagnosed by mucosal biopsy at the anastomosis site.

Statistical analysis

Statistical analysis was conducted using the NCSS version 2007 software (NCSS LLC, Kaysville, UT, USA). Continuous data were expressed as mean±standard deviation (SD) or median (minimum–maximum), whereas categorical data were expressed as numbers and frequency. Categorical variables were analyzed using the chi-square or Fisher's exact test. Univariate and multivariate regression analyses were performed to identify risk factors. The backward stepwise logistic regression analysis was also conducted to determine the relationship between the statistically significant factors and recurrence. The Kaplan–Meier method was used to estimate survival regarding the T stage, and the log-rank test was used for the statistical comparison of the groups. A p value of <0.05 was considered statistically significant.

RESULTS

Of 883 patients with CC who underwent curative resection, 160 met the inclusion criteria. The median age of the cohort was 61 (range, 30–84) years, and 96 patients (60%) were men. The mean age at the time of surgery was 60.1±11.8 (range, 30–84) years. The mean follow-up period was 42.1 (range, 3–71) months, and 25 (15.6%) patients had only IR at the anastomosis site. The mean time to IR was 21.3 (range, 3–71) months. More than half of the tumors were located in the sigmoid colon (53.8%). Laparoscopic surgery was performed in 107 (66.9%) patients. Anterior resection (n=72) was the most common form of surgery type in both laparoscopic and open surgery, followed by right hemicolectomy (n=58). Anastomoses using a circular stapler (n=92, 57.5%) and linear stapler (n=57, 35.6%) were more common than handsewn anastomoses (n=11, 6.9%) (Table 1).

Table 1: Demographic characteristics and surgical data of patients

Variable		
Age (years)	Median (min–max)	61 (30–84)
		n (%)
Sex	Female	64 (40.0)
	Male	96 (60.0)
Tumor location	Sigmoid colon	86 (53.8)
	Right colon (cecum and ascending colon)	57 (35.6)
	Descending colon	14 (8.7)
	Transverse colon	3 (1.9)
Surgery type	Laparoscopic anterior resection	54 (33.7)
	Laparoscopic right hemicolectomy	40 (25.0)
	Laparoscopic low anterior resection	9 (5.6)
	Laparoscopic left hemicolectomy	4 (2.5)
	Anterior resection	18 (11.3)
	Low anterior resection	4 (2.5)
	Right hemicolectomy	18 (11.3)
	Left hemicolectomy	9 (5.6)
	Subtotal colectomy	3 (1.9)
	Total colectomy	1 (0.6)
Surgical approach	Open	53 (33.1)
	Laparoscopic	107 (66.9)
Anastomosis type	Circular stapler	92 (57.5)
	Linear stapler	57 (35.6)
	Handsewn anastomosis	11 (6.9)

Data are expressed as median (min–max) or numbers and percentage, unless otherwise stated.

According to the TNM classification, the most common T and N stages were T3 (n=103, 64.4%) and N0 (n=100, 62.5%), respectively. The majority of patients (n=80, 50%) had Stage II CC (Table 2).

We compared patients with IR with those without locally recurrent or metastatic disease and found that age (p=0.662), sex (p=0.182), tumor location (p=0.771), and

Table 2: Histopathological features of study group

		n (%)
Histopathological type	Adenocarcinoma	138 (86.3)
	Mucinous adenocarcinoma	19 (11.8)
	Signet ring cell adenocarcinoma	2 (1.3)
	Medullary adenocarcinoma	1 (0.6)
Histological grade (n=140)	Well–moderately	133 (95.0)
	Poorly	7 (5.0)
T stage	T1	7 (4.4)
	T2	21 (13.1)
	T3	103 (64.4)
	T4	29 (18.1)
N stage	N0	100 (62.5)
	N1	33 (20.6)
	N2	25 (15.6)
	Nx	2 (1.3)
Stage	I	22 (13.8)
	II	80 (50.0)
	III	58 (36.2)
Lymph nodes	Min–Max (Median)	5–83 (27)
	Total	4664
Lymph node metastatic patients		55 (34.4)
Metastatic lymph node #	Min–Max (Median)	1–24 (12.5)
	Total	298
Tumor border	Infiltrative	107 (66.9)
	Expansive	53 (33.1)
Tumor diameter (cm)	Min–Max (Median)	1–19 (4)
Proximal distance (cm)	Min–Max (Median)	1–114 (10.5)
Distal distance (cm)	Min–Max (Median)	1–38 (9)
Peritoneal distance (mm)	Min–Max (Median)	0–20 (2)
Angiolymphatic invasion		87 (54.4)
Venous invasion		19 (11.9)
Peritumoral budging		59 (36.9)
Perineural invasion		39 (24.4)
Mesenteric tumor nodules		24 (15.0)
Surgical margin	Negative	160 (100.0)
Adjuvant treatment		82 (51.3)
Time until anastomotic recurrence (months) (n=25)	Min–Max (Median)	3–71 (17)

SD: Standard deviation, Nx: Regional lymph nodes cannot be evaluated. Data are expressed as mean±SD or numbers and percentage, unless otherwise stated

surgery type (p=0.502) exerted no effect on the IR rate. However, the preferred anastomosis technique exerted a statistically significant effect on the IR rate (p=0.003, $p < 0.01$). The rate of IR also varied depending on the tumor type. A significant difference was observed in the IR rate between poorly and moderately well-differentiated tumors (p=0.012). The T stage was identified as a significant factor for IR (p=0.017). As anticipated, patients with stage T4 had a higher recurrence rate than those with stages T1, T2, and T3. However, there was no significant

difference in lymph node metastasis and disease stage between the groups (p>0.05) (Table 3).

We also found no significant differences in the tumor diameter, total number of dissected lymph nodes, angiolymphatic and venous invasion rates, presence of mesenteric nodules, and proximal and distal margin distance values between the groups. Nevertheless, having a higher number of metastatic lymph nodes (p=0.025), closer radial margin values (p=0.006), infiltrative surgical margins (p=0.048), peritumoral budding (p=0.009), perineural invasion (p=0.013), and receiving adjuvant therapy (p=0.024) exerted a statistically significant effect on the IR rate (Table 4).

In the backward stepwise logistic regression analysis, we evaluated the reconstruction technique, histology, T stage, N stage, tumor margins, distal distance, distance to the peritoneal surface, status of peritumoral budding, perineural invasion, presence of mesenteric tumor nod-

Table 3: Risk factors for anastomotic recurrence

Variable		No IR	IR	p value
Age (years)	Min–Max (Median)	32–82 (61)	27–84 (61)	[a]0.662
Sex, n (%)	Female	57 (89.1)	7 (10.9)	[b]0.182
	Male	78 (81.3)	18 (18.7)	
Tumor location, n (%)	Sigmoid colon	70 (81.4)	16 (18.6)	[c]0.771
	Right colon	50 (87.7)	7 (12.3)	
	Descending colon	12 (85.7)	2 (14.3)	
	Transverse colon	3 (100)	0 (0)	
Surgery type, n (%)	Anterior resection	69 (81.2)	16 (18.8)	[c]0.502
	Hemicolectomy	62 (87.3)	9 (12.7)	
	Total / subtotal colectomy	4 (100)	0 (0)	
Anastomosis type, n (%)	Circular stapler	75 (81.5)	17 (18.5)	[c]0.003**
	Linear stapler	54 (94.7)	3 (5.3)	
	Hand sewn	6 (54.5)	5 (45.5)	
Histopathological type, n (%)	Adenocarcinoma	118 (85.5)	20 (14.5)	[c]0.006**
	Mucinous adenocarcinoma	17 (89.5)	2 (10.5)	
	Signet cell carcinoma	0 (0)	2 (100)	
	Medullar carcinoma	0 (0)	1 (100)	
Histological type, n (%) (n=140)	Well–moderately	115 (86.5)	18 (13.5)	[d]0.012*
	Poorly	3 (42.9)	4 (57.1)	
T stage; n (%)	T1	7 (100)	0 (0)	[c]0.017*
	T2	20 (95.2)	1 (4.8)	
	T3	89 (86.4)	14 (13.6)	
	T4	19 (65.5)	10 (34.5)	
N stage; n (%)	N0	86 (86.0)	14 (14.0)	[c]0.106
	N1	30 (90.9)	3 (9.1)	
	N2	17 (68.0)	8 (32.0)	
	Nx	2 (100)	0 (0)	
Stage; n (%)	I	21 (95.5)	1 (4.5)	[c]0.305
	II	67 (83.8)	13 (16.3)	
	III	47 (81.0)	11 (19.0)	

[a]: Student's t-test, [b]: Pearson Chi-square test, [c]: Fisher-Freeman-Halton Test, [d]: Fisher's exact test, *: p<0.05, **: p<0.01, IR: Intraluminal recurrence, SD: standard deviation, Nx: Regional lymph nodes cannot be evaluated

ule, and adjuvant therapy. The multivariate analysis revealed handsewn anastomoses (odds ratio [OR]: 45.532; 95% confidence interval (CI): 5.278–392.778), T stage (OR: 3.593; 95% CI: 1.378–9.371), and presence of tumor budding (OR: 3.912; 95% CI: 1.306–11.715) as independent risk factors for IR. The risk of IR increased 12,479 times in the presence of anastomosis performed using a circular stapler compared with that performed using a linear stapler (95% CI: 2.435–63.945). The risk of recurrence increased 45,532 times in the presence of handsewn anastomosis (95% CI: 5.278–392.778) (Table 5).

DISCUSSION

Experience with only IR at the anastomosis site after curative resection for CC is limited due to the relatively low number of cases observed. The literature lacks risk

Table 4: Anastomotic recurrence and histopathological characteristics

Variable		No IR	IR	p value
Lymph nodes	Min–Max (Median)	10–83 (27)	5–72 (27)	[e]0.895
Lymph node metastasis, n (%)	Negative	92 (87.6)	13 (12.4)	[b]0.118
	Positive	43 (78.2)	12 (21.8)	
Metastatic lymph node #	Min–Max (Median)	0–22 (11)	0–24 (12)	[e]0.025*
Tumor diameter (cm)	Min–Max (Median)	1–19 (4)	2–15 (5)	[e]0.261
Proximal distance (cm)	Min–Max (Median)	1–114 (10)	4–35 (11)	[e]0.728
Distal distance (cm)	Min–Max (Median)	0–38 (10)	1–25 (6)	[e]0.092
Peritoneal distance (mm)	Min–Max (Median)	0–20 (2)	0–7 (1)	[e]0.006**
Tumor margin, n (%)	Infiltrative	86 (80.4)	21 (19.6)	[b]0.048*
	Expansive	49 (92.5)	4 (7.5)	
Angiolymphatic invasion, n (%)	Negative	63 (86.3)	10 (13.7)	[b]0.539
	Positive	72 (82.8)	15 (17.2)	
Venous invasion, n (%)	Negative	121 (85.8)	20 (14.2)	[d]0.183
	Positive	14 (73.7)	5 (26.3)	
Peritumoral budging, n (%)	Negative	91 (90.1)	10 (9.9)	[b]0.009**
	Positive	44 (74.6)	15 (25.4)	
Perineural invasion, n (%)	Negative	107 (88.4)	14 (11.6)	[b]0.013*
	Positive	28 (71.8)	11 (28.2)	
Mesenteric tumor nodules, n (%)	Negative	118 (86.8)	18 (13.2)	[d]0.065
	Positive	17 (70.8)	7 (29.2)	
Adjuvant treatment, n (%)	Negative	71 (91.0)	7 (9.0)	[b]0.024*
	Positive	64 (78.0)	18 (22.0)	

[b]: Pearson Chi-square test, [d]: Fisher's exact test, [e]: Mann–Whitney U Test, *: p<0.0, **: p<0.01, IR: Intraluminal recurrence, SD: Standard deviation.

Table 5: Logistic regression analysis of factors affecting anastomotic recurrence

	p value	OR	95% CI	
			Lower	Upper
Anastomosis type (linear stapler)	0.002**			
Anastomosis type (circular stapler)	0.002**	12.479	2.435	63.945
Anastomosis type (handsewn)	0.001**	45.532	5.278	392.778
T stage	0.009**	3.593	1.378	9.371
Peritumoral budding	0.015*	3.912	1.306	11.715

*: p<0.05, **: p<0.01, OR: odds ratio, CI: confidence interval

factor demonstration of prospective studies reporting outcomes of large cohorts. Herein, we report our results of only IR at the anastomosis site on follow-up and the associated risk factors. We detected 25 (15.6%) patients with IR at the anastomosis site on an average follow-up of 42 months. The median time to IR was 21.3 months. The univariate analysis revealed the histopathological type, histological grade, T stage, number of metastatic lymph nodes, tumor margins, presence of tumor budding, perineural invasion, and anastomosis type as risk factors for IR. The multivariate analysis revealed handsewn anastomoses (OR: 45.532), T stage (OR: 3.593), and presence of tumor budding (OR: 3.912) as independent risk factors. Adjuvant chemotherapy did not affect the IR rate.

Although the definition of local recurrence in rectal cancer is well established, it is complex for CC. Peritoneal recurrence is considered a metastatic disease in the 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual. In contrast, the peritoneal carcinomatous condition is accepted as locoregional recurrence in several studies, which precludes an accurate interpretation of the findings. Furthermore, it is not possible to distinguish macroscopically or microscopically whether peritoneal tumors (implant) are locally recurrent or peritoneal metastatic entities.

Locoregional or local recurrence occurs in the resection area or near the anastomosis site. The currently proposed reasons for local recurrence are inadequate surgical technique, aggressive tumor biology, and the inability to remove tumor cells that are sewn during the resection. Galandiuk et al. described the first classification of CC local recurrence in 1992 (14), which consists of four patterns of recurrence, venous invasion, anastomosis, mesenteric/nodal, retroperitoneal, and peritoneal. Because several studies have investigated recurrences of colon and rectal cancer together, it is difficult to comment only on CC recurrences. The published studies describe a very wide range (0.4%–34%) for locoregional recurrence of CC (6, 15-21).

In the present study, we explored the predictors of IR occurrence after primary surgery according to oncological principles, although the surgical margins in all patients were negative. We excluded other intraabdominal recurrences, such as peritoneal, retroperitoneal, and nodal recurrences, which are considered local, and identified factors that only directly affect IR. Our results revealed the anastomosis technique (p=0.003), histopathological type (p=0.006), histological grade (p=0.012), T stage (p=0.017), peritoneal distance (p=0.006), tumor margin (p=0.048), peritumoral budding (p=0.009), perineural invasion (p=0.013), and receiving adjuvant therapy (p=0.024) as risk factors that exerted a significant or almost significant effect on IR. Nevertheless, in the multivariate analysis, the anastomosis technique (p=0.001), T stage (p=0.009), and peritumoral budding (p=0.015) were identified as independent risk factors for IR.

The current knowledge regarding the localization of primary colonic tumors is that the right-sided tumor has a worse prognosis in advanced stages, whereas right- versus left-sided tumors exert no effect on local recurrence, consistent with our findings (22, 23). Moreover, in laparoscopic/open surgery, more than one operator was among the features investigated in previous studies, and none of them were associated with IR. Nevertheless, because we could not evaluate these variables in our study, we cannot conclude the presence of causality in these correlations.

Liska et al. reported a higher locoregional recurrence rate in patients with lymphovascular invasion (p<0.001), positive surgical margin (p<0.001), and local tumor invasion (p<0.001) among 1397 patients over an average follow-up of 7.8 years (24). The recurrence rate was also higher in poorly differentiated tumors (p=0.012). Unlike that in previous reports, the study demonstrated no effect of angiolymphatic invasion on recurrence (23, 25). Other parameters, such as peritumoral budding and perineural invasion, have not yet been investigated. Therefore, our study is the first to explore these variables in CC. Despite the low proportion of patients with a tumor pathology of medullary carcinoma or signet ring cell carcinoma in the study group, the authors demonstrated a higher recurrence rate (23, 24). Another feature not investigated in previous studies is the tumor margin (infiltrative/expansive). In our study, the recurrence rate was higher in patients with infiltrative characteristics (p=0.048), indicating an aggressive tumor feature.

As mentioned in the 8th edition of the AJCC Staging Manual, at least 12 lymph nodes must be removed from colon specimens for formal resection (26). In our study, the mean number of lymph nodes dissected was 29.2±12.1, because all patients underwent radical surgery and wide resection to the tumor margin with an average of 13.3 and 11.2 cm for proximal and distal to the tumor margin, respectively. This wide resection refers to complete mesocolic excision under the threat of lymphatic spread. The number of lymph nodes dissected in our study, which can be counted among surgical-related factors, did not directly affect the development of recurrence, which is consistent with previous studies (24). More intriguingly, positive lymph nodes and the N stage did not increase the IR rate, whereas the higher number of positive lymph nodes in dissection increased the probability of IR. The preferred anastomosis type was another surgeon-related factor, where we found that recurrence was significantly higher in handsewn anastomoses than in stapled anastomoses. This high rate can be attributed to the prolonged duration of contamination with tumor cells during man-

ual anastomosis after the surgeon's contact with the tumor tissue. Tsikitis et al. investigated the clinicopathological-specific predictors of recurrence for Stage II–III CC and reported a higher recurrence rate in the group receiving adjuvant therapy and no effect of chemotherapy on IR (27), which is similar to our findings.

Nonetheless, there are some limitations in this study. The single-center, retrospective design of the study may have inevitably resulted in an inherent bias. Moreover, because we excluded patients without long-term follow-up or those who underwent surveillance colonoscopy at an external center, the recurrence rate in our series could not be described accurately. The number of cases in the control group (nonrecurrent) was relatively low, because we included only patients who were followed up at our clinic and had long-term data. The effect of genetic mutations (*i.e.*, *RAS* or *RAF* gene mutations, or MSI) on recurrence at the anastomosis site could not be investigated because of the high cost of these kits as they are not routinely performed for every patient.

CONCLUSION

CC remains an important health issue with a high mortality rate. Our study demonstrated a relationship between tumor biology and IR, and the results were comparable with those from other centers. Although a sufficient distance of clear margins or dissection of a large number of lymph nodes is of utmost importance for both distant metastases and local recurrences, whether they are a predictor of IR remains unclear. In local relapses, the tumor histopathology, stage, and tumor biology are critical factors that should be considered. In the light of our study, as the T stage of the disease increases and in the presence of peritumoral budding, the frequency of follow-up with postoperative colonoscopy should be tightened or revised. Tumor stage and tumor biology may increase the probability of early treatment of high-risk patients without the need for late-diagnosed recurrences necessitating multivisceral resections. Further studies are required to better understand IR in CC and identify its possible predictors.

Ethics Committee Approval: The study has ethical approval from the Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethics Committee (Date: 22.02.2019, No: 04).

Informed Consent: Informed consent was obtained from all the patients included in our study.

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PREDICTIVE VALUE OF THE NEUROPSYCHOLOGICAL IMPAIRMENT SCALE IN PATIENTS WITH TRAUMATIC BRAIN INJURY

TRAVMATİK BEYİN HASARI OLAN HASTALARDA NÖROPSİKOLOJİK BOZULMA ÖLÇEĞİNİN PREDİKTİF DEĞERİNİN BELİRLENMESİ

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ABSTRACT

Objective: Various studies have used different measures to predict the outcome of patients with traumatic brain injury, including the Glasgow Coma Scale (GCS), Disability Rating Scale, and length of hospital stay, among others. This study was conducted to determine the predictive significance of the Neuropsychological Impairment Scale (NIS) concerning the mortality rate, recovery, and discharge outcomes of patients who have been presented to the emergency department.

Material and Method: This descriptive–analytical cross-sectional study was conducted on 100 individuals aged ≥18 years who have experienced traumatic brain injuries. A checklist was prepared incorporating items from the NIS and patient information such as age, gender, and injury mechanism details. The checklist was completed during bedside examinations.

Result: The prevalence of traumatic brain injuries was higher in men (78%) in the age group of 21–40 years. The average age of patients was 41.63 years. Falling from a height was identified as the predominant cause of brain trauma, followed by two-car accidents. The mean primary GCS score was 13.47 for men and 14.63 for women. Hospitalization occurred in 67% of cases, followed by discharge in 30% and surgical intervention in 3% of cases. An inverse correlation was observed between the examined sample's NIS standard score and the initial GCS score.

Conclusion: An inverse relationship between NIS and initial GCS scores suggests that lower NIS scores are associated with better outcomes, indicating its utility as a predictive factor.

Keywords: Traumatic brain injury, Neuropsychological Impairment Scale, Glasgow Coma Scale

ÖZET

Amaç: Glasgow Koma Ölçeği (GKÖ), Engellilik Derecelendirme Ölçeği ve hastanede kalış süresi (HKS) dahil olmak üzere travmatik beyin hasarı olan hastaların sonuçlarını tahmin etmek için çeşitli çalışmalarda farklı ölçümler kullanılmıştır. Bu çalışma, acil servise travmatik beyin hasarı nedeniyle başvuran hastaların ölüm oranı, iyileşme ve taburculuk sonuçları açısından Nöropsikolojik Bozukluk Ölçeği'nin (NBÖ) prediktif değerinin belirlemesi amaçlanmaktadır.

Gereç ve Yöntem: Tanımlayıcı-analitik kesitsel yöntem kullanılarak yapılan bu araştırma, travmatik beyin hasarı geçirmiş 18 yaş ve üzeri 100 kişiden oluşan bir çalışma grubunu içermektedir. Araştırma, NBÖ maddeleri, yaş, cinsiyet ve yaralanma mekanizması ayrıntıları gibi hasta bilgilerini içeren bir kontrol listesi ile yapıldı. Kontrol listesi, yatak başı muayeneleri sırasında tamamlandı.

Bulgular: Bulgular, 21 ila 40 yaş grubundaki erkeklerde (%78) travmatik beyin hasarı prevalansının daha yüksek olduğunu göstermektedir. Hastaların ortalama yaşı 41,63 yıldır. Yüksekten düşme, beyin travmasının en önemli nedeni olarak ortaya çıkmakta ve bunu araç içi trafik kazaları takip etmekteydi. Ortalama birincil GKÖ skoru erkekler için 13,47, kadınlar için 14,63'tü. Vakaların %67'sinde hastaneye yatış, %30'unda taburcu edilme ve %3'ünde cerrahi müdahale yapıldı. Çalışmada, incelenen örneğin NBÖ standartı ile başlangıçtaki GKÖ puanı arasında ters korelasyon olduğu saptandı.

Sonuç: Çalışma sonuçları, NBÖ skoru ile başlangıç GKÖ skoru arasındaki ters ilişki, daha düşük NBÖ değerlerinin daha iyi sonuçlarla ilişkili olduğunu ve bunun öngörücü bir faktör olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Travmatik beyin hasarı, Nöropsikolojik Bozukluk Ölçeği, Glasgow Koma Ölçeği

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INTRODUCTION

Traumatic brain injury (TBI) is a prominent global contributor to disability and mortality, often referred to as a silent epidemic. It is caused by an external force that changes brain function or is another evidence of brain pathology (1, 2).

Worldwide, the annual occurrence of TBI is approximated to range from 27 to 69 million cases. Numerous individuals who survive experience substantial disabilities, causing a substantial social and economic burden. In 2010, the financial consequences of TBIs in the United States amounted to $76.5 billion (3).

In 2014, the Society for Patient Control and Prevention conducted a study on 2.53 million patients who sought emergency care for TBIs. The findings revealed 288,000 hospitalizations and 56,800 fatalities within this group, encompassing both children and adults. Remarkably, adults aged ≥75 years exhibited the highest rate of emergency visits, with 1682 individuals per 100,000. This was followed closely by children aged 0–4 years, with 1618.6 individuals per 100,000, whereas the age group of 15–24 years recorded the lowest number of visits (4).

In Europe, the occurrence of hospitalization after TBI is approximately 262 instances per 100,000 injuries, and the annual fatality rate is approximately 15 cases per 100,000 injuries (5). TBI extends beyond a pathophysiological event, encompassing a range of intricate issues that induce both functional and structural harm through primary and secondary mechanisms of damage (6).

The lesions formed are not confined to the site of the initial trauma but expand progressively. Hence, the initial damage occurs at the moment of impact, and over hours, weeks, months, and ultimately throughout one's life, the secondary damage is triggered by the host's response to the initial injury (7).

The primary damage arises from the immediate impact mechanism on the brain tissue caused by an external force, encompassing contusion, blood vessel damage, bleeding, and nerve damage (8, 9).

Secondary damage occurs from minutes to months after the primary injury, involving a sequence of molecular, cellular, and metabolic events that culminate in brain cell death, tissue damage, and atrophy (10).

Therefore, identifying individuals at risk of unfavorable neuropsychological outcomes after TBI holds significant importance. This study was conducted to evaluate the predictive value of the Neuropsychological Impairment Scale (NIS) in terms of mortality rate, recovery, and discharge of patients who have been admitted to the emergency department at Imam Reza Hospital in Tabriz due to TBI.

MATERIALS and METHODS

This descriptive–analytical cross-sectional investigation included a sample of 100 individuals aged ≥18 years who had experienced TBIs. Ethical approval and the research code were obtained from the Tabriz University of Medical Sciences's Research and Ethical Committee (Date: 04.07.2023, No: R.IAU.TABRIZ.REC.1402.142). Participants were recruited from among those seeking medical attention at the emergency department of Imam Reza Hospital in Tabriz between the beginning of July and the end of August 2023.

Inclusion criteria were patients with TBIs during the specified time frame. Exclusion criteria encompassed individuals who refused to participate or had preexisting physical or mental disabilities before the traumatic injury.

After the acquisition of required approvals, 100 eligible patients were included in this study. These involved individuals aged 18 years and older who visited the emergency department of Imam Reza Hospital in Tabriz between the specified dates. Those who did not wish to participate or had preexisting physical or mental disabilities (before the traumatic event) were excluded from the study.

Subsequently, a checklist was prepared incorporating items from the NIS and patient information such as age, gender, and injury mechanism details. The checklist was completed during bedside examinations and investigations, and subsequent reviews were conducted to align the collected data with the study objectives.

Statistical analysis

The collected data were processed using the SPSS ver.26 (IBM Corp., Armonk, NY, USA) statistical software. Normality of the data was evaluated using the Smirnov–Kolmogorov test. Qualitative data were expressed as frequency (percentage). Quantitative data were expressed as mean (standard deviation) when the data exhibited a normal distribution; otherwise, the median (interquartile range 25–75) value was used. Qualitative data were analyzed using the χ^2 test in two groups, and if certain conditions were met, Fisher's exact test was used.

Moreover, the independent t-test was used for analyzing quantitative data in two groups if the data followed a normal distribution. One-way ANOVA was used to determine whether any significant differences existed in the mean values of a continuous dependent variable across the groups. A p value of <0.05 was considered statistically significant.

RESULTS

In this cross-sectional study employing a descriptive–analytical approach and involving 100 patients with TBI, the obtained statistical outcomes are as follows.

Of the 100 patients with TBI, 78 (78%) were men, and 22 (22%) were women. Regarding age distribution, the mean age of all patients with TBI was 41.36 (range: 18–87) years, and 12% were aged <20 years, 47% were aged 21–40 years, 20% were aged 41–60 years, and 21% were aged ≥61 years, Table 1).

As shown in Table 2, the predominant form of trauma was falling from an elevated position, which accounted for 28% of cases, followed by two-car accidents that accounted for 20% of cases. Regarding the outcome, a majority of patients with TBI underwent hospitalization (67%), followed by discharge (30%), whereas 3% of patients unfortunately died due to the injury (Table 1).

The distribution of male and female patients across the various age groups did not show a significant difference (p=0.247). In all the four age categories under examination, the number of men exceeded that of women, indicating no variance in gender distribution across age groups.

Regarding the nature of trauma, there was no significant difference in distribution between men and women (p=0.063). The prevalent type of trauma for men was falling from a height, which accounted for 21 patients, whereas it was two-car accidents for women, which accounted for 9 patients.

Table 1: Frequency distribution of patients with TBI according to gender and age group

Variable	Variable category	Frequency (%)
Gender	Male	78
	Female	22
Age group (years)	<20	12
	21-40	47
	41-60	20
	>61	21
Trauma type	Two-car accident	20
	Accidents with pedestrians	9
	Car with motorcycle accident	7
	Falling from a height	28
	Car rollover	9
	Motorcycle rollover	8
	Motorcycle with pedestrian accident	3
	Falling from a level height	16
Outcome	Hospitalized	67
	Discharged	30
	Deceased	3

The distribution of men and women based on the outcome showed a remarkable difference (p=0.020). Specifically, the outcomes of hospitalization, discharge, and death for men were 85.1%, 60%, and 100%, respectively (Table 2).

According to the findings of the independent samples t-test that compared the mean ages of two separate groups, there was no statistically significant difference in the average age of male and female patients (p=0.055). The mean ages were 39.46 (±18.47) years for male patients and 48.09 (±18.28) years for female patients (Table 3).

The results of one-way ANOVA that compared the mean values across several independent groups revealed a significant difference in the average age among various types of trauma (p=0.022). The trauma type with the lowest average age was a "car with a motorcycle accident." Further analysis using the least significant difference (LSD) post hoc test revealed significant differences in the average age between "two-car accident" and "accident with pedestrian" traumas (p=0.024), as well as between "accident with pedestrian" and "car with motorcycle accident" traumas (p=0.006).

Moreover, there were significant differences between the average ages of "accident with pedestrian" and "motorcycle rollover" traumas (p=0.006) and "accident with pedestrian" and "other" traumas (p=0.022), as well as between "car with motorcycle accident" and "falling from a height" traumas (p=0.014). Furthermore, the differences in mean age were significant between "falling from a height" and "motorcycle rollover" traumas (p=0.013), as well as between "falling from a height" and "falling from a level height" traumas (p=0.049). Conversely, there was no significant difference in the average age across the outcomes of hospitalization, discharge, and death (p=0.0665).

Nonetheless, it is worth noting that the average age in the death outcome was higher than that in the outcomes of discharge and hospitalization (Table 3).

The independent samples t-test revealed a significant difference in the average NIS score between male (6.17±5.38) and female (3.37±3.66) patients (p=0.035).

The one-way ANOVA revealed a statistically significant difference in the mean NIS scores across various types of trauma (p=0.011). The mean NIS for car rollover trauma was remarkably lower than that for other traumas, amounting to 4.38. Further analysis using the two-by-two mean

Table 2: Comparison of the frequency distribution of gender across age groups, types of trauma, and primary Glasgow Coma Scale (GCS) based on the results obtained from Fisher's exact test

Variable	Variable category	Gender		p value
		Male n (%)	Female n (%)	
Age group (years)	<20	11 (91.7)	1 (8.3)	0.247
	21-40	38 (80.9)	9 (19.1)	
	41-60	16 (80.0)	4 (20.0)	
	>60	13 (61.9)	8 (38.1)	
Trauma type	Two-car accident	11 (55.0)	9 (45.0)	0.063
	Accident with pedestrian	7 (77.8)	2 (22.2)	
	Car with motorcycle accident	7 (100.0)	0 (0.0)	
	Falling from a height	21 (75.0)	7 (25.0)	
	Car rollover	7 (77.8)	2 (22.2)	
	Motorcycle rollover	8 (100.0)	0 (0.0)	
	Motorcycle with pedestrian accident	2 (66.7)	1 (33.3)	
	Falling from a level height	15 (93.8)	1 (3.6)	
Outcome	Hospitalized	57 (85.1)	10 (14.9)	0.020
	Discharged	18 (60.0)	12 (40.0)	
	Deceased	3 (100.0)	0 (0.0)	

Table 3: Average age according to the variables of gender, type of trauma, and outcome in patients with TBI

Variable	Variable category	Frequency	Age average	Standard deviation	Min	Max	p value
Gender	Male	78	39.46	18.47	18	87	0.055[1]
	Female	22	48.09	18.28	18	87	
Trauma type	Two-car accident	20	38.25	16.29	18	72	0.022[2]
	Accidents with pedestrians	9	54.67	20.95	18	87	
	Car with motorcycle accident	7	29.71	9.43	18	44	
	Falling from a height	28	48.46	18.68	20	87	
	Car rollover	9	41.00	16.29	21	68	
	Motorcycle rollover	8	30.50	14.54	19	64	
	Motorcycle with pedestrian accident	3	34.33	24.91	18	63	
	Falling from a level height	16	37.38	19.50	18	73	
Outcome	Hospitalized	67	41.15	18.79	18	87	0.665[2]
	Discharged	30	40.87	17.52	18	87	
	Deceased	3	51.00	31.95	26	87	

1: Independent samples t-test, 2: One-way ANOVA

comparison test (LSD) revealed significant differences in mean NIS scores between "two-car accident" and "accident with pedestrian" traumas (p=0.001), "accident with pedestrian" and "falling from a height" traumas (p=0.003), "accident with pedestrian" and "car rollover" traumas (p=0.004), "accident with pedestrian" and "motorcycle

Table 4: Comparison of mean NIS and GCS scores according to the variables of gender, type of trauma, and outcome in patients with TBI (nine patients were excluded during NIS score evaluation because of toxicology suspicious finding) (one patient's GCS score was variable and altered and then excluded during evaluation in this table).

Variable	Variable category	Frequency	Initial GCS score Median	Min	Max	p value
Gender	Male	77	15	3	15	0.305[1]
	Female	22	15	10	15	
Trauma type	Two-car accident	20	15	3	15	0.129[2]
	Accidents with pedestrians	9	14	3	15	
	Car with motorcycle accident	7	14	11	15	
	Falling from a height	27	15	4	15	
	Car rollover	9	15	4	15	
	Motorcycle rollover	8	14	13	15	
	Motorcycle with pedestrian accident	3	14	14	15	
	Falling from a level height	16	15	14	15	
Outcome	Hospitalized	66	14	4	15	<0.001[2]
	Discharged	30	15	15	15	
	Deceased	3	3	3	4	

Variable	Variable category	Frequency	NIS mean	Standard deviation	Min	Max	p value
Gender	Male	72	6.17	5.38	0	30	0.035[1]
	Female	19	3.37	3.66	0	13	
Trauma type	Two-car accident	18	4.44	3.59	0	11	0.011[2]
	Accidents with pedestrians	7	11.86	10.54	0	21	
	Car with motorcycle accident	6	7.50	6.16	0	18	
	Falling from a height	25	5.56	4.00	0	15	
	Car rollover	8	4.38	5.34	0	12	
	Motorcycle rollover	8	6.88	3.72	2	12	
	Motorcycle with pedestrian accident	3	7.67	5.03	3	13	
	Falling from a level height	16	3.00	3.03	0	10	
Outcome	Hospitalized	59	6.61	4.77	0	21	<0.001[2]
	Discharged	30	2.53	1.98	0	8	
	Deceased	2	21.00	12.73	3	12	

[1]: Independent samples t-test, [2]: One-way ANOVA, GCS: Glasgow Coma Scale, NIS: Neuropsychological Impairment Scale

rollover" traumas (p=0.05), as well as between "accident with pedestrian" and "other" traumas (p>0.001).

Furthermore, a significant difference was observed in mean NIS scores among the outcomes of hospitalization, discharge, and death (p<0.001). The mean NIS scores for hospitalization, discharge, and death outcomes were 6.61, 2.53, and 21.00, respectively. Further analysis using the LSD test indicated significant differences in mean NIS scores between hospitalization and discharge (p<0.001) and hospitalization and death (p<0.001), as well as between discharge and death (p>0.001) (Table 4).

The independent samples t-test revealed no statistically significant differences in the median initial Glasgow Coma Scale (GCS) scores between male and female patients (p=0.305), with the median initial GCS score for both groups being 15.

The one-way ANOVA also revealed no significant differences in the median initial GCS scores across various types of trauma (p=0.129).

However, the mean GCS scores exhibited a significant difference among the outcomes of hospitalization, discharge, and death (p<0.001), with the scores being 14, 15, and 3, respectively. Further analysis using the two-by-two mean comparison test (Tukey's post hoc test) revealed significant differences in mean GCS scores between hospitalization and discharge (p=0.004) and hospitalization and death (p<0.001), as well as between discharge and death (p>0.001) (Table 4).

Moreover, the Pearson correlation coefficient test revealed a significant negative correlation between the NIS and initial GCS scores (r=−0.534; p<0.001).

DISCUSSION

Mild TBI, characterized by a GCS score of ≥13, constitutes the majority of traumatic injuries, especially in the domain of sports (3). In a comprehensive study involving 17,470 patients with TBI, 57% initially experienced a loss of consciousness, which decreased to 12% after the implementation of initial treatment interventions. By the end of the treatment period, 98% of these patients had fully regained consciousness, indicating significant improvement (11).

After prolonged hospitalization, most people with TBI require a prolonged period of rehabilitation and may face problems such as physical, cognitive, psychological, and behavioral difficulties (12).

Research suggests that various factors, including age and initial GCS score, play a significant role in predicting the outcome of individuals with TBI (13).

Various criteria, such as the initial GCS score, disability criteria, and length of hospital stay, are used to predict the prognosis of individuals with TBI.

The NIS is a standard assessment tool for screening adults' neuropsychological symptoms. It comprises various sections addressing general and specific disorders, encompassing symptoms that might go unnoticed during diagnostic and treatment processes or may not be explicitly communicated by the patient. The NIS serves as a valuable instrument for a detailed evaluation of neuropsychiatric complications. It helps in identifying the affected areas and determining whether the patient benefits from treatment by aligning with treatment goals.

The NIS questionnaire includes 95 items and covers aspects related to motor function, tone and joint movement limitations, sensory perception, perceptual function, speech abilities, cognitive function, behavior, mood, vision and hearing, pain sensation, and fatigue perception (14).

Although movement issues resulting from brain trauma tend to improve in the majority of patients, neuropsychological and behavioral challenges persist for months and even years. Therefore, these issues are labeled as invisible disabilities, affecting various facets of an individual's life, including personal, social, familial, and professional dimensions, ultimately impacting both career and overall quality of life (15, 16).

In our study, the mean age of the patients was 41.36 years, and there was a remarkable gender disparity, with the majority (78%) being men in terms of relative frequency.

Skaansar et al. conducted a study in 2020 to investigate TBI across various age categories, including 1571 patients aged ≥15 years, and showed that the average age was 58 years, with a remarkable predominance of men, constituting 70% of the participants. Furthermore, 39% of the examined population was aged ≥65 years (17).

Subsequently, in a systematic study, Mollayeva et al. examined 58 registered articles on TBIs, which included 1,265,955 patients, to determine the frequency of age and gender in these patients. They reported that 67% of the study patients were men (18). This gender predominance is consistent with the findings of the two abovementioned studies.

Zia et al. evaluated 3749 patients with TBI whose average age was 28 years. The highest occurrence was observed in the age group of 19–45 years, and men were predominant. They found that the primary cause of TBI in the examined population was road accidents (42.1% involving pedestrians and 28.1% involving motorcyclists), followed by falls from heights (11.1%) (19).

Although the average age and proportion of occurrence in various age groups documented in comparable studies

vary from those observed in our study, this disparity is affected by the demographic characteristics specific to distinct geographic regions of the analyzed samples (17, 19).

The predominant cause of TBI in our investigation, with the highest incidence, was associated with falls from elevated surfaces (28%), followed by involvement in two-vehicle accidents (20%).

Lee et al. evaluated the prognostic factors in individuals with TBIs. Over three months, they examined 42 patients with TBIs whose average age was 44.5 years (range: 8–73 years). The primary cause of injury in 45.2% of the patients was falling from a height, followed by motorcycle, pedestrian, bicycle, and car accidents (20). This finding was in contrast to those reported by Zia et al., where falling from a height was the third most prevalent mechanism, following a car accident involving a pedestrian and an accident when riding as a motorcycle passenger (19).

Ponsford et al. conducted a study in 2008 involving 60 patients with mild-to-severe TBIs, wherein they reported a correlation between suboptimal The Extended Glasgow Outcome Scale (GOS-E) criteria and impaired performance in processing speed, attention, memory, and executive function (21).

The mean initial GCS score in our study patients was 14.3 for men and 13.74 for women. Although there was no significant difference across various trauma mechanisms, there was a clear correlation with the outcome. Specifically, the mean GCS scores for discharged, hospitalized, and deceased patients were 15, 13.85, and 3.33, respectively. When comparing this outcome with the findings of Lee et al. and Ponsford et al, it appears that a low GCS score is related to a poorer short-term outcome and an inferior long-term prognosis (20, 21).

Our study also indicated a negative correlation between GCS and NIS scores, consistent with the findings of Ponsford et al who showed a correlation between low GCS scores and suboptimal neuropsychological performance (21).

Comparable studies have yielded multiple results on the relevance of neuropsychological factors in predicting the outcomes of patients with TBIs. For instance, Sigurdardottir et al. reported that improved neuropsychological functioning, including higher verbal, reasoning, visual, spatial, and perceptual skills, and reduced short-term memory impairment are associated with a more favorable prognosis (22).

Although our study contributes valuable insights for understanding TBIs, it is essential to acknowledge certain limitations inherent in the research design. First, our sample size may limit generalizability, and caution should be exercised when extrapolating these findings to broader populations. Furthermore, the retrospective study design introduces the potential for recall bias, as reliance on medical records may capture only some relevant details.

Moreover, our study's focus on specific demographic and neuropsychological factors necessarily excludes consideration of other potentially influential variables. Future studies could benefit from a more expansive approach to include a broader array of factors that contribute to a more comprehensive understanding of TBIs.

CONCLUSION

TBI is more prevalent among men and women aged 21–40 years. Falls from a height constitute the primary cause. Most patients exhibit a GCS score of >13, signifying mild traumatic injuries. An inverse relationship between NIS and initial GCS scores suggests that lower NIS scores are associated with better outcomes, indicating its utility as a predictive factor.

Ethics Committee Approval: The study has ethical approval from the Tabriz University of Medical Sciences's Research and Ethical Committee (Date: 04.07.2023, No: R.IAU.TABRIZ.REC.1402.142).

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THE COURSE OF BEHÇET'S DISEASE FOLLOWING COVID-19 DIAGNOSIS: A LARGE RETROSPECTIVE COHORT STUDY

COVID-19 TANISINI TAKİBEN BEHÇET HASTALIĞININ SEYRİ: GENİŞ BİR RETROSPEKTİF KOHORT ÇALIŞMASI

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ABSTRACT

Objective: Behçet's disease (BD) is a chronic vasculitic disease with mucocutaneous and systemic involvements. This study aims to examine the effects of COVID-19 on the symptoms and course of BD.

Material and Method: The BD patients who were followed up in our department with a history of COVID-19 were evaluated regarding the course of infection and the course of BD post-COVID diagnosis, assessed by Behçet's Disease Activity Index (BDAI).

Result: Among 449 BD patients, 68 (15.1%) had contracted COVID-19. The mean age of the patients was 42.7±11.8 years of whom 63.2% (n=43) were female. While most (n=48, 70.6%) had only mucocutaneous symptoms, others also had systemic symptoms (n=20, 29.4%) during their BD course; 86.8% (n=59) had received colchicine only at the time of infection. Prior to infection, 85.3% (n=58) had been in remission (BDAI score of 0), while 14.7% (n=10) already had active BD (BDAI score between 1-3). Post-COVID-19 diagnosis, activation or exacerbation of activation was seen in 39.7% (n=27) of BD patients, as characterized by increased BDAI scores (BDAI score between 1-4). No change was recorded in 60.3% (n=41) of the patients. Disease activation in the BD patients was mostly mucocutaneous (n=21, 30.9%). Comparison of the BD patients' BDAI scores pre- and post-COVID-19 diagnosis revealed the scores to be significantly elevated ($p<0.001$; $z=-4.691$), demonstrating the possible effects of COVID-19 on BD severity.

ÖZET

Amaç: Behçet hastalığı mukokutanöz ve sistemik bulgularla seyredebilen kronik vaskülitik bir hastalıktır. Bu çalışmada COVID-19 enfeksiyonunun Behçet hastalığı semptomları ve seyri üzerine etkisini incelemeyi amaçladık.

Gereç ve Yöntem: Üçüncü basamak bir Dermatoloji merkezinde takip edilen Behçet hastaları COVID-19 enfeksiyonu açısından sorgulandı. Daha önce PCR ile kanıtlanmış COVID-19 geçiren hastalarda Behçet hastalığı seyri ve aktivasyonu Behçet Hastalığı Aktivite İndeksi (BHAİ) ile retrospektif olarak değerlendirildi.

Bulgular: Bu çalışma sürecinde poliklinik kontrolüne başvuran 449 Behçet hastasının 68'inin (%15,1) COVID-19 enfeksiyonu geçirme öyküsü mevcuttu. Hastaların yaş ortalaması 42,7±11,8 yıl olup, %63,2'si (n=43) kadındı. Behçet hastalarının çoğunda (n=48, %70,6) Behçet hastalığı sadece mukokutanöz tutulum ile seyrederken, bir kısmında (n=20, %29,4) çeşitli sistemik tutulumlar hastalık sürecinde gelişmişti. Bu hastaların büyük bir çoğunluğu (n=59, %86,8) enfeksiyon öncesinde sadece kolşisin tedavisi almaktaydı. COVID-19 enfeksiyonu öncesi BHAİ skorlama sistemine bakıldığında hastaların %85,3'ü (n=58) remisyondaydı (BHAİ-0), %14,7'si (n=10) ise halihazırda aktivasyondaydı (BHAİ-1-3). COVID-19 enfeksiyonunu takiben hastaların %39,7'sinde (n=27) aktivasyon veya hastalık şiddetinde artış görüldü (BHAİ-1-4). Behçet hastalarımızda enfeksiyonu takiben görülen hastalık aktivasyonu çoğunlukla mukokutanöz (n=21, %30,9) lezyonlar ile karakterizeydi. Hastaların %60,3'ünde (n=41) ise hastalık seyrinde herhangi bir değişiklik kaydedilmedi. Behçet hastalarında COVID-19 enfeksiyonu öncesi ve enfeksiyon sonrası BHAİ skor-

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Conclusion: COVID-19 infection may lead to activation of BD symptoms, with new organs being involved in some patients, which should be confirmed by prospective large series.

Keywords: Behcet's disease, COVID-19, disease activity

ları karşılaştırıldığında anlamlı düzeyde (p<0,001 ve z=-4,691) Behçet hastalık şiddetinde artış tespit edildi.

Sonuç: COVID-19 enfeksiyonu bazı hastalarda Behçet hastalığı semptomlarının aktivasyonuna, alevlenmesine ve hatta yeni organ tutulumuna yol açabilmektedir. Bu durum prospektif geniş serilerle doğrulanmalıdır.

Anahtar Kelimeler: Behçet hastalığı, COVID-19, hastalık aktivitesi

INTRODUCTION

Hulusi Behçet first described Behçet's disease (BD) in 1937 as a triple-symptom disorder characterized by oral aphthae, genital ulceration, and uveitis. Although particularly prevalent in countries on the Silk Road, BD can be seen worldwide. Türkiye is one of the countries where the disease is most commonly encountered, with a prevalence between 20:100,000 and 420:100,000. Immunological and environmental factors such as bacterial and viral infections are thought to play an important role in the pathogenesis of the disease. BD is a chronic, inflammatory, and systemic vasculitic disease with various mucocutaneous and multiorgan involvements. The ocular and vascular involvements of the disease that adversely affect prognosis are more commonly seen in males (1).

The coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus hit the world in December 2019 and was declared a pandemic by the World Health Organization (WHO) in March 2020. While affecting all age groups, individuals with chronic diseases have been implicated as being at greater risk (2). BD patients under immunosuppressive and immunomodulatory treatments have been previously suggested to be in the high-risk spectrum for COVID-19. However, only a few studies have evaluated the course of BD post-COVID-19 diagnosis in large groups of BD patients (3–16). This study aims to delineate the effects of COVID-19 on BD and to describe the course of COVID-19 infection in a large BD patient group in an experienced tertiary center in Türkiye.

MATERIALS and METHODS

The study retrospectively evaluates the data from consecutive patients who'd been diagnosed with BD, focusing on the routine follow-up visits between February-August 2021 with a history of COVID-19 diagnosis. Patients without a polymerase chain reaction (PCR) confirmation were not included in the study group, regardless of whether their symptoms or radiological findings were compatible with COVID-19. Moreover, the study group consists of unvaccinated patients due to lack of community COVID-19 vaccination program in Türkiye during this period. Data regarding patients' symptoms, including the need for hospitalization and oxygen support, were also collected.

The study records individual data regarding clinical status and ongoing treatments and gives the BD patients' Behçet's Disease Activity Index (BDAI; Table 1) scores based on their symptoms three months prior to and one month after their COVID-19 diagnosis. According to this scoring system, patients with a score of 0 were accepted as being in remission, while any score above 0 was treated as an active BD case. When assessing the status of the disease following COVID-19, any increase in the BDAI score was considered an activation or exacerbation of activation of BD.

Statistical analysis

The study expresses the data as averages, means, medians, and percentages and uses the program SPSS (Version 22; IBM, SPSS Corp., Armonk, NY, USA) for all statistical analyses, with $p<0.05$ being considered significant. The study uses the Wilcoxon Sign Rank Test to evaluate the patients pre- and post-COVID-19 BDAI scores.

The patients gave written informed consent to publishing their case details. This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the İstanbul University, İstanbul Faculty of Medicine local ethical committee (Date: 10.09.2021; No: 16).

RESULTS

Among the 449 BD patients (42.3% males; 57.7% female; mean age = 42.9 years) who underwent follow-up

Table 1: Behçet's disease activity index (BDAI) score

BDAI-0	Less than two oral aphthae per month
BDAI-1	More than two oral aphthae, pseudofolliculitis, and/or erythema nodosum per month
BDAI-2	The same findings as BDAI-1, as well as genital ulcer, joint involvement, and/or superficial thrombophlebitis
BDAI-3	The same findings as BDAI-2, as well as eye involvement, vascular involvement, neurological involvement, and/or other system involvements
BDAI-4	New organ involvement

visits throughout the study, 68 (15.1%) had COVID-19 infection that was verified with PCR. These patients' mean age was 42.7±11.8 years, of whom 63.2% (n=43) were female. The median treatment duration for BD was 11.7±6.8 years before COVID-19 diagnosis. These BD patients either had only mucocutaneous symptoms (n=48, %70.6) or developed systemic symptoms (n=20, 29.4%) during their course of BD (Table 2). In managing BD during at the time of COVID-19 diagnosis, 59 (86.8%) patients only received colchicine, 5.9% (n=4) received azathioprine and colchicine, and one patient received adalimumab. One patient received no treatment due to pregnancy. Three patients (4.4%) were followed up without any medication due to BD being in remission (Table 2). When evaluating the patients based on their pre-COVID-19 BDAI scores, 85.3% (n=58) had been in remission with a BDAI score of 0, while 14.7% (n=10) had already had an active BD status. Of these latter patients, seven (10.3%) had a score of 1, two (2.9%) had a score of 2, and one had a score of 3 (Table 3). The following pre-COVID-19 BDAI scores were found for BD patients who'd undergone any systemic involvement in the course of BD (n=20) that had been managed with various immunosuppressives including systemic corticosteroids (n=15), azathioprine (n=10), and/or adalimumab (n=2): BDAI-0 (n=14), BDAI-1 (n=4), BDAI-2 (n=1), and BDAI-3 (n=1).

Of note, only one female patient (57 years old) with a BDAI-0 was hospitalized due to COVID-19 symptoms. All other BD patients had COVID-19 with mild or moderate symptoms. Arthralgia (n=30) was the most common COVID-19 clinical finding recorded in the BD patients, followed by myalgia (n=28), headache (n=24), cough (n=17), shortness of breath (n=14), fatigue (n=13), loss of smell (n=9), and loss of taste (n=5). Furthermore, 15 patients had asymptomatic COVID-19. Of these patients, 29 were treated with favipiravir, two with favipiravir and enoxaparin, three with hydroxychloroquine, and two with enoxaparin. Five patients received no treatment, while information regarding the COVID-19 treatments

Table 2: The clinical features and treatments of patients with Behçet's disease before COVID-19 infection

Variables	n (%)	Male/Female
BD course		
- Only mucocutaneous involvement	48 (70.6)	13/35
- Mucocutaneous and systemic involvement	20 (29.4)	12/8
Mucocutaneous involvements		
Recurrent aphthous stomatitis	68 (100)	25/43
Genital ulcer	35 (51.5)	9/26
Erythema nodosum	51 (75)	17/34
Pseudofolliculitis	30 (44.1)	7/23
Extragenital ulcer	1 (1.5)	0/1
Systemic involvements		
Joint involvement	17 (25)	5/12
Vascular involvement	14 (20.6)	9/5
- Deep venous thrombosis	12 (17.6)	7/5
- Superficial thrombophlebitis	4 (5.9)	4/0
Uveitis	7 (10.3)	4/3
- Uveitis and vascular involvement	1 (1.5)	1/0
Neurological and vascular involvement	1 (1.5)	0/1
Treatment		
Colchicine only	59 (86.8)	19/40
Immunosuppressive	5 (7.4)	4/1
- Azathioprine and colchicine	4 (5.9)	4/0
- Adalimumab	1 (1.5)	0/1
No treatment	4 (5.9)	2/2
- Pregnancy	1 (1.5)	0/1
- Remission	3 (4.4)	2/1

BD: Behcet's disease

Table 3: Comparison of clinical features of Behçet's disease in patients before and after COVID-19 infection

Pre-COVID-19 clinical status (BDAI)	Post-COVID-19 clinical status	n (%)	Male/Female
Remission (n=58, 85.3%), BDAI-0 (n=58)	**Activation** BDAI-1 (n=17), BDAI-2 (n=5), BDAI-3 (n=2), BDAI-4 (n=1)	**25 (36.8)**	**8/17**
	Systemic involvement	4 (5.9)	3/1
	-Deep venous thrombosis	1 (1.5)	1/0
	-Superficial thrombophlebitis	1 (1.5)	1/0
	-Uveitis	1 (1.5)	0/1
	-Joint involvement	2 (2.9)	2/0
	Mucocutaneous involvement	21 (30.9)	5/16
	-Recurrent aphthous stomatitis	17 (25.0)	4/13
	-Genital ulcer	5 (7.4)	1/4
	-Erythema nodosum	5 (7.4)	1/4
	-Pseudofolliculitis	3 (4.4)	0/3
	No change BDAI-0 (n=33)	**33 (48.5)**	**13/20**
Activation (n=10, 14.7%), BDAI-1 (n=7), BDAI-2 (n=2), BDAI-3 (n=1)	**Exacerbation of activation** BDAI-3 (n=1); BDAI-4 (n=1)	**2 (2.9)**	**1/1**
	Systemic involvement		
	- Deep venous thrombosis	1 (1.5)	0/1
	- Deep venous thrombosis and epididymo-orchitis	1 (1.5)	1/0
	No change BDAI-1 (n=6), BDAI-2 (n=1), BDAI-3 (n=1)	**8 (11.8)**	**3/5**

BDAI: Behçet's Disease Activity Index

for other patients was unobtainable due to the study's retrospective nature. According to current recommendations and the authors' clinical experience, when the patients had positive PCR result, the immunosuppressive treatments of this patient group were stopped, whereas the colchicine treatment was continued (17).

Post-COVID-19 diagnosis, activation (n=25) or exacerbation of activation (n=2) was noted in 27 (39.7%) patients as a result of increased BDAI scores, while no change in BDAI scores was recorded in 41 (60.3%) patients (Table 3). Disease activation in the BD patients was mostly mucocutaneous (n=21, 30.9%), of whom 16 were female. Systemic activation was recorded in only six (8.8%) patients, which manifested as deep venous thrombosis (n=3), superficial thrombophlebitis (n=1), joint involvement (n=2), uveitis (n=1), or epididymo-orchitis (n=1). One patient with BD who was in remission (BDAI-0) had their first genital ulcer attack post-COVID-19 diagnosis. Another patient with active BD (BDAI-2) had their first epididymo-orchitis episode and a recurrence of deep venous thrombosis post-COVID-19 diagnosis. Finally, the BDAI scores of all post-COVID-19 BD patients in the cohort were as follows: BDAI-0 (n=33), BDAI-1 (n=23), BDAI-2 (n=6), BDAI-3 (n=4), and BDAI-4 (n=2; Table 3). The patients who had no change in symptoms (n=41) had the following BDAI scores: BDAI-0 (n=33), BDAI-1 (n=6), BDAI-2 (n=1), and finally BDAI-3 (n=1; Table 3).

Of the 27 BD patients showing disease activation or exacerbation in activation post-COVID-19 diagnosis, 25 had been in remission (BDAI-0) pre-COVID-19 diagnosis, one had had a score of BDAI-1, and one a score of BDAI-2. Post-COVID-19 diagnosis, most of the patients had mild BD activation, with a BDAI score of BDAI-1 (n=17) or BDAI-2 (n=5), as well as severe disease activation observed in five patients, with BDAI-3 (n=2) or BDAI-4 (n=1, first genital ulcer attack). Moreover, BDAI scores increased from 1 to 3 in one patient and from 2 to 4 in another (the patient with their first epididymo-orchitis episode), accompanied by aggravated BD activation (Table 3).

Furthermore, when comparing the patients pre- and post-COVID-19 BDAI scores using the Wilcoxon signed rank test, the results are found to be highly significant ($p<0.001$; $z=-4.691$), proving COVID-19 to likely have an impact on the course of BD.

DISCUSSION

Limited data is found in the literature on the complications of COVID-19 in patients with BD and COVID-19's effects on the course of BD (3-16). The present study has

aimed to use a larger patient series to identify the changes in the progression of BD post-COVID-19 diagnosis. The article retrospectively analyzed 449 BD patients, of whom 68 had a PCR-confirmed COVID-19 diagnosis that resulted in a statistically significant activation and exacerbation of the disease as graded by BDAI. Therefore, the study's results imply COVID-19 likely leads to BD activation and/or exacerbation in some BD patients. The current study group had a 15.1% prevalence of COVID-19. Unlike the current study that only included BD patients whose COVID-19 diagnosis was confirmed through PCR, some previous studies had also included BD patients who were highly suspected of COVID-19 in addition to confirmed diagnoses (11, 14). Previous studies have also controversially discussed the prevalence of COVID-19 among BD patients. One study reported a higher prevalence of COVID-19 in BD patients, while another study described the opposite (11, 15). Yet another study found the prevalence of COVID-19 in BD patients to be 4.2% and concluded no increased risk of COVID-19 infection or complication to exist in BD patients compared to the general population, similar to other studies (4, 6). One study that included 10 BD patients reported BD patients to be much younger and appear to have an increased risk of severe outcome due to COVID-19 (8), a finding not supported by the current study. In addition, another study in Türkiye with a small number of patients reported the relative risk of COVID-19 infection and complications in BD patients to be higher than the other inflammatory diseases (3). However, another study in France evaluated 117 patients with autoinflammatory diseases, 21 of whom had been diagnosed with BD, and found no significant difference in the severity of COVID-19 between patients with systemic autoinflammatory disease and those without. Furthermore, the study in France suggested patients with systemic autoinflammatory disease under corticosteroid treatment should be considered at high risk for severe COVID-19 (14). Yet another study also found receiving glucocorticoids and cytotoxic drugs to be associated with an increased hospitalization rate (11). In contrast, a recent systemic review and meta-analysis concluded administering glucocorticoids have no significant correlation with hospitalization risk in patients with rheumatic diseases, as well as the risk of severe COVID-19 in this population was similar to that observed in the reference population (18). The current study found only one BD patient to have been hospitalized due to COVID-19. All other BD patients' COVID-19 infections had mild or moderate symptoms.

While this study found deep venous thrombosis to be the most common form of systemic activation, recurrent aphthous stomatitis was the most common form of mucocutaneous activation. Remarkably, one patient post-COVID-19 experienced their first epididymo-orchitis attack, with another experiencing their first genital ulcer attack; these attacks also significantly raised their BDAI scores. This study found systemic activation to be more common in males whereas mucocutaneous activation to be more common in females.

The higher rates of deep venous thrombosis in BD patients post-COVID-19 may be due to high levels of IL-1a in BD patients' serum. IL-1a is a cytokine responsible for susceptibility to vascular inflammation and clot formation. Similarly, IL-1a release and inflammasome formation have also been observed in COVID-19 infection. IL-1a released into the blood causes endothelial damage and inflammatory thrombosis formation, which might explain the exacerbation of activation observed in some BD patients (19-21). Contrary to previous results, a recent study in Türkiye reported COVID-19 not appearing to exacerbate thrombotic events during or after infection in a large series (10). Colchicine is widely used in BD worldwide to prevent inflammasome formation and suppress IL-1 levels, which may have a protective role against COVID-19. However, another study in Türkiye reported evaluating the frequency and severity of COVID-19 in patients with various rheumatic diseases (including BD) who'd been treated regularly with colchicine or hydroxychloroquine; these treatments did not result in preventing COVID-19 or ameliorating its manifestations (12). Another study showed BD patients to have asymptomatic COVID-19 and also suggested anti-tumor necrosis factor agents to be able to protect BD patients from severe COVID-19 (9). Biological agents were delayed following COVID-19 cases in many countries that were used to manage many diseases. During this period of treatment cessation, uveitis attacks were reported in BD patients using infliximab and adalimumab. Of the current work's study group, only one patient under adalimumab treatment had experienced an uveitis attack post-COVID-19. In addition, other systemic involvements have been reported, such as a Neuro-Behçet's disease being exacerbated by COVID-19 (22). Although the present study has only one patient with Neuro-Behçet's disease, no aggravation or change in her condition was observed. Furthermore, the fact that most of the current study's patients suffered exacerbations regarding BD, with mostly mucocutaneous symptoms in addition to mild COVID-19 symptoms, may be related to the protective role of colchicine.

This study's main limitations are its retrospective nature and the fact that it has been conducted in a single tertiary center. Furthermore, a direct comparison of activation ratios could not be performed due to the need for a control group of BD patients who'd not had COVID-19.

CONCLUSION

This study represents a large case series of BD patients who'd also contracted COVID-19 and shows BD acti-

vation or exacerbation of activation occurred mostly with mucocutaneous symptoms, in addition to the systemic involvements recorded in a few patients post-COVID-19 diagnosis. The findings suggest a complex relationship between COVID-19 and BD, however the underlying pathogenic mechanisms needs to be further studied.

Ethics Committee Approval: The study was approved by the İstanbul University, İstanbul Faculty of Medicine local ethical committee (Date: 10.09.2021; No: 16).

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
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THE RELATION OF SERUM NESFATIN-1 LEVELS WITH DISEASE SEVERITY AND COMPLICATIONS IN PATIENTS WITH LIVER CIRRHOSIS*

KARACİĞER SİROZLU HASTALARDA SERUM NESFATİN-1 DÜZEYLERİNİN HASTALIK ŞİDDETİ VE KOMPLİKASYONLARI İLE İLİŞKİSİ

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ABSTRACT

Objective: Nesfatin-1 is an anorectic polypeptide that plays important roles in regulating appetite and energy intake. Cachexia and malnutrition are common in individuals with cirrhosis. We examined the relationship between serum nesfatin-1 levels and stage of cirrhosis, with the hypothesis that an increase in nesfatin-1 levels in patients with cirrhosis may be related to this catabolic process.

Material and Method: The study includes 51 patients with cirrhosis and 30 healthy volunteers. Nesfatin-1 levels in serum samples were compared using the enzyme-linked immunosorbent assay (ELISA). We calculated the Child-Pugh stages and Model for End-Stage Liver Disease (MELD) scores of patients with cirrhosis and examined their relationship with nesfatin-1. We've also investigated the relationship between cirrhosis complications and nesfatin-1.

Results: We found nesfatin-1 levels to be significantly higher in the cirrhosis patient group compared to the control group (p=0.001). The patient group was divided into those with compensated and those with decompensated liver cirrhosis

ÖZET

Amaç: Nesfatin-1, iştahın ve enerji alımının düzenlenmesinde önemli rolleri olan anorektik bir polipeptittir. Kaşeksi ve malnutrisyon sirozlu bireylerde yaygındır. Nesfatin-1 düzeylerinin sirozlu hastalardaki artışının sirozdaki katabolik süreçle alakalı olabileceği hipoteziyle nesfatin-1 serum düzeyleri ve siroz aşamaları ilişkisini inceledik.

Gereç ve Yöntem: Çalışmamıza 51 sirozlu hasta ve 30 sağlıklı gönüllü alındı. Serum örneklerinden nesfatin-1 düzeyleri ELISA kullanılarak karşılaştırıldı. Sirozlu hastaların Child-Pugh evreleri ve multifaktöriyel son dönem karaciğer hastalığı modeli (MELD) skorları hesaplanarak nesfatin-1 ile ilişkileri incelendi. Ek olarak siroz komplikasyonları ile nesfatin-1 arasındaki ilişki araştırıldı.

Bulgular: Nesfatin-1 düzeyleri sirozlu hasta grubunda kontrol grubuna göre anlamlı olarak yüksek bulundu (p=0,001). Hasta grubu kompanse ve dekompanse olarak ayrılıp, kontrol grubu ile karşılaştırıldı. Kompanse siroz grubunda nesfatin-1 düzeyleri anlamlı olarak yüksek bulundu (p=0,01). Sirozlu hastalar Child-Pugh

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and compared with the control group. Nesfatin-1 levels were found to be significantly higher in the compensated liver cirrhosis group (p=0.01). When classifying the patients with cirrhosis based on their Child-Pugh stages and MELD scores, no significant relationship was detected between these groups and their nesfatin-1 levels.

Conclusion: Nesfatin-1 may have antioxidant, anti-inflammatory, and anti-apoptotic effects in maintaining the state of patients with compensated liver cirrhosis. Low levels of nesfatin-1 in decompensated liver cirrhosis may result from defense mechanisms and inadequate production.

Keywords: Nesfatin-1, liver, cirrhosis, decompensation, anti-inflammatory, antioxidant

evrelerine ve MELD skorlarına göre sınıflandırıldığında bu gruplar ile nesfatin-1 düzeyleri arasında anlamlı bir ilişki saptanmadı.

Sonuç: Sirozlu hastalarda kompanze halin sürdürülmesinde nesfatin-1'in antioksidan, antiinflamatuar ve antiapoptotik etkileri olabilir. Dekompanse sirozda nesfatin-1 düzeylerinin düşük olması savunma mekanizması ve yetersiz üretimden kaynaklanabilir.

Anahtar Kelimeler: Nesfatin-1, karaciğer, siroz, dekompansasyon, antienflamatuvar, antioksidan

INTRODUCTION

Cirrhosis develops as a result of hepatocellular damage leading to extensive fibrosis and nodular regeneration in the liver. Advanced cirrhosis usually involves anorexia. Taste and smell disorders in patients increase anorexia. Weight loss, cachexia, sarcopenia, and chronic disease are common. Body mass index (BMI) has been previously shown to be insufficient at revealing malnutrition, especially in patients with overt ascites (1, 2).

Nesfatin-1 is a polypeptide that has important roles in the regulation of food intake, energy homeostasis, and water intake. Nesfatin-1 is the amino-terminal portion of nucleobindin-2 (NUCB2) detected in the hypothalamic nuclei and has proven to be effective in appetite control in rats. Intracerebroventricular (ICV) injection of nesfatin-1 dose-dependently reduced food intake in rats with leptin receptor mutation (3). Nesfatin-1 has been shown to be expressed in many peripheral tissues other than the central nervous system (4, 5). Recent studies have highlighted the anti-inflammatory, antioxidant, and anti-apoptotic effects of nesfatin-1 (6).

We aim to determine the level of nesfatin-1 in the cirrhosis and control groups in order to investigate its relationship with the severity and complications of cirrhosis and also to determine whether nesfatin-1 has a role in decompensated liver cirrhosis by considering how the anorectic nesfatin-1 peptide might be responsible for the frequently encountered cachexia and malnutrition in patients with cirrhosis.

MATERIALS and METHODS

Study group

Our study involves a patient group consisting of 51 patients with cirrhosis and 30 healthy volunteers who were admitted to the internal medicine clinic in our hospital. We determined sample size according to a power analysis based on previous articles about nesfatin-1. Volunteers over 18 years old who'd been diagnosed with cirrhosis of the liver and followed up in our clinic and who'd been supported by clinical, laboratory, and radiologic data were included in the study as the patient group. Volunteers over 18 years old without liver cirrhosis were included in the study as the control group. Persons under 18 years of age, who did not give consent, who were pregnant, who have a BMI > 35 kg/m^2, who'd been diagnosed with schizophrenia and anorexia nervosa, or who are patients with a malignancy were excluded from the study.

We noted the age, sex, height, and weight of the participants after obtaining informed consent from all volunteers, then questioned them about comorbidities and drug use before measuring basic biochemical parameters, international normalized ratio (INR), C-reactive protein (CRP), and glycated hemoglobin (HbA1c) levels. We then asked about any complications of cirrhosis and the etiology of cirrhosis. The only complications of cirrhosis in the patient group were hepatic encephalopathy, ascites, and variceal bleeding. The Model for End-Stage Liver Disease (MELD) scores and Child-Pugh stages were also calculated. The primary endpoint of the study is to determine and compare the levels of nesfatin-1 in the patient and control groups. The secondary endpoint of our study is to determine the relationship between cirrhosis complications and nesfatin-1 levels. Our study was approved by Türkiye's University of Health Sciences Prof. Dr. Cemil Taşçıoğlu City Hospital Ethics Committee (Date: 05.03.2019, No: 1143).

Measuring nesfatin-1

A blood sample was taken from the participants in one biochemistry tube and centrifuged. The supernatant portion was stored at -80°C. All stored blood samples were thawed only once on the day of analysis. Nesfatin-1 levels were measured using a human nesfatin-1 enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp. ELIZA Kit for Nesfatin 1 [NES1] CEA242Hu 96 Tests), with nesfatin-1 levels able to be detected between 617.3-50,000 pg/mL.

Data analysis

The descriptive statistics use mean, standard deviation, median, and 25%-75% values for the numerical variables, with numbers and percentages being used for the cat-

egorical variables. The independent samples t-test was used to analyze the differences between the two groups of variables through parametric distribution, while the Mann-Whitney U test was used to analyze differences between the two groups of variables with non-parametric distribution and the Kruskal-Wallis test to analyze the differences between more than two groups of variables with non-parametric distribution. Pearson's chi-square test was used for intergroup comparisons of the categorical variables. When a significant difference was detected in the comparisons between more than two groups, a post-hoc analysis was performed to understand from which group the difference had originated. Correlation analysis was performed using Spearman's non-parametric correlation test, with the confidence interval set at 95% and the significance level being accepted as $p < 0.05$.

RESULTS

The patient group includes a total of 51 patients (21 females, 30 males), while the control group includes a total of 30 participants (17 females, 13 males). The groups are similar in terms of age (p=0.201) and sex (p=0.177, Table 1).

When examining the etiology of cirrhosis, liver cirrhosis was seen to be due to cryptogenic liver disease in 15 (29.4%) patients, alcoholic liver disease in 12 (23.5%), nonalcoholic steatohepatitis (NASH) in nine (17.6%), cardiac causes in five (9.8%), hepatitis B virus (HBV) infection in four (7.8%), hepatitis C virus (HCV) infection in three (5.9%), and other causes in three (6%) patients. Nesfatin-1 levels were found to be significantly higher in the NASH group (p=0.003). BMI was higher in the NASH group, but not at a statistically significant level.

The levels of nesfatin-1 were compared between the cirrhosis and control groups and found to be statistically significantly higher in the cirrhosis group (p=0.001, Table 1).

A significant difference was found among the control group, the compensated liver cirrhosis group, and the decompensated liver cirrhosis group in terms of nesfatin-1 levels (p=0.001). Post-hoc analysis was performed to examine from where the difference had originated. Accordingly, the levels of nesfatin-1 in the compensated group were found to be significantly higher than in the control group (p=0.010, Table 2).

Table 1: A comparison of some parameters between the cirrhosis and control groups

	Cirrhosis group (min-max) (n=51)	Control group (min-max) (n=30)	p value
Age (year)	65.8±12.5	61.9±13.9	0.201
Female, n (%) Male, n (%)	21 (42) 30 (58)	17 (56) 13 (44)	0.177
DM (+), n (%) DM (-), n (%)	24 27	17 13	0.404
HT (+), n (%) HT (-), n (%)	23 28	15 15	0.669
IHD (+), n (%) IHD (-), n (%)	11 40	4 26	0.357
BMI (kg/m^2)	27.4±4.2	26.6±3.6	0.439
Nesfatin-1 (ng/mL)	11.3 (8.4-13.1)	7.2 (6-10.1)	**0.001**
Fasting blood glucose (mg/dL)	110 (96-149)	97 (88-174)	0.152
Creatinine (mg/dL)	0.94 (0.6-1.4)	0.73 (0.5-1.2)	0.286
Sodium (mmol/L)	138 (133-140)	139 (137-143)	0.089
ALT (U/L)	20 (14-30)	15 (11.7-19)	**0.007**
AST (U/L)	29 (22-40)	18 (14.7-23.2)	**< 0.001**
Platelet (10^3/uL)	98 (68-125)	211.5 (175.5-271)	**< 0.001**
Total Bilirubin (mg/dL)	1.5 (0.9-2.2)	0.4 (0.3-0.5)	**< 0.001**
INR	1.2 (1.1-1.3)	1.0 (0.9-1.1)	**< 0.001**
Albumin (g/dL)	3.0 (2.4-4.1)	3.7 (3.1-4.1)	**0.004**
HbA1c (%)	6.2 (5.4-7.8)	7 (5.7-8.6)	0.079
CRP (mg/L)	14 (5-63.5)	15.6 (5.2-35.6)	0.835

ALT: Alanine transaminase, AST: Aspartate transaminase, CRP: C reactive protein, DM: Diabetes mellitus, HbA1c: Hemoglobin A1C, HT: Hypertension, INR: International correction ratio, IHD: Ischemic heart disease, BMI: Body mass index

Patients with cirrhosis were grouped as Child A, Child B, Child C (for the Child-Pugh stage) and as having MELD scores equal to or greater than 15 or less than 15. No significant difference was found between the groups in terms of nesfatin-1 levels (Table 3).

Table 2: Nesfatin-1 level comparisons among the control, compensated, and decompensated cirrhosis groups

Nesfatin-1 (ng/mL)	Control A (n=30)	Compensated cirrhosis B (n=20)	Decompensated cirrhosis C (n=31)	A-B-C p value	A-B p value	A-C p value	B-C p value
Median	7.2 (6-10.1)	11.9 (10.2-14.8)	10.4 (6.6-12.6)	**0.001**	**0.010**	0.077	0.218
BMI		(28.01±4.09)	(26.9±4.27)				0.38

BMI: Body mass index

Table 3: Nesfatin-1 level comparison according to the Child A, B, and C as well as MELD <15 and MELD ≥15 groups

Nesfatin-1 (ng/mL)	CHILD A (n=14)	CHILD B (n=28)	CHILD C (n=9)	p value
Mean	11	12.4	11.9	0.667
Median	11.3 (10.1-13.1)	11 (8.1-14.6)	10.4 (6.4-12.6)	
	Cirrhotic patients with MELD ≥15 (n=17)		**Cirrhotic patients with MELD score <15 (n=34)**	
Mean	11.6		12.1	0.589
Median	12.4 (8-14.8)		10.9 (8.3-13.1)	

CHILD: Child-Pugh Score, MELD: Model for End-Stage Liver Disease score

We examined the relationship between nesfatin-1 levels and the amount of ascites (absent, minimal, overt; p=0.727), the presence of encephalopathy (absent, present; p=0.499), and variceal bleeding (absent, present; p=0.902) and found no significant difference.

We then examined the relationship between complications and nesfatin-1 levels and again found no significant correlation (ascites: r=-0.096, p=0.504; encephalopathy: r=-0.096, p=0.505; variceal bleeding: r=0.017, p=0.903).

DISCUSSION

Our study has found serum nesfatin-1 levels to be significantly higher in the cirrhosis of the liver patient group than in the control group. Increased nesfatin-1 levels may be one of the mechanisms of malnutrition and cachexia in patients with cirrhosis. When making a triple comparison among the compensated liver cirrhosis, decompensated liver cirrhosis, and control groups, a significant difference was found in terms of nesfatin-1 levels. When comparing the groups with one another in pairs, a significant difference was found only between the compensated liver cirrhosis group and the control group.

Nesfatin-1 has been shown to be an anorectic polypeptide and to be expressed in peripheral tissues such as the liver and the hypothalamus (4, 7). In the decompensated period, hepatic expression of nesfatin-1 may decrease with the deterioration of liver functions. Again, in patients with encephalopathy, central expression of nesfatin-1 may decrease due to possible central nervous system involvement. These mechanisms may explain the relatively lower release of nesfatin-1 in decompensated liver cirrhosis than in compensated liver cirrhosis. On the other hand, when comparing the control group and the decompensated cirrhosis group, p was found to be 0.070, which is not significant; however, we might have reached a significant difference if we'd had more patients. Clearly, more studies with larger patient groups are needed on this subject.

The Child-Pugh classification and MELD are prognostic scoring systems created using biochemical parameters and the complications of cirrhosis. With its antioxidant and anti-inflammatory properties, nesfatin-1 can be thought to be more prominent in cirrhosis. Although not statistically significant, the relative elevation of nesfatin-1 in the Child B group supports our view that this may be due to antioxidant and anti-inflammatory activities of nesfatin 1 in compensated liver cirrhosis.

Ogiso et al. found serum nesfatin-1 levels to be lower in patients with anorexia nervosa restricting type when compared to controls (8). When considering this information alongside our results, we can say that the possible defense mechanisms and insufficient production in decompensated liver cirrhosis suppress nesfatin-1 values. Aydın et al. studied 97 patients with cirrhosis in their study on prolidase, urotensin-2, and nesfatin-1 levels. Their study found the serum nesfatin-1 levels to be significantly higher in the

cirrhosis group and the decompensated cirrhosis group when comparing these to the control group (9). The difference between our results and those of Aydın et al. may be due to the patients having comorbid chronic diseases and the differences in the etiologic causes of cirrhosis. Due to the insufficient number of patients, we were unable to make a separate etiologic evaluation. Our study also examined the relationship between complications of cirrhosis and nesfatin-1 and found no significant difference.

The production of free oxygen radicals such as nitric oxide (NO), which plays an active role in the hemodynamic changes developing in liver failure and the regulation of hepatocyte function (10), can be stimulated by endotoxins formed in liver failure, by a portosystemic shunt, by decreased reticuloendothelial cell function, and by bacterial products of gastrointestinal origin being cleared less (11). Úbeda et al. study on rats found increased concentrations of activated helper T cells, monocytes, and proinflammatory cytokines in the peripheral blood of rats with cirrhosis that had not yet developed ascites. They concluded the activation of the immune system to have occurred before the development of ascites in experimental cirrhosis, as well as bacterial DNA fragments to have reached the mesentery lymph nodes and caused local inflammation in compensated cirrhosis (12). Recent studies support nesfatin-1 as having anti-inflammatory, antioxidant, and anti-apoptotic effects (13-16). Another study has also shown nesfatin-1 levels to decrease in sepsis (17). Nesfatin-1 may balance the negative effects of inflammatory cytokines, NO, and free oxygen radicals through its antioxidant and anti-inflammatory mechanisms in those with compensated liver cirrhosis. A decrease in nesfatin-1 levels may accelerate the decompensation process by the decrease in anti-inflammatory, antioxidant, and anti-apoptotic activities.

Mean arterial blood pressure increase induced by nesfatin-1 had been shown to be abolished through the melanocortin-3/4 receptor antagonist or phentolamine (18). Another study showed the blood pressure raising effect of nesfatin-1 to be blocked by the oxytocin receptor antagonist ornithine vasotocin (19). Another study examining the relationship among nesfatin-1, blood pressure, and sympathetic activity showed ICV nesfatin-1 injections to increase the sympathetic activity of the melanocortin system in kidneys and to cause an increase in blood pressure (20). Hyperdynamic circulation occurs in cirrhosis. In hepatorenal syndrome (HRS) and ascites in particular, sympathetic nervous system activation is known to occur due to effective arterial volume reduction. Increased nesfatin-1 levels may play a role in the pathogenesis of cirrhosis and its complications, and the maintenance of compensation through the activation of the sympathetic nervous system.

Nesfatin-1 is a pleiotropic molecule with different effects in many tissues. Many unknown factors could change the results of the study. In addition, the etiology of cirrhosis may also affect nesfatin-1 levels. Therefore, studies with larger numbers of patients are needed to demonstrate the effects of the nesfatin-1 molecule. Our study found no significant difference between BMIs in the control and cirrhosis groups, whereas the nesfatin-1 levels of NASH patients were found to be significantly higher than in the control group. Controversial results about this subject are found in the literature (21, 22). One study showed nesfatin-1 levels to be low in patients with NASH, while another showed them to be high, albeit not a statistically significant level. While our study did find nesfatin-1 levels to be significantly higher in patients with NASH, we refrain from making a definitive interpretation due to their cirrhotic stage and the low number of patients. In patients who develop decompensated ascites, relative weight gain secondary to ascites may be misleading because it may hide malnutrition and sarcopenia when evaluated using BMI alone. The limitations of our study are the lack of additional evaluations for malnutrition and the small sample size.

CONCLUSION

Our study has found nesfatin-1 levels to be significantly higher in the cirrhosis group and compensated liver cirrhosis group compared to in the control group. Nesfatin-1 may be involved in the maintenance of compensation with its antioxidant, anti-inflammatory, and anti-apoptotic effects. Based on the control group, the decrease in serum nesfatin-1 levels in decompensated liver cirrhosis compared to compensated liver cirrhosis may be due to its defense mechanisms and insufficient production. Nesfatin-1 has blood pressure increasing effects on the central and sympathetic nervous system, which may contribute to the maintenance of homeostasis in patients with cirrhosis involving hyperdynamic circulation.

Ethics Committee Approval: The study has ethical approval from the Türkiye's University of Health Sciences Prof. Dr. Cemil Taşçıoğlu City Hospital Ethics Committee (Date: 05.03.2019, No: 1143).

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IS ONLY REQUESTING AN ANTI-HCV TEST SUFFICIENT FOR HEPATITIS C SCREENING?

HEPATİT C TARAMASI İÇİN SADECE ANTİ-HCV TESTİ İSTEMEK YETERLİ MİDİR?

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ABSTRACT

Objective: Unless treated, a hepatitis C virus (HCV) infection is associated with high morbidity and mortality. The study investigates anti-HCV screening efficacy and treatment access rates for patients.

Material and Method: This cross-sectional study screened all anti-HCV tests requested between January 2014-June 2017 from hospital records. Patient interviews were conducted by telephone-based interview.

Result: The overall number of anti-HCV tests requested was 77,783, 1,373 of which were positive. Among these, the study interviewed 488 patients (266 females, 222 males; mean age=52.81±16.5 years) and analyzed their tests. Further investigation with HCV-RNA had not been done in 69 (14.1%) anti-HCV positive patients. HCV-RNA was positive in 309 patients, 268 of whom were treated (86%), while 41 were not (14%). The main reasons for remaining untreated are: unknown (21%), no patient follow up (28%), or physician didn't indicate (19%).

Conclusion: In order to successfully eliminate HCV, the anti-HCV test alone is not enough. Informing patients about the results of the anti-HCV test and, if positive, referring them for the HCV-RNA test are important. When considering the high amount of untreated patients, linkage to care should be encouraged in HCV-RNA positive patients unless an absolute contraindication is present.

Keywords: Hepatitis C virus infection, anti-HCV antibody, HCV-RNA, screening

ÖZET

Amaç: Hepatit C Virüs (HCV) enfeksiyonu, tedavi edilmediği sürece yüksek morbidite ve mortalite ile ilişkilidir. Bu çalışmada anti-HCV tarama etkinliği ve tedaviye erişim oranları araştırılmıştır.

Gereç ve Yöntem: Bu kesitsel çalışmada Ocak 2014 ile Haziran 2017 tarihleri arasında istenen tüm anti-HCV testleri hastane kayıtlarından tarandı. Hasta görüşmeleri telefon ortamında gerçekleştirildi.

Bulgular: İstenilen toplam anti-HCV testi sayısı 77,783 olup, bunların 1,373'ü pozitif çıkmıştır. Bunlardan 266'sı kadın, 222'si erkek; yaş ortalaması 52,81±16,5 yıl olan 488 hastayla görüşme yapılmıştır. Anti-HCV pozitif hastaların 69'una (%14,1) HCV-RNA ile ileri araştırma yapılmadığı saptandı. Üç yüz dokuz hastada HCV-RNA pozitifti ve 268'i tedavi almışken (%86), 41'i (%14) tedavi edilmemişti. Tedavisiz kalmanın temel nedenleri ise bilinmeyen (%21), takip edilmeyen hasta (%28) ve hekimin endikasyon göstermemesi (%19) olarak belirlendi.

Sonuç: HCV'nin başarılı bir şekilde eradike edilmesi için anti-HCV testi tek başına yeterli değildir. Hastaların anti-HCV testi sonuçları hakkında bilgilendirilmesi; pozitif ise HCV-RNA testine başvurulması önemlidir. Tedavi edilmeyen hasta sayısının yüksek olduğu göz önüne alındığında, mutlak bir kontrendikasyon olmadığı sürece HCV-RNA pozitif hastalarda tedaviye yönlendirilme teşvik edilmelidir.

Anahtar Kelimeler: Hepatit C enfeksiyonu, anti-HCV antikoru, HCV RNA, tarama

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INTRODUCTION

Acute hepatitis C virus (HCV) infection has a chronicity rate of 75-80%, and is one of the most common reasons for chronic liver disease and hepatocellular carcinoma (1, 2). Chronic HCV infection is a common public health issue associated with high all-cause morbidity and mortality if left untreated (1). Around 71 million cases of HCV infection are found all around the world (2). The introduction of direct-acting antiviral agents (DAA) makes HCV a curable disease in a shorter time with less adverse effects and more than 95% sustained viral response (SVR) rates (3). Thus, the World Health Organization (WHO) announced the target of eliminating HCV by 2030 (4). To achieve this 2030 target, around 300,000 viremic HCV patients have been waiting to be diagnosed and treated since 2016 in Türkiye (5). However, the success of this elimination plan will only be possible if infected patients are detected and get treatment on time, thus indicating the importance of HCV screening. An effective screening and treatment policy is not only important for diagnosis and treatment success but also for decreasing disease incidence and prevalence by avoiding transmission among individuals (4).

To investigate the effectiveness of the HCV screening policy regarding hospital care settings and treatment administration rates, the study evaluates the patient pathway, which starts with anti-HCV monitorization and continues with the treatment of HCV RNA positive patients, and identifies key markers for each step.

MATERIALS and METHODS

This study was conducted cross-sectionally and identifies all patients screened for HCV between January 1, 2014-June 30, 2017 at a single tertiary center based on the medical records of the hospital archive, with a total of 77,783 patients being included. To gather data, a telephone-based interview was performed with the patients themselves.

Virologic tests were performed using the routine enzyme-linked immunosorbent assay (ELISA) method for the anti-HCV test (Innogenetics HCV Ab IV; Innogenetics N.V, Belgium) and quantitative real-time polymerase chain reaction (qt RT-PCR) for the HCV-RNA test in a microbiology laboratory (COBAS Ampliprep/COBAS Taqman HCV Quantitative Test V2.0, Roche Diagnostics Mannheim, detection range 15-100 000 000 IU/mL).

All participants gave informed consent and volunteered to be interviewed. The study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.09.2023, No: 19). Data are presented as mean values for continuous variables and as percentages for qualitative variables.

Statistical analysis

Due to only patient group data being defined, mean and standard deviations have been given for the quantitative data and frequencies for the qualitative variables. Statistical analyses were performed using SPSS Statistics for Windows, version 28.0 (IBM SPSS Corp., Armonk, NY, USA).

RESULTS

Study population characteristics

A total of 77,783 patients were enrolled in the study. Among these, 1,373 had tested positive for anti-HCV (1.76%). Duplicated requests (n=430, 31.3%) were removed, and the remaining 943 patients were reviewed. After obtaining consent, the 943 patients were interviewed. Among these, 200 patients' telephone numbers could not be found, 157 did not respond to repeated calls, 73 patients had died, and 25 refused to give information. As a result, the study interviewed 488 patients (266 females, 222 males; mean age = 52.81±16.5 years) via a telephone-based interview.

Among the 488 anti-HCV positive patients, 69 (14.1%) had not been monitored for HCV-RNA testing. Among the 419 HCV-RNA results, 110 were negative (false positive for anti-HCV), while 309 were confirmed positive through qt RT-PCR (Figure 1). No significant differences were observed regarding age or gender for the patients tested positive for anti-HCV and HCV-RNA.

Reasons for HCV screening and transmission route according to groups

Based on the patient interviews, Table 1 presents the major reasons for anti-HCV screening for the HCV-RNA tested (n=419) and non-tested (n=69) groups. The main indication for screening was general screening in study population (32%), and pre-operative screening was the most common reason in patients who'd not been referred for HCV-RNA testing (42%).

The main routes of HCV transmission were declared as unknown (37%), blood transfusion (23%), surgery (12%), dental care (9%), hemodialysis (14%), tattoo-piercing (1%), risky sexual behavior (1%), intravenous drug abuse (1%), and positive family history for viral hepatitis (1%).

Treatment status

Positivity for HCV-RNA was detected in 309 patients, of whom 268 received treatment (86%) and 41 who did not (14%). The given treatments involved interferon-based regimen (46%), DAA (27.4%), multiple therapy (21.5%), and unknown (5.1%). Patients declared the reasons for remaining untreated as: unknown (21%), no patient follow up (28%), clinician's decision to not consider treatment without a contraindication (19%), physician perceiving the patient as too young or too old for treatment (12%),

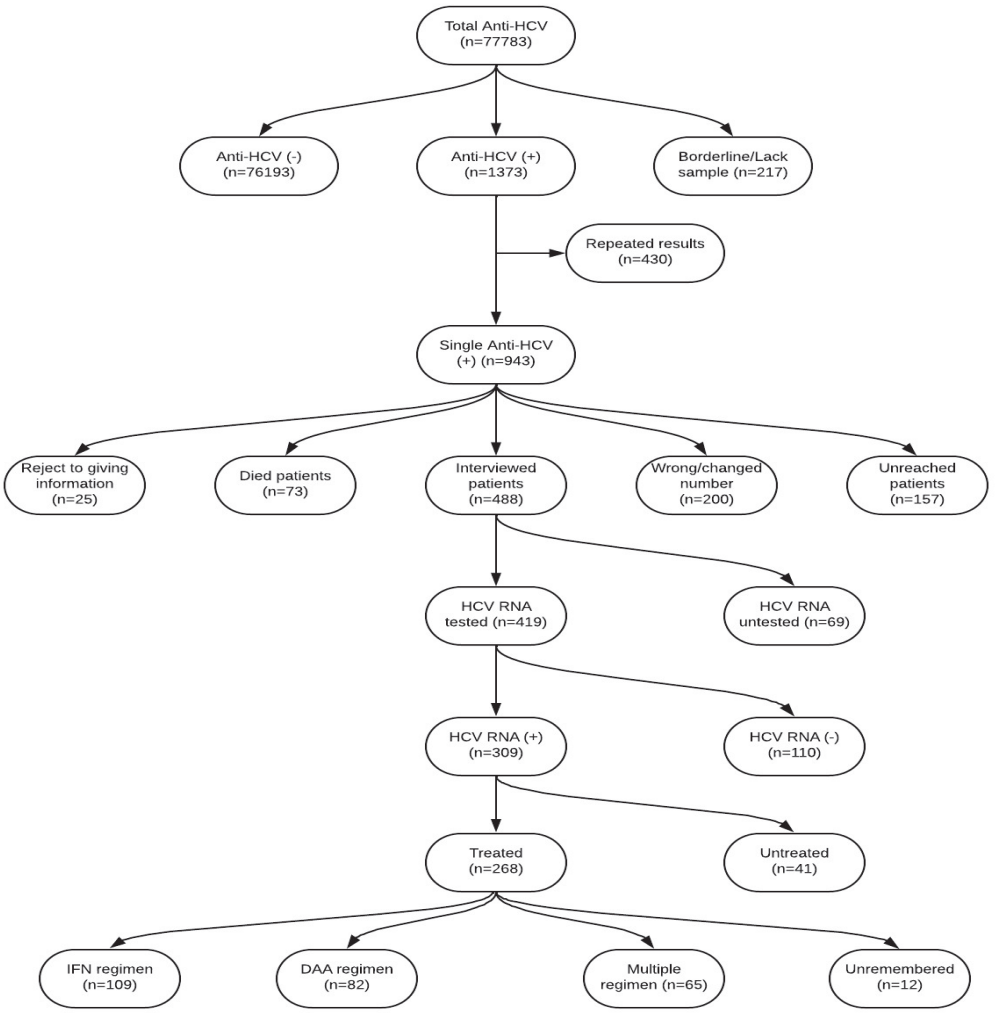

Figure 1: Study flowchart
HCV: Hepatitis C virus, IFN: Interferon, DAA: Directly acting antivirals

Table 1: The reasons for HCV screening overall and by patient group

Screening indications	All patient groups (n=488), %	Patients not screened for HCV-RNA (n=69), %	HCV-RNA positive patients not referred and untreated (n=41), %
General screening	32	32	43
Surgery	26	42	22
Impaired liver biochemistry	21	10	15
Hemodialysis	7	3	N/A
Don't remember	5	9	10
Blood donation	3	3	2
Self-interest	2	N/A	N/A
Risky behavior	1	N/A	3
Job application	1	N/A	3
Marriage screening	1	1	N/A
Family history	1	N/A	2

improper general health status (5%), patient rejected (10%), and being under a treatment plan (5%). Therefore, 29 (71%) of the 41 untreated HCV-RNA positive patients (i.e., the ones excluding those as non-indicated by physicians, with improper health status, and under a treatment plan) were also eligible for treatment. Overall, 14% of anti-HCV positive patients (n=69) and 13% of HCV-RNA positive patients (n=41), namely a total of 110 of the 488 interviewed patients at the time of that research, were unable to access treatment.

DISCUSSION

Chronic hepatitis C virus (HCV) infection is a worldwide public health problem and one of the reasons for preventable morbidity and mortality if left untreated (1). Therefore, early screening, diagnosis, and linkage to care have crucial importance. The generally accepted method for HCV screening is the testing of anti-HCV antibodies in peripheral venous blood using the enzyme-linked immunosorbent assay (ELISA) method. Anti-HCV positivity frequency varies from region to region and also from the general population to special groups, including HIV comorbidity and among people who inject drugs (PWID) (2, 4, 6). WHO data showed anti-HCV positivity in Western countries as 1.10%, while studies from Eastern regions demonstrated even higher anti-HCV rates. Naz et al. declared 1.56% in Pakistan, while Turkish HCV epidemiology data reveal an anti-HCV positivity rate of 1% (7, 8). This study found an anti-HCV positivity rate of 1.76%, higher than in most studies that include the general population. However, because this study was performed in a tertiary hospital, high-risk subgroups were probably recruited at a greater frequency than in the general population as a one-center study bias. This is consistent with previous hospital-based studies that found a higher prevalence of anti-HCV positivity than expected for the general population (9-11). During the calculation of anti-HCV positivity, those who had negative HCV RNA tests (n=110) were not excluded; they had either been treated previously or had a false positive anti-HCV test with no actual infected status. However, even in the patients requesting the HCV RNA test, being tested once is necessary to exclude active infection and need for treatment.

The high number of repeated tests and the gap between anti-HCV positivity and a referral for the HCV-RNA test were the main results of low awareness among physicians. Repeated tests not only increase the cost of managing the disease but also the emotional stress for patients with false positives or who've been cured owing to the lifelong positivity of anti-HCV in both situations. Although studies have shown an increased effort to screen special groups, such as patients with risky behaviors, HCV-HBV, HCV-HIV coinfections, and PWIDs, they also show screening, referrals for HCV-RNA testing, and active infection rates to be lower in the general population (6, 12). However, studies have also shown cost effectivity for screening high-risk populations vs general populations owing to decreased transmission and higher treatment success rates with DAAs (13). In addition, the lack of evaluation regarding the HCV-RNA test results for proper linkage to care and the lack of education among patients about disease outcomes and the importance of being treated decreased the rate of treatment initiation. The results from this study have revealed a lack of knowledge on how to manage HCV infection to be present among patients and physicians, such as informing anti-HCV positive patients who'd already been indicated as positive in the healthcare databases about test results, as well as the need to refer these patients for HCV-RNA testing to determine viremia.

One of the limitations of this study is its retrospective design, which has led to an important loss for the cohort due to the inability to contact patients. Another limitation is the basis on a single tertiary center. Data from tertiary centers may include more risky and complicated patient groups compared to the general population.

CONCLUSION

In conclusion, requesting an anti-HCV test is not adequate as a single screening tool with regard to the strategy for eliminating HCV. Further investigation of HCV-RNA in positive patients is so crucial and a complementary portion of the screening strategy. Therefore, physicians' awareness of screening and linkage to care become more important. The wide use of electronic patient record systems and digital warnings may decrease the number of unnecessary duplicated test requests. Despite being easily treated with DAAs, approximately one in four patients lose the chance of being successfully referred to treatment. Therefore, the healthcare system should be evolved to treat every patient who tests positive for HCV-RNA unless an absolute contraindication is present.

Ethics Committee Approval: The study has ethical approval from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.09.2023, No: 19).

Informed Consent: All participants gave informed consent and volunteered to be interviewed.

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MID-TERM OUTCOMES OF MID-SHAFT CLAVICULAR NON-UNIONS TREATED BY PLATE FIXATION WITH AUTOLOGOUS BONE GRAFTING: DOES THE TYPE OF INITIAL TREATMENT HAVE AN INFLUENCE ON THE SURGICAL RESULTS?

OTOLOG KEMİK GREFTİ İLE PLAK FİKSASYONU İLE TEDAVİ EDİLEN MİD-ŞAFT KLAVİKULA KAYNAMAMALARININ ORTA DÖNEM SONUÇLARI: BAŞLANGIÇ TEDAVİSİNİN TİPİNİN CERRAHİ SONUÇLARA ETKİSİ VAR MIDIR?

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ABSTRACT

Objective: The recent literature is scarce regarding the outcomes of mid-shaft clavicular non-union treated by open reduction and internal fixation (ORIF) with autologous bone grafting, and the influence of the type of initial treatment has not yet been investigated. The study aims: (1) to present the mid-term surgical results of mid-shaft clavicular non-unions treated by plate fixation with autologous bone grafting and (2) to determine the effect the type of initial treatment has on the surgical results.

Material and Method: The study reviews 14 patients (eight females) who'd undergone ORIF with autologous bone grafting due to atrophic mid-shaft clavicular nonunion where clinical and radiographical outcomes were recorded. The study divides the patients into two groups based on their initial treatment: Group A (eight with non-surgical treatment) and Group B (six with surgical treatment).

Result: The mean QuickDASH score at final follow-up was 22. The Constant Score significantly improved from 40 to 87 postoperatively. The mean Preoperative Visual Analogous Scale (VAS) score went down from 7 to 2 ($p<0.001$). A solid union was achieved in all patients. In the preoperative between-group comparison, no significant differences were observed for any of the clinical outcome. At final follow-up, Group A exhibited significantly higher Constant Scores.

Conclusion: Regardless of the type of initial treatment, plate fixation with autologous bone grafting is effective in obtaining

ÖZET

Amaç: Otolog kemik grefti ile açık redüksiyon ve internal fiksasyon (ORIF) ile tedavi edilen mid-şaft klavikula kaynamamasının sonuçlarıyla ilgili güncel literatür azdır. Bu çalışmanın amaçları şunlardı: (1) otolog kemik grefti ile plak fiksasyonu ile tedavi edilen mid-şaft klaviküler kaynamamaların orta dönem cerrahi sonuçlarını ardışık bir vaka serisinde sunmak ve (2) başlangıç tedavi yönteminin klinik sonuçlara olan etkisini araştırmaktır.

Gereç ve Yöntem: Atrofik mid-şaft klavikula kaynamaması nedeniyle otolog kemik grefti ile ORIF uygulanan 14 hasta (sekiz kadın) retrospektif olarak incelendi. Genel çalışma popülasyonunda çeşitli klinik ve radyografik sonuçlar kaydedildi. Hastalar daha sonra başlangıç tedavisinin türüne göre iki gruba ayrıldı: Grup A (sekizi cerrahi olmayan tedavi) ve Grup B (altısı cerrahi tedavi).

Bulgular: Son değerlendirmede ortalama QuickDASH skoru 22 idi. Constant skoru ameliyat sonrası 40'tan 87'ye anlamlı olarak arttı. Ameliyat öncesi ortalama görsel analog skala (VAS) skoru son değerlendirmede 7'den 2'ye ($p<0,001$) geriledi. Tüm hastalarda kaynama görüldü. Ameliyat öncesi dönemde gruplar arası karşılaştırmada, klinik sonuçları arasında anlamlı bir fark gözlenmedi. Son değerlendirmede gruplar arası karşılaştırmada grup A anlamlı derecede daha yüksek Constant puanları sergiledi.

Sonuç: Başlangıç tedavisinin türü ne olursa olsun, otolog kemik grefti ile plak tespiti, mid-şaft klaviküler kaynamaması olan hastalarda füzyon elde etmede ve klinik durumu iyileştirmede etkilidir.

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solid fusion and improving the clinical status of patients with mid-shaft clavicular non-union. Additionally, this technique can provide shorter time to union and greater improvements in specific shoulder function in patients who've undergone non-surgical treatment.

Keywords: Mid-shaft clavicular non-union, initial treatment, plate fixation, autologous bone grafting

Ek olarak, bu teknik, başlangıçta konservatif tedavi gören hastalarda kaynamanın daha kısa sürede gerçekleşmesini ve spesifik omuz fonksiyonlarında daha büyük iyileşmeler sağlayabilir.

Anahtar Kelimeler: Mid-şaft klavikula kaynamama, başlangıç tedavisi, plak ile osteosentez, otolog kemik greftleme

INTRODUCTION

Clavicular fractures are common injuries accounting for up to 10% of all fractures in adults and most often occur at the middle-third segment (1, 2). Mid-shaft clavicular non-union is a rare but potentially serious complication that may require surgical intervention due to pain and functional impairment in the upper extremity (3-6).

Although different surgical techniques, involving intramedullary, external and internal fixation have been introduced in the management of mid-shaft clavicular nonunions, little consensus is found on which technique is the ideal method of fixation (5-8). Open reduction and internal fixation (ORIF) with autogenous bone graft have been reported as an acceptable method for treating this rare disorder in older case series, with favorable results (5, 6, 9-11). However, according to a literature review, more recent published studies investigating the outcomes of ORIF with plate and screws in such patients are lacking.

Mid-shaft clavicular non-union can occur following initial surgical or non-surgical treatment (5). Although clinical and radiological outcomes after treatment by ORIF in such patients have been evaluated, the influence of the type of initial treatment on the surgical results has not yet been investigated in the literature as far as is known. This study assumes the choice of initial treatment to be a possible critical factor in the success of treatment by ORIF in mid-shaft clavicular non-union.

The primary aim of this study is to present the mid-term surgical results of mid-shaft clavicular non-unions treated by plate fixation with autologous bone grafting in a consecutive case series. The secondary aim is to determine the influence of the type of initial treatment on the surgical results.

MATERIALS and METHODS

The study has retrospectively identified 17 patients with atrophic mid-shaft clavicular nonunion who were diagnosed and treated at an institution using ORIF with autologous bone grafting between 2009-2015. The study has evaluated all patients based on the eligibility criteria (Table 1). After excluding three patients (one missed the follow-up, and two had inadequate medical records), the study enrolled the remaining 14 patients (six males, eight females; 14 clavicles) who met the inclusion criteria and invited them to a final follow-up appointment. All patients had been initially treated surgically or non-surgically at an outside hospital and admitted to the institute's department with a painful clavicular nonunion and limitations in daily activities. All participants gave informed consent, and the study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 26.01.2024, No: 02).

Table 1: Eligibility criteria for inclusion and exclusion of the study participants

Inclusion criteria	Exclusion criteria
A diagnosis of mid-shaft clavicular non-union	Missed follow-up
Complete medical records and radiographic images	Inadequate medical records
Willingness to participate in the study	Pathological fracture
	Concomitant fracture in the same extremity
	Unwillingness to participate in the study

Study protocol

Part I: Clinical and radiographic assessment of the entire study population

Demographic and clinical data were obtained from the hospital's database, as well as the patients' medical records including gender, age, mechanism of injury, initial treatment, the period from injury to the non-union surgery, follow-up period, occupational history and presence of pre- and post-operative neurological symptoms (brachialgia). Complications were also recorded.

Clinical outcome measures

The shortened Disabilities of the Arm Shoulder and Hand Questionnaire (QuickDASH) scores were measured at the patients' final follow-ups (12). The Constant Score and Visual Analogous Scale (VAS) were both measured immediately before the non-union surgery and again at the final follow-up (13).

The Constant Score is a validated shoulder-specific outcome measure consisting of two objective and two subjective individual parameters that add up to an overall

maximum of 100 points: range of motion (40 points), strength (25 points), pain (15 points), and activities of daily living (20 points). The QuickDASH score is a functional outcome measure for evaluating overall upper-extremity disability and involves an 11-item scale with possible scores ranging from 0 (no disability) to 100 (severe disability). VAS is used to assess changes in pain intensity. The VAS score used in the current study is a simplified measure where pain intensity during daily activity is rated on a scale of 0–10, with 0 showing no pain and 10 showing the highest pain (14).

Radiographical outcome measures

Non-union is defined as persistent pain and no radiographic sign of a bridging callus at the fracture site six months after the initial treatment (8). Clavicular length is measured from the anterolateral angle of the acromion to the sternal notch on standard anterior-posterior clavicle radiographs immediately before the non-union surgery and at the final follow-up. A clavicular reconstruction ratio was utilized to evaluate the restoration of clavicular length, with a reconstruction ratio of 1 implying the reconstructed length of the non-united clavicle to be equal to that of the contralateral uninjured clavicle. Union is defined as evidence of continuity of cortex or bridging callus on standard radiographs of the clavicle (anterior-posterior 30° cephalad and anterior-posterior perpendicular to cassette).

Part II: Assessing the initial treatment method's effect on the surgical results

All the patients included in the study were first divided into two groups based on the type of initial treatment: Group A (8 patients with non-surgical treatment; 4 AO type 15.2 A1, 2 AO type 15.2A2, and 2 AO type 15.2 B1) and Group B (6 patients with surgical treatment; 2 AO type 15.1 A1, 3 AO type 15.2 A2, and 1 AO type 15.2 B2). The parameters described above were then compared between the two groups.

In Group A, the initial treatment was sling immobilization in three patients and figure-of-eight bracing in five patients (Figure 1). In Group B, all patients initially underwent ORIF with plate and screws for their clavicle fractures, with non-union having developed as a result of loosening of the osteosynthesis (Figure 2).

Surgical technique

The main indication for non-union surgery was pain and functional impairment in all the patients. All operations were performed by 1 of 2 experienced orthopedic surgeons. A standardized protocol for the surgical technique was used in all patients. Patients were placed on a radiolucent table in a beach-chair position with a folded towel under the involved shoulder. The involved upper extremity and iliac crest were draped free to respectively enable manipulation and harvesting of the autologous bone graft. The standard anterior approach to the middle-third of the

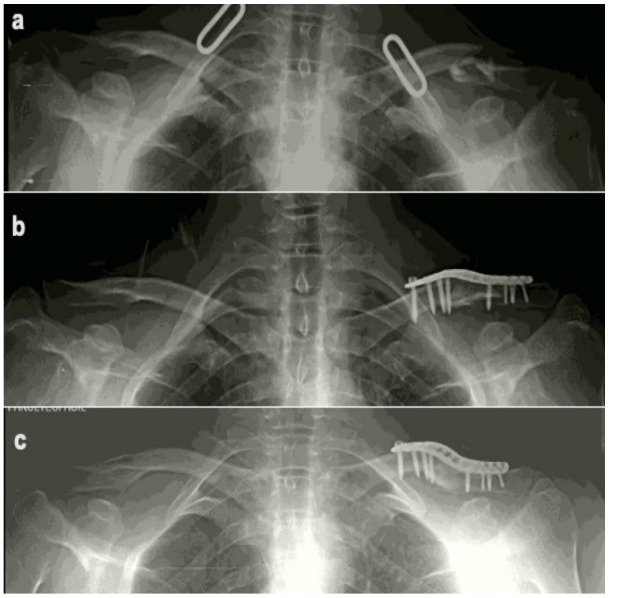

Figure 1: The preoperative radiograph shows a nonunion case of mid-shaft clavicle following the initial non-surgical treatment (a). The postoperative standard radiographs demonstrate continuity of cortex as evidence of union at the nonunion site (b and c).

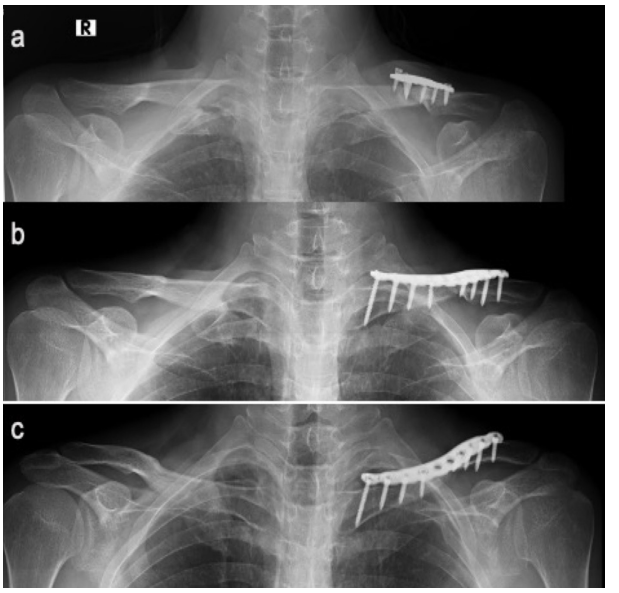

Figure 2: The preoperative radiograph displays a case of nonunion of the mid-shaft clavicle following initial surgical treatment. Note that loosening of the osteosynthesis with the pull-out of the screws (a). The postoperative standard radiographs show continuity of cortex as evidence of union at the nonunion site (b and c).

clavicle was used. In Group B, all previously inserted implants and screws were first removed, and then the nonunion site was exposed. In both groups, pseudoarthrotic tissue was removed, and the sclerotic bony ends of the atrophic non-union area were refreshed using curettes,

rongeurs, and osteotomes until cortical bone bleeding was observed (the paprika sign). The medullar canal was then opened on both sides of the nonunion area using a high-speed drill. In the later stage, a cancellous autogenous bone graft was routinely taken from the iliac crest and inserted onto the non-union area in all cases. One patient from Group A and two patients from Group B developed a gap at the non-union site after resection of the sclerotic bony ends, which was then reconstructed with an intercalary iliac-crest graft. In the final stage, ORIF was performed using a 3.5 mm superior anterior limited-contact dynamic-compression plate (Synthes, LCP clavicle plate®, Bettlach, Switzerland) under an image intensifier. Reconstruction and stability were evaluated via direct observation and fluoroscopic imaging intraoperatively. After confirming stability, wound closure was performed.

Postoperative rehabilitation protocol

All patients had undergone the same rehabilitation protocol. The shoulder joint was immobilized for comfort with an arm-sling for 4-6 weeks postoperatively. During this period, the patients were allowed to execute active elbow flexion and extension. After six weeks, the arm-sling was removed, and active-assisted physical therapy was initiated until union was demonstrated radiographically. After bony union was achieved, progressive strengthening exercises were performed. Return to recreational and occupational activities were allowed three months post-operation.

Statistical analysis

Statistical analyses were performed using SPSS ver. 25.0 software (IBM Corp, Armonk, NY, USA). A $p<0.05$ was considered significant. The Shapiro–Wilk test and histogram graphics were used for normality tests. Data were presented as maxima, minima, and arithmetic means. Student's t-test and the Mann–Whitney U test were used for the between-group analysis. Fisher's exact test was used for categorical variables. For intra-group differences from before to final follow-up, the paired samples t-test was used, with the Mann-Whitney U test used for between-group differences at baseline and the final follow-up assessment, both measuring significance at $p<0.05$.

RESULTS

Part I: Baseline characteristics and results for the entire study population

Baseline characteristics

The study examined five females and nine males with a mean age of 45 years (range=31-65 years). The right side was involved in six patients and the left side in eight patients. The dominant side was involved in seven patients. The mean period from injury to non-union surgery was 10 months (range=6-24 months), and the mean follow-up was 44 months (range=24-72 months). Preoperative brachialgia was present in one patient, which resolved fully after the non-union surgery (Table 2).

Table 2: Demographic characteristics of the study participants.

Number of patients	14
Gender (Female/Male)	5/9
Age (years), mean (min–max)	45 (31-65)
Left/Right side	8/6
Involvement of the dominant side	7 cases
Follow-up (month), mean (min-max)	44 (24-72)
Period from injury to non-union surgery (month), mean (min-max)	10 (6-24)
Mechanism of injury, n (%)	
Simple fall	9 (64**%**)
Car accident	3 (21**%**)
Motorcycle accident	2 (15**%**)
Occupations, n (%)	
Office worker	6 (42**%**)
Housewife	4 (30**%**)
Teacher	2 (14**%**)
Hairdresser	1 (7**%**)
Police officer	1 (7**%**)

Clinical results

With favorable functional outcomes, all patients returned to their daily activities without difficulties or much effort. The mean QuickDASH score was measured as 22 (range=12-33) at the final follow-up. The Constant Score significantly improved from 40 (range=28-52) preoperatively to 87 (range=78-99) postoperatively. The mean preoperative VAS score significantly reduced from 7 (range=5-9) to 2 (range=0-4; $p<0.001$) at the final follow-up (Table 3). Four patients experienced complete relief from pain, with the remaining patients reporting substantial relief from pain.

Radiographic results

The reconstruction ratio significantly improved from 0.79 (range=0.74-0.84) preoperatively to 0.94 (range=0.87-0.99) postoperatively ($p<0.001$). Solid union was achieved in all patients, with the mean time to union being eight months (range=4-13 months; Figures 1 and 2; Table 3).

Part II: Comparing the results between the two groups

Group A is comprised of three females and five males with a mean age of 47 (range=34-65) years, and Group B consists of two females and four males with a mean age of 40 (range=31-59) years. The comparison of the baseline characteristics between the two groups is demonstrated in Table 4.

Table 5 details the comparison of the clinical and radiographical outcomes at pre-op and final follow-up assessments between the two groups. In the intra-group comparisons, all the clinical outcome measures were sig-

Table 3: Preoperative and final follow-up clinical and radiographical outcomes of the study participants

Variables	Preoperative	Postoperative	p values
Clinical outcomes			
QuickDASH score, (mean) (min-max)		22 (12-33)	0.002*
Constant score, (mean) (min-max)	40 (28-52)	87 (78-99)	0.003*
VAS score, (mean) (min-max)	7 (5-9)	2 (0-4)	<0.001*
Radiographical Outcomes			
Reconstruction ratio, (%) mean (min-max)	0.79 (0.74-0.84)	0.94 (0.99-0.87)	<0.001*
Time to union (months) mean (min-max)		8 (4-13)	

*p<0.05

nificantly improved preoperatively after non-union surgery compared to preoperatively. In the preoperative between-group comparison, no significant differences were observed among any of the clinical outcome measures.

Table 4: Comparison of the demographic characteristics between the two groups

Variables	Group A (n=8)	Group B (n=6)	p values
Mean age (years), mean (min–max)	47 (34–65)	40 (31–59)	0.131[a]
Gender (female/male)	3/5	2/4	0.516[b]
Period from injury to non-union surgery (months), mean (min-max)	9 (6–12)	12 (6–24)	0.231[a]
Follow-up (months), mean (min-max)	42 (24–70)	46 (24–72)	0.623[a]

[a] Mann-Whitney U test, p<0.05; [b] Fisher's exact test, p<0.05

Table 5: Comparison of clinical and radiographical outcomes at preoperative and final follow-up assessments

Variables	Group A			Group B			Follow-up comparisons between groups	
	Pre-operative	Final follow-up	p values[a]	Preopera-tive	Final follow-up	p values[a]	Preoperative p values[b]	Final fol-low-up p values[b]
Clinical outcomes								
QuickDASH score, mean (min-max)	-	19 (12-30)		-	25 (18-35)	0.004	-	0.07
Constant score, mean (min-max)	42 (36-52)	90 (82-99)	0.001*	39 (28-47)	83 (81-93)	0.001	0.49	0.01*
VAS score, mean (min-max)	8 (5-9)	2 (0-3)	0.001*	7 (4-9)	3 (1-4)	0.001	0.81	0.51
	Radiographical outcomes							
Reconstruction ratio, (%) mean (min-max)	0.79 (0.75-0.86)	0.95 (0.92-0.99)	0.001*	0.77 (0.74-0.80)	0.93 (0.87-0.97)	0.001*	0.07	0.06
		Preoperative			Final follow-up			
Time to union, (months) mean (min-max)		7 (4-12)			10 (6-13)		0.01*	

VAS: Visual Analogous Scale; [a] For within-group differences from pre- to final follow-up, the paired samples T test was used, p<0.05; [b] For between-group differences at baseline and the final follow-up assessment, Mann–Whitney U test was used, p<0.05; * p<0.05

At the final follow-up between-group comparison, no significant differences were observed in the QuickDASH or VAS scores, while Group A exhibited significantly higher Constant Scores.

With respect to radiographical outcomes, the reconstruction ratio significantly increased in the respective Groups A and B from 0.79 (range=0.75-0.86) and 0.77 (range=0.74-0.80) preoperatively to 0.95 (range=0.92-0.99) and 0.93 (range=0.87-0.97) at the final follow-up. Otherwise, no significant difference was observed in the between-group comparisons either at baseline or at the final follow-up. Time to union was significantly shorter in Group A (mean=7 months, range=4-12) than in group B (mean=10 months, range=6-13).

DISCUSSION

Earlier case series have shown plate fixation with autologous bone grafting to be an acceptable method for treating patients with mid-shaft clavicular nonunion by providing favorable clinical results and higher rates of bony union (5, 6, 9-11). However, the more recent literature as best is known contains little information apart from a few retrospective case series supporting the use of this technique (8,15,16). Accordingly, the study has primarily aimed to investigate the mid-term outcomes of plate fixation with autologous bone grafting in a consecutive case series of mid-shaft clavicular non-union. Current data from the study have confirmed the feasibility and effectiveness of this technique.

In one of several recent clinical studies on the topic, Huang et al. achieved radiographic consolidation in all patients (n=21) using AO reconstruction plate at a mean time of 13.6 (range=11-27) weeks (8). All patients were subjectively satisfied with the outcome of their operation. They used an autologous iliac bone graft for only atrophic non-unions. Baker and Mullet later reported a case series of 15 patients treated by a pre-contoured locking plate without the use of distant bone graft from the iliac crest at a mean follow-up of 12.4 months (15). All patients in their case series achieved radiographical union along with favorable clinical outcomes. The authors concluded the distant bone graft to perhaps be unnecessary for clavicular non-union and even atrophic types. More recently, Beirer et al. reported the results of 14 patients with clavicular non-union (n=11) and/or malunion (n=3) treated by an LCP with autologous iliac bone graft at a short-term follow-up (mean=27 months, range=12-44 months) (16). All but one patient in their series had mid-shaft non- or mal-union. The authors achieved union in all patients and observed significant improvement in the relative Constant Scores.

This study examined ORIF consecutively performed with an LCP and autologous bone grafting for the treatment of atrophic non-union of the mid-shaft clavicle in 14 patients. The primary goal with this treatment approach was to obtain solid fusion, relieve symptoms, and improve clinical status. In this study's case series and consistent with the recent literature above, all patients achieved radiographical union at a mean time of eight months (range=4-13) without implant failure. An autologous bone graft originating from the iliac crest was routinely used. While an intercalary bone graft was required in three cases with a large defect, a cancellous bone graft was adequate to fill the defect area in the remainders. In contrast to Baker and Mullet, who determined distant bone graft from the iliac crest as unnecessary, this study considers this procedure to represent a safer option that generates high rates of bony union for the treatment of clavicular non-union, especially for those with a large defect (15).

Discussing the quality of life and functional status of the patients involved in this study is also necessary. In the present case series, four patients reported complete relief from pain, with the remaining patients experiencing a substantial relief from pain. The present study population consists of young and middle-aged patients with a mean age of 45 (range=31-65) years. Thus, symptomatic clavicular nonunion had imposed a significant socioeconomic burden and affected the quality of life of the study's patients who had physically demanding occupations prior to non-union surgery due to pain and inability to use their affected extremities. In contrast, this study considers the substantial improvement in Constant Scores and satisfactory QuickDASH scores at the final follow-up after non-union surgery to correspond to higher rates of returning to work with an enhanced quality of life in the study population. Comparable to the recent literature mentioned above, the current study's results have demonstrated ORIF using an LCP with autologous bone grafting to be effective in providing pain relief and improving clinical status.

As reviewed above, the existing literature has mainly focused on the treatment outcomes of ORIF with plate and screws for mid-shaft clavicular non-unions. Different from previous studies, this article has also explored as a secondary outcome whether the type of initial treatment received has an influence on the surgical results, with a significantly shorter time to union being found in patients who'd initially received non-surgical treatment. Performing non-union surgery as a revision surgery appears to have longer consolidation times as an expectation. With respect to clinical status, while both groups exhibited poor results in terms of Constant Score and VAS score before the non-union surgery, post-operative outcomes demonstrated each group's clinical status to be satisfactory. Nonetheless, despite no differences in QuickDASH and VAS scores at the final follow-up, patients who'd initially undergone surgical treatment exhibited better postoperative Constant Scores. Constant Score spe-

cifically measures the shoulder joint function, whereas QuickDASH is a general tool for assessing overall upper extremity function. Therefore, these findings may be interpreted to mean that plate fixation with autologous bone grafting is able to provide favorable overall clinical status regardless of the type of initial treatment. However, patients who initially received non-surgical treatment can experience significantly greater improvements in specific shoulder function.

Finally, several limitations need to be considered. The major limitations of the study are its retrospective nature and limited sample size. Nevertheless, compared to most of the studies cited herein, the present study has included a larger patient cohort with longer follow-up (8, 15, 16). Lastly, the existing literature on the topic has mostly been limited to retrospective case series. Thus, further prospective controlled studies are needed to confirm which technique is the ideal method of fixation in the management of mid-shaft clavicular nonunion.

CONCLUSION

In conclusion, evidence from this study has shown that, regardless of the type of initial treatment, plate fixation with autologous bone grafting is effective in obtaining solid fusion, relieving pain, and improving clinical status in patients with mid-shaft clavicular non-union at mid-term follow-up. Nonetheless, this technique can confer a shorter time to union and greater improvements in specific shoulder function in patients who'd initially undergone non-surgical treatment for a mid-shaft clavicle fracture.

Ethics Committee Approval: The study has ethical approval from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 26.01.2024, No: 02).

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

Peer Review: Externally peer-reviewed.

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

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EVALUATING LONG-TERM EXTERNAL DOSES AT SHORT RANGE IN PATIENTS RECEIVING ^{131}I TREATMENT FOR DIFFERENTIATED THYROID CARCINOMA

DİFERANSİYE TİROİD KARSİNOMUNDA ^{131}I TEDAVİSİ ALAN HASTALARDA KISA MESAFEDE UZUN DÖNEM EKSTERNAL DOZUN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Iodine-131 (^{131}I) treatment offers imaging advantages due to its emission of high-energy gamma radiation, despite concerns being raised regarding radiation safety. Various rules and standards for radiation protection have been established internationally and nationally. Patients undergoing this treatment can present a risk of external radiation exposure to the people around them. The purpose of this study is to measure the radiation dose rates around patients who have undergone treatment and to assess the effects of cumulative doses received by those who have been exposed.

Material and Method: The study includes a total of 44 patients who had undergone radioactive iodine therapy for differentiated thyroid cancer and measures their thyroid stimulating hormone (TSH) levels and 24-hour radioiodine uptake. The ^{131}I doses administered to the patients were recorded. Following treatment, external dose rates (EDR) were measured at distances of 30 cm and 1 m from the patients at 0, 4, 24, 48, 72, 144, and 240 h post ^{131}I treatment. Radiation exposure was calculated by considering three scenarios for those accompanying the patient.

Result: The study calculated the median TSH values of the 44 patients as 81.74±41.98 mIU/L, while the median of their 24-hour uptake values was determined as 6.39±8.42%. The mean administered treatment dose was 5.28 GBq (±1.3). A correlation was observed between the initial 24-hour measurements and the

ÖZET

Amaç: İyot-131 (^{131}I) tedavisi, yüksek enerjili gama radyasyonu yayması nedeniyle görüntüleme avantajları sunmakla birlikte, radyasyon güvenliğine ilişkin endişeleri de artırmaktadır. Radyasyondan korunmaya yönelik uluslararası ve ulusal düzeyde çeşitli kurallar ve standartlar oluşturulmuştur. Bu tedaviyi gören hastalar, etraflarındaki kişilerin dışarıdan radyasyona maruz kalma riski oluşturabilir. Bu çalışmanın amacı tedavi gören hastaların yakınındaki radyasyon doz oranlarını ölçmek ve maruz kalanların aldığı kümülatif dozları değerlendirmektir.

Gereç ve Yöntem: Diferansiye tiroid kanseri nedeniyle radyoaktif iyot (RAI) tedavisi gören toplam 44 hasta çalışmaya dahil edildi. Tiroid uyarıcı hormon (TSH) düzeyleri ve 24 saatlik radyoiyot alımları ölçüldü. Hastalara uygulanan RAI dozları kaydedildi. Tedaviyi takiben ^{131}I tedavisinden sonraki 0, 4, 24, 48, 72, 144 ve 240. saatlerde hastalardan 30 cm ve 1 m mesafeden eksternal doz oranları (EDR) ölçüldü. Hastaya eşlik edenlerin radyasyon maruziyetleri üç senaryo dikkate alınarak hesaplandı.

Bulgular: Kırk dört hastanın ortanca TSH değeri 81,74±41,98 mIU/L olarak hesaplanırken, 24 saatlik alım değerlerinin ortancası %6,39±8,42 olarak belirlendi. Ortalama uygulanan tedavi dozu 5,28 GBq (±1,3) idi. İlk 24 saatlik ölçümler ile uygulanan doz arasında korelasyon gözlendi. Ayrıca 24. saatten sonra alınan ölçümler ile 24. saatteki alım değeri arasında korelasyon bulun-

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administered dose. Furthermore, another correlation was found between the measurements taken after the 24th hour and the 24-hour uptake value. The radiation exposure for accompanying individuals was estimated to range between 0.4-1.62 mSv across three scenarios.

Conclusion: Patients receiving ^{131}I treatment should be given comprehensive information about the importance of radiation protection after treatment and the precautions to be taken during isolation.

Keywords: Radiation safety, differentiated thyroid carcinoma, ^{131}I , external dose rate, radiation protection

du. Eşlik eden kişilerin radyasyona maruz kalma oranının üç senaryoda 0,4 ila 1,62 mSv arasında olduğu tahmin edildi.

Sonuç: RAI tedavisi gören hastalara tedavi sonrasında radyasyondan korunmanın önemi ve izolasyon sırasında alınması gereken önlemler konusunda kapsamlı bilgi verilmelidir.

Anahtar Kelimeler: Radyasyon güvenliği, diferansiye tiroid karsinomu, ^{131}I , harici doz hızı, radyasyon koruması

INTRODUCTION

Since the first applications of nuclear medicine, radionuclides have been increasingly utilized in clinical diagnosis and treatment (1-3). Radioactive iodine (^{131}I) is widely used in the diagnosis and treatment of diseases, especially in the context of thyroid cancer therapy. The effectiveness of ^{131}I treatment has been proven and successfully applied for ablation and adjuvant therapy following total thyroidectomy in patients diagnosed with thyroid carcinoma. This treatment increases the survival rate and reduces the risk of recurrence and metastasis after total thyroidectomy (1).

The ^{131}I ablation treatment, commonly administered using empirical methods, is given in doses ranging from 1850 MBq-7400 MBq in line with the latest guidelines. The effective beta radiation emitted in the treatment also has 364 keV of gamma energy to provide an imaging advantage; however, this presents challenges concerning radiation safety, the importance of which remains significant due to the long half-life, high energy, and the quantity of activity administered in nuclear medicine applications. Because of the high gamma energy, patients undergoing treatment act as open sources, exposing nearby individuals to external radiation. Therefore, the International Commission on Radiological Protection (ICRP) has established strict rules for radiation protection in its ICRP Publication 60. In 2006, the International Atomic Energy Agency (IAEA) suggested a guideline for discharging patients, recommending an external dose rate (EDR) of 1200 MBq or 70 μSv/h at a distance of 1 meter from patients as a directive level. However, the specific regulations vary by country. According to European Union (EU) guidelines, the release of patients undergoing ^{131}I treatment is specified to be permissible only if the retained body activity (RBA) is < 400 MBq and the EDR at a distance of 1 meter is < 20 μSv/h (3). According to the laws of the Turkish Energy, Nuclear and Mineral Research Agency (TENMAK) and the Turkish Ministry of Health, patients who are to be treated at levels exceeding 600 MBq must be treated in specially designed rooms in clinics possessing a radionuclide therapy license as per the rule for inpatient care. For patients to be discharged, the requirement is that the EDR must have fallen below 30 μSv/h at a distance of 1 meter from the abdominal region (4, 5). Even if the EDR falls below these levels, patients remain an open source of radiation until the activity in their bodies has been completely neutralized and thus are a source of external radiation (6-9). Close-range exposure to doses from patients undergoing treatment may lead to undesirable situations. This is particularly challenging in large cities with dense populations and cultures with limited living spaces, as isolating patients treated for radiation can pose dangers. Despite education provided to patients and their families regarding radiation protection and isolation prior to treatment, instances do occur where these recommendations are not followed. Understanding the exposure levels of individuals subjected to close-range dose exposure is crucial for various reasons, particularly regarding those who do not comply with radiation isolation rules. Many studies have been carried out concerning patients' EDRs, and these studies have provided results regarding the decrease in radiation in the patient's body (10-13). However, most of these studies are based on measurements taken at the end of the treatment up until the patient is discharged (13-17). Furthermore, the approach in long-term studies has involved taking measurements from 1-2 m away from the patient (13, 18). Both the individuals who are exposed as well as health authorities must possess a basic understanding of the exposure risks associated with close contact with a patient who has undergone ^{131}I treatment.

This study aims to assess patients' short-range dose rates and calculate the cumulative doses to which family members or those in close contact are exposed.

MATERIALS and METHOD

Patient population

This study includes a total of 44 patients (6 males, 38 females) who received ^{131}I treatment at the Istanbul University Faculty of Medicine Department of Nuclear Medicine between 2005-2006 are included in this study. The

average age of these patients is 42±13.8 years. This study was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 15.12.2023, No: 25).

Treatment procedure

All patients had previously undergone total thyroidectomy, and ^{131}I therapy was administered after a minimum waiting period of 6 weeks following surgery. Additionally, all patients adhered to a low-iodine diet for at least two weeks before therapy, with LT4 therapy being discontinued for 4–6 weeks prior to receiving ^{131}I therapy in order to prevent iodine contamination and to ensure maximum iodine retention. Before treatment, a ^{131}I uptake test was performed to ensure that the TSH levels of the patients admitted to the clinic for treatment were >30 mIU/L. Furthermore, female patients underwent hCG testing to rule out pregnancy. To optimize ^{131}I absorption in patients, they were required to fast for 3 hours before treatment. The dose of the ^{131}I capsule to be administered to the patient was measured using a dose calibrator and then administered with 200 ml of water.

The measurements

The measurements were performed using a Ludlum Model 3 survey meter (measurement range 0-2 R/h; Ludlum Measurements, Inc., Sweetwater, TX, USA), calibrated by the national Secondary Standard Dosimetry Laboratories (SSDL). To reduce the margin of error in the measurements following the administration of ^{131}I, readings were taken using a specially designed setup at distances of 30 cm and 1 meter from the abdominal region at 0, 4, 24, 48, 72, 144, and 240 h post-treatment. Upon considering the localization of ^{131}I uptake in the thyroid region, additional measurements were also recorded from the neck area at distances of 0 meters and 30 cm over the 240 h period with the survey meter.

Scenarios

The radiation exposure experienced by individuals who were in close contact with the patient as companions after receiving treatment was calculated for the total periods between 48 hours and 240 hours in accordance with the following three different scenarios:

Scenario 1 (S1): At 48 h post-discharge, a scenario was devised assuming that the patient sleeps in the same bed as the companion at a distance of 30 cm for eight hours in a day.

Scenario 2 (S2): At 48 h post-discharge, a second scenario was devised assuming that the patient sleeps in a different bed than the companion in an adjacent room and separated by a 10 cm concrete wall (at 30 cm distance from the patient) for eight hours in a day. In accordance with radiation protection rules, the reduction effect of the concrete wall was calculated mathematically. It was calculated that a 10 cm concrete wall absorbed 20% of ^{131}I radiation (19).

Scenario 3 (S3): At 48 h post-discharge, a third scenario was devised assuming that the patient slept in separate beds in the same room, with a 50 cm distance between them (1 m distance between patient and companion), for eight hours in a day (Figure 1).

For these three scenarios, calculations were made to determine the radiation exposure dose for the companion of the treated patient. A decay graph of the EDR values measured from the patients was plotted based on these calculations. The cumulative dose value was determined by calculating the area under this graph. After 240 hours, the activity regarding the dose calculations was assumed to only decrease physically within the patient's body.

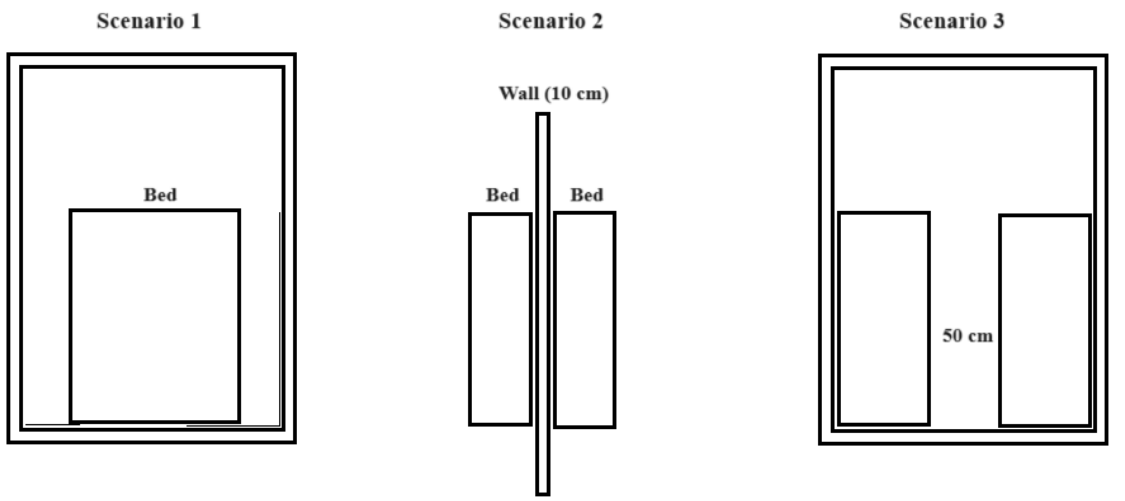

Figure 1: Positions of rooms and beds for the three scenarios used in the study.

Statistical analysis

IBM SPSS Statistics for Windows (Ver. 28.0; IBM Corp., Armonk, NY, USA) was used for the statistical analyses. The Pearson correlation test was used to analyze the relationship between TSH and EDR, uptake at 24 h and EDR, and dose and EDR, with a $p<0.05$ being considered statistically significant.

RESULTS

Table 1 presents the TSH, uptake value at 24 h, and EDR measurements for all patients. When considering these values, the median TSH value for the 44 patients was calculated as 81.74±41.98 mIU/L, and their median uptake value at 24 h was calculated as 6.39±8.42%.

The mean administered treatment dose was 5.28 GBq (±1.3). Treatment dosages were distributed as follows: 14 patients received 3.7 GBq, 23 received 5.55 GBq, six received 7.4 GBq, and one received 8.51 GBq. Of these patients, five were undergoing repeated treatment.

The 30 cm EDR values at 0 h, 4 h, 24 h, 48 h, 72 h, 144 h, and 240 h were measured and found respectively as 67.2±15.02 mR/h, 66.59±14.95 mR/h, 19.76±8.74 mR/h, 7.92±4.87 mR/h, 4.14±4.35 mR/h, 1.23±1.40 mR/h, and 0.45±0.74 mR/h (Figure 2).

The 1 m EDR values at 0, 4, 24, 48, 72, 144, and 240 h were measured and found respectively as 16.26±3.80 mR/h, 16.01±3.88 mR/h, 4.94±1.99 mR/h, 2.02±1.32 mR/h, 1.08±1.18 mR/h, 0.40±0.76 mR/h, and 0.07±0.21 mR/h.

Pearson correlation tests were performed for the EDR measurements with TSH, uptake at 24 h, and the applied treatment dose. No significant relationship was found between the patients' pre-treatment TSH values and EDR measurements ($p>0.05$). While no significant relationship was found between uptake result at 24 h and the EDR measurements at 0, 4, and 24 h ($p>0.05$), significant relationships were found for subsequent EDR measurements at 48, 72, 144, and 240 h ($p<0.05$).

A significant relationship was observed to exist between the ^{131}I dose that was applied to the patients and EDR measurements at 0, 4, and 24 h ($p<0.05$). However, the subsequent measurements showed no significant relationship ($p>0.05$). Dose calculations were made using the EDR results based on the three different scenarios. The radiation exposure dose for the patients' companions was calculated as 1.62 mSv for scenario 1, 1.29 mSv for scenario 2, and 0.4 mSv for scenario 3 (Figures 3 and 4).

Scenario 1: Calculations were made based on the average EDR measured at a distance of 30 cm from patients undergoing treatment. According to these calculations, a person who spends eight hours in the same bed at 30 cm from the patient would be exposed to approximately

Table 1: Patients' thyroid stimulating hormone (TSH), uptake values at 24 h, external dose rates (EDR) mR/h taken from distances of 30 cm and 1 m

Patient	TSH (mIU/L)	Iodine Uptake at 24 h (%)	30 cm EDR (mR/h)							1 m EDR (mR/h)						
			0 h	4 h	24 h	48 h	72 h	144 h	240 h	0 h	4 h	24 h	48 h	72 h	144 h	240 h
1	97.3	1.00	50	49	16.0	9.0	4.2	0.9	0.40	12.0	12.0	3.4	1.6	1.0	0.2	0.0
2	70.9	2.97	44	44	20.0	9.0	4.6	1.1	0.22	11.0	11.0	4.2	2.7	1.0	0.3	0.0
3	65.0	1.10	49	50	9.0	3.5	1.0	0.4	0.20	12.0	12.0	3.3	1.3	0.4	0.1	0.0
4	75.0	6.41	45	45	8.0	3.1	0.9	0.5	1.00	10.5	10.5	2.6	0.9	0.3	0.2	0.1
5	74.0	9.35	48	48	11.0	4.0	2.1	1.1	0.20	12.0	12.0	2.8	1.1	0.5	0.1	0.0
6	69.0	4.07	50	48	12.0	4.8	2.9	1.0	0.08	12.0	10.0	2.7	1.2	0.9	0.1	0.0
7	130.5	0.75	46	46	12.0	2.6	1.2	0.4	0.12	12.0	12.0	3.2	0.9	0.3	0.0	0.1
8	65.9	26.79	41	41	15.0	8.0	4.3	1.7	0.54	11.0	11.0	3.3	1.8	1.1	0.4	0.2
9	71.9	8.60	45	45	14.0	8.0	4.8	1.0	0.20	12.0	12.0	3.1	2.0	1.1	0.3	0.0
10	13.2	4.19	49	48	22.0	12.0	4.0	0.9	0.15	13.0	13.0	4.8	2.0	1.2	0.2	0.0
11	154.0	0.26	48	48	10.0	1.6	0.9	0.3	0.19	12.0	12.0	2.8	0.5	0.3	0.0	0.0
12	19.9	33.22	49	49	22.0	11.0	5.6	2.1	0.40	11.0	11.0	4.3	2.3	1.3	0.4	0.0
13	63.0	6.20	72	72	46.0	11.0	6.0	2.1	0.70	12.0	12.0	8.0	2.6	1.5	0.6	0.2

14	36.8	6.54	72	72	24.0	9.0	4.8	1.1	0.47	16.0	16.0	4.5	2.2	1.2	0.5	0.0
15	46.5	1.56	76	76	26.0	13.0	5.8	1.3	0.35	17.0	17.0	7.0	2.5	1.3	0.5	0.2
16	225.4	11.35	75	75	27.0	9.0	4.9	1.6	0.34	19.0	19.0	8.0	2.1	1.2	0.6	0.1
17	136.8	11.20	74	74	20.0	10.0	5.0	1.5	0.23	17.0	17.0	4.2	2.2	1.0	0.5	0.1
18	38.6	9.10	70	70	18.0	9.0	4.5	1.6	0.80	16.0	16.0	4.8	2.2	1.0	0.4	0.0
19	97.5	2.16	68	68	13.0	3.7	1.3	0.6	0.12	17.0	17.0	4.2	0.8	0.35	0.1	0.0
20	76.1	3.78	75	75	18.5	6.0	3.6	0.8	0.40	20.0	19.0	4.5	1.6	0.9	0.3	0.0
21	92.4	2.45	72	72	14.0	3.8	1.6	0.6	0.15	17.0	17.0	3.3	1.2	0.41	0.1	0.0
22	79.0	4.20	74	74	19.0	6.0	3.4	0.9	0.46	18.0	18.0	4.5	1.5	0.9	0.2	0.1
23	64.8	1.05	73	73	23.0	7.5	3.0	1.2	0.80	18.0	18.0	6.0	1.7	0.6	0.3	0.2
24	89.0	1.49	78	78	15.0	7.5	3.9	1.6	0.90	18.0	16.0	3.3	1.8	0.7	0.3	0.1
25	145.0	0.95	72	72	21.0	13.0	4.6	0.5	0.22	24.0	24.0	4.6	2.7	1.1	0.4	0.0
26	72.5	0.66	75	75	11.0	3.3	1.2	0.4	0.24	18.0	18.0	3.7	1.2	0.3	0.1	0.0
27	33.0	14.30	72	72	28.0	18.0	9.0	2.8	0.40	16.0	16.0	8.0	3.8	2.0	0.4	0.2
28	117.0	0.39	78	78	24.0	7.0	4.1	0.3	0.12	16.0	16.0	6.0	1.6	1.2	0.3	0.0
29	48.7	3.76	78	78	17.0	6.0	3.2	0.8	0.20	21.0	21.0	5.0	1.7	0.8	0.3	0.0
30	54.7	6.00	72	72	20.0	6.5	3.2	1.3	0.38	16.0	16.0	4.2	1.6	0.7	0.2	0.0
31	16.0	37.77	75	74	40.0	30.0	30.0	9.5	5.00	19.0	18.0	10.0	8.0	8.0	5.2	1.4
32	129.0	4.20	72	71	17.0	6.0	3.1	0.9	0.40	15.0	15.0	3.6	1.1	0.7	0.2	0.0
33	72.7	7.60	72	72	19.0	10.0	5.2	1.9	0.90	19.0	18.0	4.1	2.3	1.2	0.5	0.2
34	42.0	14.19	76	75	23.0	9.0	3.3	1.4	0.45	18.0	18.0	6.0	1.8	1.2	0.4	0.2
35	162.0	10.07	89	89	30.0	9.0	4.4	1.6	0.35	20.0	19.0	7.0	2.1	1.2	0.2	0.1
36	94.4	1.40	88	87	29.0	8.5	4.3	1.1	0.30	19.0	19.0	7.0	2.4	1.0	0.4	0.0
37	44.6	15.79	83	76	29.0	13	5.9	1.8	0.30	22.0	22.0	7.0	3.5	1.4	0.5	0.0
38	96.8	1.57	80	76	32.0	6.0	2.9	0.6	0.30	21.0	21.0	9.0	1.9	0.9	0.3	0.0
39	72.9	1.00	85	82	15.0	6.0	2.7	0.9	0.20	21.0	21.0	4.5	2.2	0.8	0.2	0.0
40	64.9	0.24	49	43	10.5	4.1	1.5	0.4	0.20	12.0	11.0	3.4	1.4	0.5	0.1	0.0
41	70.0	0.19	86	86	38.0	6.0	2.1	0.5	0.16	20.0	20.0	9.0	1.5	0.6	0.2	0.0
42	85.8	0.20	45	45	6.5	1.6	1.0	0.3	0.06	11.0	9.0	2.0	0.4	0.2	0.1	0.0
43	124.0	1.08	96	96	13.0	3.2	1.1	0.4	0.05	23.0	23.0	3.6	1.1	0.3	0.0	0.0
44	98.6	0.38	71	71	12.0	10	5.0	0.4	0.10	19.0	19.0	7.0	6.0	3.0	0.9	0.0
Median ±SD	81.74 ±41.98	6.39 ±8.42	67.2 ±15.02	66.59 ±14.95	19.76 ±8.74	7.92 ±4.87	4.14 ±4.35	1.23 ±1.40	0.45 ±0.74	16.26 ±3.80	16.01 ±3.88	4.94 ±1.99	2.02 ±1.32	1.08 ±1.18	0.40 ±0.76	0.07 ±0.21

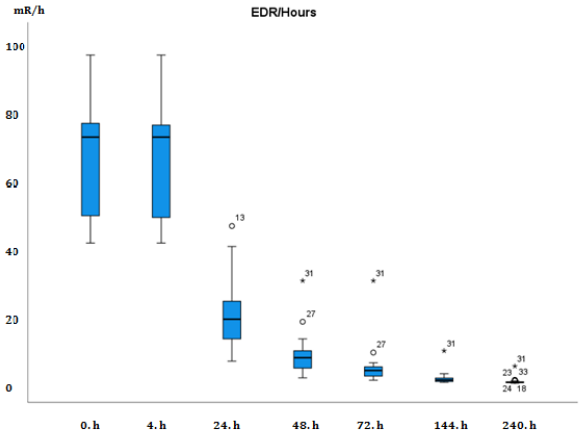

Figure 2: Distribution of patients' decrease in external dose rates (EDR) in milliroentgen per hour (mR/h) at 30 cm.

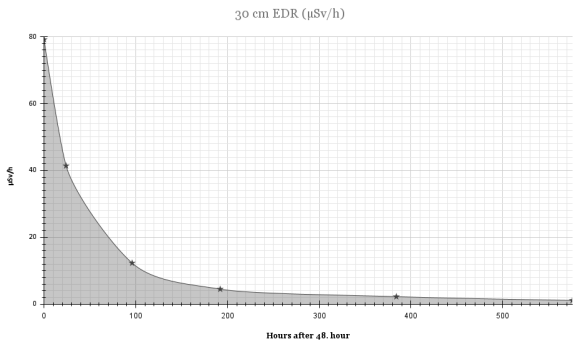

Figure 3: Cumulative companion dose measured using the external dose rates (EDR) for the period of 48-240 h post-treatment at a distance of 30 cm from the patient in microsieverts per hour (µSv/h).

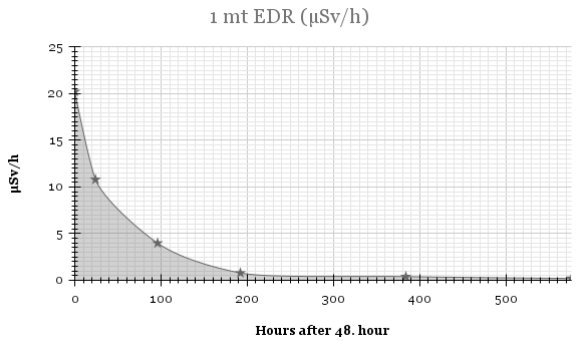

Figure 4: Cumulative companion dose measured using the external dose rates (EDR) for the period of 48-240 h post-treatment at a distance of one m from the patient in microsieverts per hour (µSv/h).

1.62 mSv of radiation. This dose exceeded the permissible exposure limit for the public (an average of 1 mSv over 5 consecutive years) (20).

Scenario 2: The calculations were performed using the average EDR measured 30 cm away from patients receiving treatment. According to these calculations, a person who spends all eight hours a day in a bed 30 cm away next to the same 10 cm concrete wall will be exposed to approximately 1.29 mSv of radiation. The exposure dose may vary depending on the construction material of the wall separating the beds. If the wall is made of brick, the exposure dose will be higher than 1.29 mSv.

Scenario 3: According to Scenario 3, even if the two beds in the same room are assumed to have 50 cm between them, the distance between the patient and the person being exposed is approximately one meter due to their position in the center of the bed. Therefore, calculations were made based on the average EDR measured at a distance of one meter from patients undergoing treatment. According to the calculations, a person who spends a total of eight hours in a separate bed in the same room as the patient would be exposed to approximately 0.4 mSv of radiation.

DISCUSSION

^{131}I ablation therapy is a proven and commonly administered treatment for thyroid cancers. Due to the high gamma energy of ^{131}I, patients who receive this treatment can be a significant source of radiation exposure to people nearby. Patients are typically kept in the hospital until the radioactivity in their body falls to a safe threshold as defined by national guidelines. However, even after discharge, external radiation emissions continue until the radioactive substance is fully depleted from the body, thus presenting a risk to individuals close to the patient.

This study found 91% (n=41) of the patients who'd received treatment to have reduced their radiation levels to below the discharge threshold (30 µSv/h or 3 mR/h) by the 48th hour. For those patients whose measurements were above 3 mR/h at the 48-hour mark, three were noted to have had high iodine uptake values at 24 hours because of remnant thyroid tissue and the third patient to have distant metastasis.

A notable correlation emerged between the treatment dose administered to a patient and the measurements recorded at 0, 4, and 24 h ($p<0.05$). However, no correlation was detected regarding the measurements taken at 48, 72, 144, or 240 h. The median EDR values obtained following the administration of 3.7 GBq, 5.55 GBq, and 7.4 GBq of ^{131}I at a distance of 30 cm were respectively 6.38 mR/h (±3.51), 10.96 mR/h (±5.75), and 8.5 mR/h (±2.87).

Notably, the association between the administered dose and the subsequent measurements deteriorated with time, particularly at 48 h and beyond. These findings suggest the physiological excretion of ^{131}I to occur within the initial 48 h post-administration, with any remaining activity

likely sequestered within residual tissue or metastatic sites.

When looking at the EDR measurements regarding the activity of patients who've received ablation therapy, the following observations are made: at 72 h post-treatment, the average EDR measurements at 30 cm was 3.04 mR/h for patients who received 3.7 GBq, 5.21 mR/h for those who'd received 5.55 GBq, and 4.04 mR/h for those who'd received 7.4 GBq. At 240 h (10 d) post-treatment, the average EDR measurements at 30 cm for these same groups were found to be 0.3 mR/h, 0.631 mR/h, and 0.29 mR/h, respectively. Based on these results, no direct relationship has been concluded to exist between the dose administered to the patient and the EDR measurements after 48 h. This suggests that factors other than just the administered dose may influence the radiation exposure levels measured from patients different times post-treatment.

When looking at the iodine uptake values at 24 h compared to the EDR measurements, no correlation was observed to be present in the measurements taken at 0, 4, and 24 h, while correlations with EDR measurements were found for uptake values at 48, 72, 144, and 240 h ($p<0.05$). After the physiological excretion had completed, the activity is thought to remain solely in remnant tissue. When considering these findings, companions of patients with higher 24-hour uptake values were observed to have increased radiation exposure post-discharge. In light of these results, patients with high 24-hour iodine uptake values should pay more attention to isolation rules after discharge.

Because of high ^{131}I uptake value of 31th patient (24-hour ^{131}I uptake value of 37.77%), patient number 31 had a 144 h post-treatment EDR measurement of 9.5 mR/h at a distance of 30 cm and a 240 h post-treatment EDR measurement of 5 mR/h at a distance of 0.3 meters.

Bhatia et al.'s study with 32 patients conducted EDR measurements following the administration of radioiodine therapy for ablating remnant tissue after a total thyroidectomy (16). The average EDR measurements taken 1 m away from the patients were 0.16 mR/h/mCi at 0 h, 0.13 mR/h/mCi at 1 h, and 0.11 mR/h/mCi at 2 h. These findings are consistent with the 0-hour EDR measurements in the current study. Moreover, Bhatia et al. also simultaneously measured EDRs at 5 cm away from the skin at the neck and stomach levels. They observed the stomach level EDRs to be higher during the first hour, with neck-level EDRs increasing and stomach EDRs decreasing in the second hour. This indicates the residual tissues in the patients to have quickly absorbed the radioactivity and the physiological excretion to also occur rapidly. Although this study provided important information about the physiology in the early post-treatment period, it did not provide any long-term EDR details.

Zhang et al.'s study with 70 patients took EDR measurements while patients were seated at distances of 1, 1.5, 2, and 2.5 m over 72 h (18). The study found a majority of the physiological excretion to finish within the first 48 h and also noted excretion rates to occur faster in subsequent treatments. This aspect aligns with the initial hour EDR measurements in the current study. However, Zhang et al.'s study did not include close-range measurements or assess the radiation exposure risks associated with close physical contact.

The outcomes of the scenarios indicated radiation exposure levels to exceed the permissible public dose limit in Scenarios 1 and 2 while falling below this threshold in Scenario 3. ^{131}I has high gamma energy, and although the building material of the wall between patient and companion in S2 reduces the dose from 1.62 mSv to 1.29 mSv, this value is higher than the annual maximum allowable dose limit for the public (1 mSv/year). As can be understood from the descending graph, radioactivity in a patient's body fully diminishes by 240 h post-treatment. Hence, ensuring patients' compliance with isolation protocols for a minimum of 10 days is imperative. ^{131}I is also known to be excreted through the respiratory system. Even if the exposure to external radiation is below the permissible dose when sharing the same room with a patient, the risk of internal radiation exposure through inhalation is still present. Internal radiation is a significant concern for individuals. Numerous studies have demonstrated the internal exposure following ^{131}I treatment due to the exhalation of patients treated with ^{131}I to result in the presence of ^{131}I in the environment. When considering both external and internal exposure, adherence to radiation safety guidelines becomes even more crucial for patients who undergo ^{131}I treatment. Therefore, patients receiving ^{131}I treatment should be thoroughly educated about the importance of post-treatment radiation protection and the specific conditions to be observed during isolation.

CONCLUSION

The companions of patients who receive ^{131}I treatment may be exposed to an external dose of over 1 mSv if the patients do not comply with isolation rules. Even in cases where the patient and companion sleep in separate rooms, maintaining a safe distance between the beds remains imperative. Consequently, giving these patients comprehensive information about the importance of protection from radiation after treatment and about the precautions to be taken during isolation is crucial.

Ethics Committee Approval: The study has ethical approval from the İstanbul University, İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 15.12.2023, No: 25).

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

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EFFECT of CURCUMIN on BREAST CANCER CELLS THROUGH miR-145-5p AND ITS TARGET GENES

KURKUMİNİN miR-145-5p VE HEDEF GENLERİ ÜZERİNDEN MEME KANSERİ HÜCRELERİNE ETKİSİ

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ABSTRACT

Objective: Curcumin is considered an epigenetic regulator with anticancer effects. The micro-RNA (miRNA) miR-145-5p is a tumor suppressor that shows low expression levels in various cancers, including breast cancer (BC). This study aims to investigate whether curcumin inhibits MCF-7 human BC cell line proliferation and migration by regulating miR-145-5p and its possible target genes.

Material and Method: MCF-7 cells were treated with curcumin and its solvent control. Additionally, cells were transfected with an miR-145-5p mimic and a non-targeting miRNA mimic. Cell viability was then evaluated, and the scratch wound assay was used to assess cell migration. The study predicts the miR-145-5p putative target genes by searching for overlapping genes in the miRNet and miRTarBase v8 databases via the overexpressed genes in the BC tissue samples in the Cancer Genome Atlas (TCGA) datasets. Expression levels of miR-145-5p and the selected genes were detected using the quantitative real-time polymerase chain reaction (qRT-PCR). The $2^{-\Delta\Delta Ct}$ method was used for the quantification analysis, with $p<0.05$ being considered statistically significant.

Result: Curcumin treatment and overexpression of miR-145-5p via the transfection of an miR-145-5p mimic significantly decreased the proliferation and migration of MCF-7 cells. Moreover, curcumin treatment significantly increased the

ÖZET

Amaç: Kurkumin anti-kanser etkileri olan bir epigenetik regülatör olarak kabul edilmektedir. miR-145-5p, meme kanseri (MK) dahil olmak üzere bir çok kanserde ekspresyon düzeyi düşük olan bir tümör baskılayıcı mikroRNA'dır (miRNA). Bu çalışmada, kurkuminin miR-145-5p'yi ve olası hedef genlerini regüle ederek MCF-7 insan MK hücre hattının proliferasyonunu ve göçünü inhibe edip etmediğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: MCF-7 hücreleri kurkumin ve onun solvent kontrolü ile muamele edildi. Ayrıca hücreler miR-145-5p mimiği ve negatif kontrol miRNA mimiği ile transfekte edildi. Hücre canlılığı değerlendirildi ve hücre göçü scratch yara (wound) testi kullanılarak değerlendirildi. miR-145-5p'nin potansiyel hedefleri, MK doku örneklerinde aşırı eksprese edilen genlerle miRNet ve miRTarBase v8 veri-tabanları ile örtüşen genlerin Kanser Genom Atlası (TCGA) datasetlerinde araştırılması yoluyla belirlendi. miR-145-5p ve seçilen genlerin ekspresyon düzeyleri, kantitatif gerçek-zamanlı polimeraz zincir reaksiyonu (qRT-PCR) ile tespit edildi. Kantifikasyon analizi için $2^{-\Delta\Delta Ct}$ yöntemi kullanıldı. p-değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Hem kurkumin ile muamele edilmiş hem de miR-145-5p mimiği ile transfekte edilmiş MCF-7 hücrelerinde, proliferasyonun ve göçün, kontrollere kıyasla azaldığı gözlenmiştir. Ayrıca, kurkumin ile muamele edilen hücrelerde miR-145-5p'nin ekspresyonunun anlamlı düzeyde arttığı görülmüştür. miR-145-5p'nin olası

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expression of miR-145-5p. The possible target genes of miR-145-5p (i.e., *MCM2, MMP1, MMP9, EEF1A2*) were downregulated in curcumin-treated MCF-7 cells. Additionally, miR-145-5p mimic-transfected cells showed low expression levels of the *MCM2, MMP1*, and *MMP9* genes.

Conclusion: Curcumin inhibits the proliferation and migration of MCF-7 cells by acting on miR-145-5p and its possible target genes.

Keywords: Breast cancer, MCF-7 cell line, curcumin, miR-145-5p, target genes

hedef genlerinin, *MCM2, MMP1, MMP9* ve *EEF1A2*, kurkumin ile muamele edilmiş MCF-7 hücrelerinde ekspresyon düzeyinin azaldığı saptanmıştır. Bunun yanı sıra, miR-145-5p mimiği ile transfekte edilmiş hücrelerde, *MCM2, MMP1* ve *MMP9* genlerinin ekspresyon düzeyinin düşük olduğu belirlenmiştir.

Sonuç: Kurkuminin, miR-145-5p ve olası hedef genleri üzerinden etki ederek MCF-7 hücrelerinin hem proliferasyonunu hem de göçünü inhibe ettiği gösterilmiştir.

Anahtar Kelimeler: Meme kanseri, MCF-7 hücre hattı, kurkumin, miR-145-5p, hedef genler

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed cancer among women, with approximately 2.26 million cases occurring in 2020. BC is the major leading cause of mortality in female cancer patients (1, 2). By 2040, more than three million new cases of BC and one million deaths are predicted to occur each year due to population growth and aging. Global efforts and public health awareness programs aimed at managing the incidence and mortality of BC should include screening, early diagnosis, treatment, and follow-up of BC patients (3). The MCF-7 cell line is a luminal A subtype of BC that expresses both the progesterone receptor (PR) and the estrogen receptor (ER) (4).

Numerous molecular mechanisms have demonstrated the impact of natural nutritional sources such as curcumin on cellular processes (5, 6). Curcumin is a yellow pigment found in *Curcuma longa Linn rhizome* (7). *In vitro* and *in vivo* studies have shown curcumin to have anticancer effects on various tumors by reducing the proliferation and migration of cells and by inducing apoptosis (8). Recent studies have suggested curcumin to be able to act as an epigenetic regulator in several cancers by acting on non-coding RNAs (5, 9).

MicroRNAs (miRNAs) are noncoding RNAs of about 22 nucleotides (10); miRNAs regulate the expression of genes as oncogenes or tumor suppressors and affect different biological processes (e.g., proliferation and apoptosis) through their involvement in tumor development and progression (2, 11).

miRNA-145-5p (miR-145-5p) is a miRNA with tumor suppressor activity and has low expression in different malignant tissues such as BC compared to normal tissues. miR-145-5p decreases tumor cell proliferation and migration, as well as invasion, and increases cell sensitivity to chemotherapy drugs by acting on a variety of genes. Some studies suggested miR-145-5p to perhaps have value as a target molecule in cancer treatment, apart from its diagnostic and prognostic benefits during the assessment of cancers (12, 13).

By regulating multiple genes, miR-145-5p can serve as a potential biomarker for risk assessment in BC patients (14, 15). miR-145-5p could be a potential predictive biomarker for BC stemness by acting on the SRY-box transcription factor 2 (*SOX2*) gene (14). Moreover, studies have significantly linked decreased miR-145-5p expression to higher Ki-67 expression levels, larger tumors, metastasis, and shorter overall survival (OS) (15). Another study has demonstrated the oncogenic hepatitis B X-interacting protein (*HBXIP*) gene as a target of miR-145 in MCF-7 cells (16). In addition, a recent study has reported that, compared to their controls, both BC cell lines and patient BC tissues significantly showed lower expression levels of miR-145-5p and a negative association with the expression of the programmed death-ligand 1 (*PDL1*) gene (17). The regulatory role of curcumin in miR-145-5p has been investigated in several cancers such as laryngeal squamous cell carcinoma (18). Many studies have also determined low expression levels of miR-145-5p in both BC cell lines and patient tissue samples, thus establishing it as a known tumor suppressor miRNA in BC. However, how curcumin affects BC cells via miR-145-5p and its target genes remains unclear. This study aims to investigate whether curcumin inhibits the proliferation and migration of the MCF-7 human BC cell line by acting on miR-145-5p and its target genes.

MATERIALS and METHODS

Cell culture

The MCF-7 human BC cell line was obtained from the İstanbul University Faculty of Sciences Department of Biology. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose (EcoTech Biotechnology, Türkiye) supplemented with 10% fetal bovine serum (FBS; Gibco, UK) and 1% antibiotic (penicillin-streptomycin; Gibco, NY, USA). Cells were incubated at 37°C, > 90% humidity, and 5% CO_2.

Curcumin treatment

High-purity curcumin (Bio Basic Inc., Canada) was dissolved in dimethyl sulfoxide (DMSO; 1mg/mL; EcoTech Biotechnology, Türkiye). Based on the recommendations of previous literature, the study performed a 10 μM curcumin treatment for 24 h (19-21).

miR-145-5p mimic transfection

A commercial miR-145-5p mimic was used for transfection with the following sequence: 5′-GUCCAGUUUUCCCAGGAAUCCCU-3′ (Thermo Fisher Scientific, USA); a non-targeting miRNA mimic (NT miRNA mimic) with validated random sequences producing no identifiable effects on known miRNA function (Ambion, USA) were used for transfection. Transfection with 30 pmol miR-145-5p mimic and NT mimic miRNA was achieved using the Lipofectamine 2000 Transfection Reagent (Invitrogen, USA) in accordance with the manufacturer's instructions. In short, 1.5 µL transfection reagent and 3 µL 5nmol miR-145-5p mimic were mixed with 125 µL Opti-MEM medium (Gibco, USA) separately and incubated for 5 min at room temperature. Both tubes were then mixed and incubated for 20 min at room temperature. The same was done for the NT mimic miRNA transfection. All transfection processes were performed for 24 h.

Cell proliferation assay

MCF-7 cells were seeded in quadruplicate (5,000 cells per well) in four columns of 96-well plates (Nest Biotechnology Co., China) and incubated under suitable conditions. After 24 h of cell culturing, cells were treated with 10 µM curcumin and its solvent control (DMSO). Additionally, the cells were transfected with 30 pmol miR-145-5p mimic and NT mimic miRNA using the Lipofectamine 2000 Transfection Reagent (Invitrogen, USA). 24 h later, 10 µL of the cell viability detection kit 8 (CVDK8) solution (NutriCulture, Türkiye) were added. After 3 h of plate incubation, absorbance was measured using a microplate reader at 450 nm. The medium was then discarded by flipping the plate. All wells were washed with phosphate-buffered saline (PBS), and cell morphology was evaluated using a phase contrast microscope to capture images (Argenit, Türkiye).

Cell migration

The wound-healing assay was used for evaluating cell migration. MCF-7 cells were seeded in six-well plates (Nest Biotechnology Co., China) and incubated. After the cells became almost completely (90%) confluent, the monolayer was scratched using a sterile 10 µl pipette tip. The wound area was recorded at 0 h and 24 h using a phase contrast microscope. The migration area was calculated as:

$$\text{Migration area (\%)} = [(M_0 - M_{24}) / M_0] \times 100 \quad (1)$$

where M_0 is the wound area at 0 h, and M_{24} is the wound area at 24 h.

Gene expression analysis

Analyzing the miR-145-5p expression level

MCF-7 cells were cultured and treated with curcumin and its solvent control. Cells were also transfected with the miR-145-5p mimic and NT miRNA mimic. 24 h later, the cells were harvested and resuspended in 1 mL TRIzol Reagent (Invitrogen, USA) for total RNA isolation. The concentration and purity of the isolated RNAs were evaluated utilizing a NanoDrop ND-2000c spectrophotometer (Thermo Fisher Scientific Inc.). Complementary DNA (cDNA) synthesis for miRNAs was performed to validate the transfection and to evaluate the relative expression levels of miR-145-5p using miRNA reverse transcription primers (Thermo Fisher Scientific Inc.) and Taqman miRNA Reverse Transcriptase Kit (Applied Biosystems, Thermo Fisher Scientific Baltics UAB, Lithuania).

The LightCycler® 480 instrument (Roche, Germany) was used for transfection validation and for evaluating the alterations in miR-145-5p expression. All reactions were conducted in duplicate using the TaqMan miRNA probes (Thermo Fisher Scientific Inc.) and TaqMan Universal Master Mix Kit (Applied Biosystems, Thermo Fisher Scientific Baltics UAB, Lithuania). RNU43 expression was used to normalize miRNA expression. The quantitative real-time polymerase chain reaction (qRT-PCR) protocol was conducted as presented in Table 1.

Analyzing the expression levels of the identified genes

The cDNA synthesis was conducted utilizing the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Baltics UAB, Lithuania). The LightCycler® 480 instrument (Roche, Germany) was used to determine the expression levels of the identified genes. All reactions were conducted in duplicate using the 5X HOT FIREPol EvaGreen qPCR Supermix (Solis Biodyne, Estonia). The β-actin (*ACTB*) gene was used for expression normalization. The sequences of the primers are presented in Table 2 (22-27). The qRT-PCR protocol was conducted as presented in Table 3.

Table 1: The qRT-PCR cycles protocol for TaqMan Universal Master Mix regarding miRNA detection

Cycle step		Temperature	Time	Cycles
Enzyme Activation		95°C	15 min	1
Quantification	Denaturation	95°C	15 s	40
	Annealing	60°C	60 s	
	Elongation	60°C	1 s	
Cooling		40°C	30 s	1

min: Minutes, s: Seconds

Table 2: Forward and reverse primer sequences of the studied genes

Gene	Forward Primer	Reverse Primer	Ref.
MCM2	5′-CTACCAGCGTATCCGAATCCA-3′	5′-CCTACAGCAACCTTGTTGTCCT-3′	(22)
MMP1	5′-ATTTGCCGACAGAGATGAAGTCC-3′	5′-GGGTATCCGTGTAGCACATTCTG-3′	(23)
MMP9	5′-TCCCTGGAGACCTGAGAACC-3′	5′-CGGCAAGTCTTCCGAGTAGTT-3′	(24)
EEF1A2	5′-GGACCATTGAGAAGTTCGAGA-3′	5′-AGCACCCAGGCATACTTGAA-3′	(25)
FN1	5′-GTGTGACCCTCATGAGGCAAC-3′	5′-CTGGCCTCCAAAGCATGTG-3′	(26)
ACTB	5′-GCCTCGCCTTTGCCGATC-3′	5′-CCCACGATGGAGGGGAAG-3′	(27)

Ref.: Reference, *MCM2*: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1, *ACTB*: actin beta

Table 3: The qRT-PCR cycles protocol for Hot FirePol EvaGreen qPCR Super mix regarding gene expression detection

Cycle step		Temperature	Time	Cycles
Enzyme activation		95°C	12 min	1
Quantification	Denaturation	95°C	15 s	40
	Annealing	60°C	25 s	
	Elongation	72°C	25 s	
Melting curve		95°C	5 s	1
		62°C	60 s	
Cooling		40°C	20 s	1

min: Minutes, s: Seconds

Bioinformatic analyses

Predicting the target genes of miR-145-5p

Different *in silico* analyses were conducted to find the possible target genes of miR-145-5p. Overexpressed genes that met the logFC>+2 and p<0.001 criteria were determined in the Cancer Genome Atlas (TCGA) BC datasets (1,135 tumor tissue samples against 114 normal tissue samples). The expression analyses were performed using GEPIA2 (28, 29), an interactive web tool for gene expression evaluation based on thousands of cancers and normal specimens from the TCGA and GTEx databases that are processed through a standard RNA sequencing workflow. The miRNet 2.0 and miRTarBase v8 databases were also used to identify the target genes of miR-145-5p (30, 31). Subsequently, the genes that overlapped between those overexpressed in TCGA BC and the miR-145-5p probable candidate targets in the miRNet and miRTarBase databases were identified.

The identified genes were searched in PubMed using words such as "gene name", "breast cancer", "BC", "overexpression", "downregulation", "oncogene", and "tumor suppressor". Other databases were used to search for these genes, including Ensembl and Genecards (32, 33). Additionally, enrichment analyses for the identified genes were performed using the Metascape and the DisGeNET databases (34, 35). Moreover, the OS rates for BC associated with the selected genes were investigated utilizing the Kaplan-Meier plotter tool (36). While gene co-expression analysis involves a number of complex processes, it is an effective approach for identifying gene partners and predicting gene function (37). The current study used the Correlation AnalyzeR tool (Bishop Lab, UT Health San Antonio), with Pearson correlation being preferred in the analysis, for performing a genome-wide co-expression correlation analysis in order to determine the genes that correlate with those studied in BC (38).

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Student's t-test was used to compare the means of the two groups. The $2^{-\Delta\Delta Ct}$ method was performed for the relative quantitation analysis. All study results were presented as mean ± SD, with p<0.05 being considered statistically significant. The figures were drawn using GraphPad Prism 5.

RESULTS

Curcumin treatment and overexpression of miR-145-5p decrease cell proliferation

Microscopic images of MCF-7 cells at 24 h after curcumin treatment and miR-145-5p mimic transfection were cap-

tured and compared to their controls (Figure 1a). Both cell viability and morphology results indicate the curcumin treatment to have statistically significantly reduced the proliferation of MCF-7 cells compared to the control group at 24 h ($p<0.01$). Additionally, the miR-145-5p mimic transfection had statistically significantly decreased the proliferation of MCF-7 cells compared to the NT miRNA mimic transfected group at 24 h ($p<0.001$; Figure 1b).

Curcumin treatment and overexpression of miR-145-5p reduce cell migration

According to the wound healing assay results, curcumin treatment statistically significantly decreased MCF-7 cell migration compared to the control cells at 24 h (Figure 1a). Moreover, miR-145-5p mimic transfection significantly reduced the migration compared to NT mimic miRNA-transfected cells at 24 h (Figure 2b).

Curcumin increases the expression level of miR-145-5p

The MCF-7 cells transfected with mimic miR-145-5p expressed significantly higher levels of miR-145-5p than the NT mimic miRNA control ($p<0.001$). This finding indicates the miR-145-5p mimic transfection into cells to have been successfully achieved. The MCF-7 cells treated with curcumin had higher levels of miR-145-5p expression than the control group ($p<0.01$; Figure 3a).

Bioinformatic analyses results

miR-145-5p candidate genes

In total, 261 overexpressed genes with logFC values greater than +2 and $p<0.001$ were chosen from the TCGA BC dataset. The miRNet and miRTarbase v8 databases were used to identify 881 potential target genes of miR-145-5p (Figure 1). The minichromosome maintenance com-

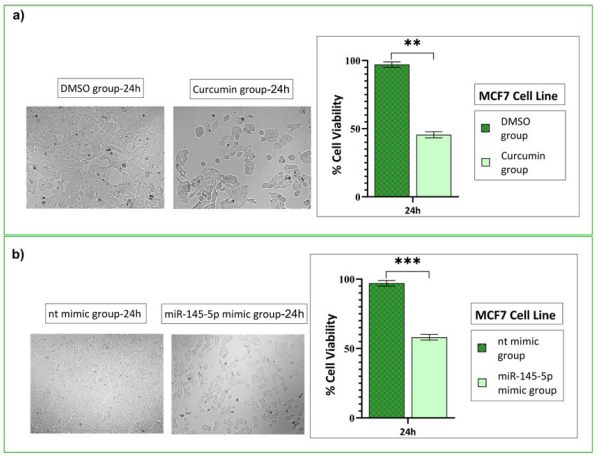

Figure 1: The effects of curcumin and miR-145-5p on MCF-7 cell proliferation. a) microscopic images for MCF-7 cells 24 h after curcumin treatment and miR-145-5p mimic transfection compared to their controls. b) Cell proliferation (%cell viability) of cells was evaluated by the CVDK8 assay 24 h after curcumin treatment and mimic miR-145-5p transfection compared to their controls (NT mimic = non-targeting mimic).
**p<0.01, ***p<0.001

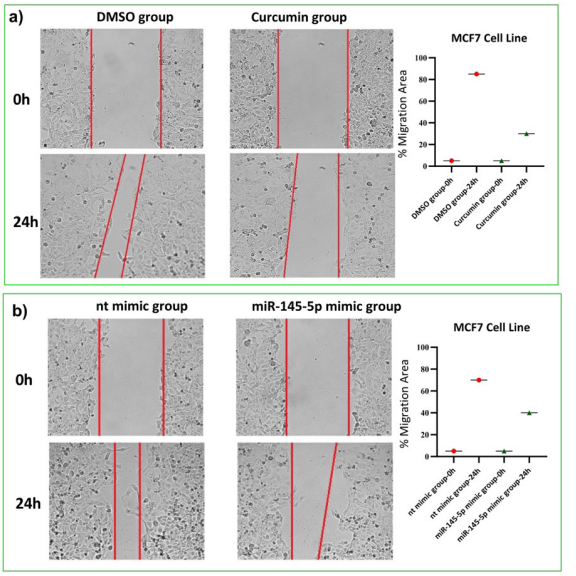

Figure 2: Scratch wound assay for evaluating the cell migration of MCF-7 cells. a) The effects on cell migration at 24 h post-curcumin treatment. b) The effects on cell migration at 24 h after miR-145-5p mimic transfection.

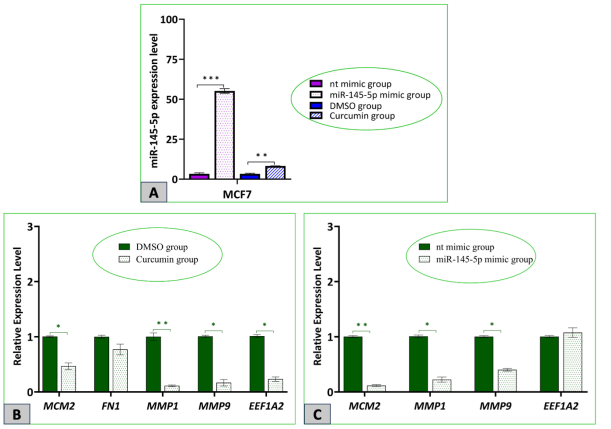

Figure 3: a) Expression level of miR-145-5p in MCF-7 cells after 24 h of curcumin treatment and miR-145-5p mimic transfection compared to their controls. b) Expression levels of the studied genes in MCF-7 cells after 24 h of curcumin treatment compared to the solvent control (DMSO) group. c) Expression levels of the studied genes in MCF-7 cells after 24 h of miR-145-5p mimic transfection compared to the NT mimic miRNA control group.
*p<0.05, **p<0.01, ***p<0.001, *MCM2*: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1

plex component 2 (*MCM2*), matrix metalloproteinase 1 (*MMP1*), matrix metalloproteinase 9 (*MMP9*), eukaryotic translation elongation factor 1 alpha 2 (*EEF1A2*), and fibronectin 1 (*FN1*) genes were found to overlap among the TCGA BC data and probable targets of miR-145-5p in the miRNet and miRTarbase v8 databases (Figure 4).

Significance of the selected genes

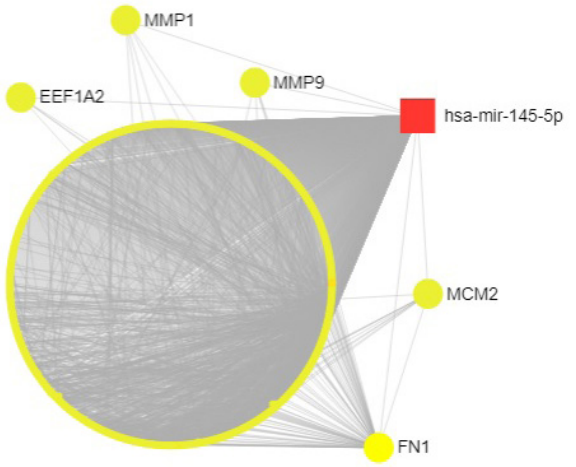

Figure 4: The miR-145-5p *in silico* target genes. Yellow nodes involve 881 genes, including *MCM2*, *FN1*, *EEF1A2*, *MMP1* and *MMP9*. The red square is miR-145-5p. Interaction edges number 2,723 (Created using miRNet).

Because all five genes are oncogenes and associated with BC, they are considered good candidates for the *in vitro* investigations, as shown in Figure 5a. In the enrichment analysis, the identified possible target genes of miR-145-5p were associated with many malignancies, with BC being one of these (Figure 5b). Upregulation of *MCM2*, *MMP1*, and *MMP9* reduced OS for BC. In contrast, expression changes in the *FN1* and *EEF1A2* genes had no influence on the OS (Figure 6). In the correlation analysis, the top genes likely to be correlated with the studied genes (i.e., *MCM2*, *MMP1*, and *MMP9*) were selected and are presented in Table 4. These genes were shown to be associated with BC through both *in silico* and *in vitro* methods.

Curcumin modulates the expression of MCM2, MMP1 and MMP9 genes by regulating miR-145-5p

Relative quantification analysis of the *MCM2*, *MMP1*, *MMP9*, *EEF1A2*, and *FN1* genes was performed using the primers described in Table 2. The *MCM2*, *MMP1*, *MMP9*, and *EEF1A2* genes were significantly downregulated in curcumin-treated MCF-7 cells compared to the controls ($p<0.05$; Figure 3b). Moreover, the *MCM2*, *MMP1*, and *MMP9* genes were significantly downregulated in the mimic miR-145-5p-transfected cells compared to the NT miRNA mimic control cells ($p<0.05$; Figure 3c). The *MCM2*, *MMP1*, and *MMP9* genes were the commonly less-expressed genes in both the curcumin-treated and miR-145-5p mimic-transfected MCF-7 cells. These findings suggest that curcumin may be a negative regulator

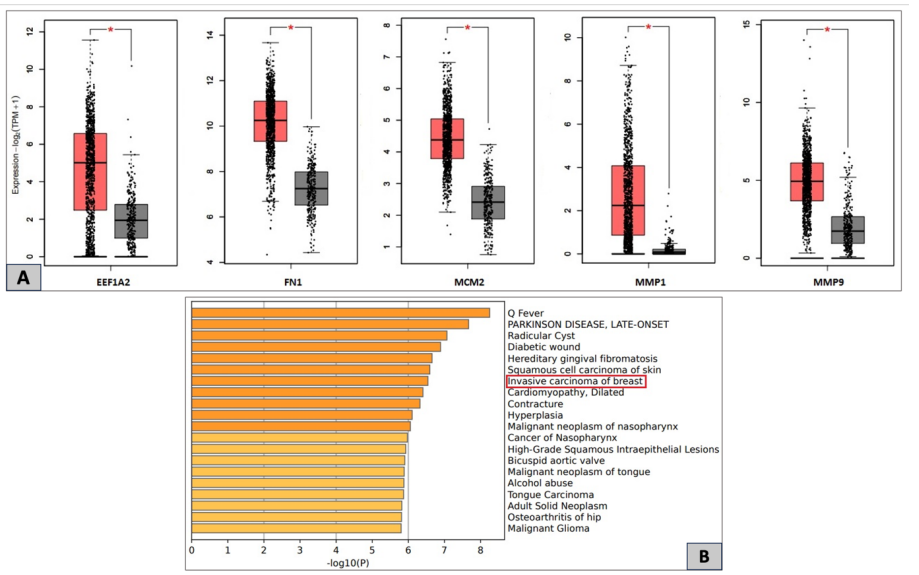

Figure 5: a) GEPIA2 relative expression analysis of the studied miR-145-5p target genes in BC samples. All five genes are overexpressed in the BC tumor tissue samples. GEPIA2 TCGA. (log2FC Cutoff = 1; p_{Cutoff}=0.01; 1085 tumor samples vs. 291 normal samples). b) The associations the five identified miR-145-5p potential targets (i.e., *MCM2, FN1, EEF1A2, MMP1*, and *MMP9*) have with different diseases. Invasive BC is one of the diseases related to these genes, as seen in the red box. According to the figure, these genes are also linked to other cancers such as squamous cell skin carcinoma, nasopharynx cancer, and tongue cancer.
MCM2: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1

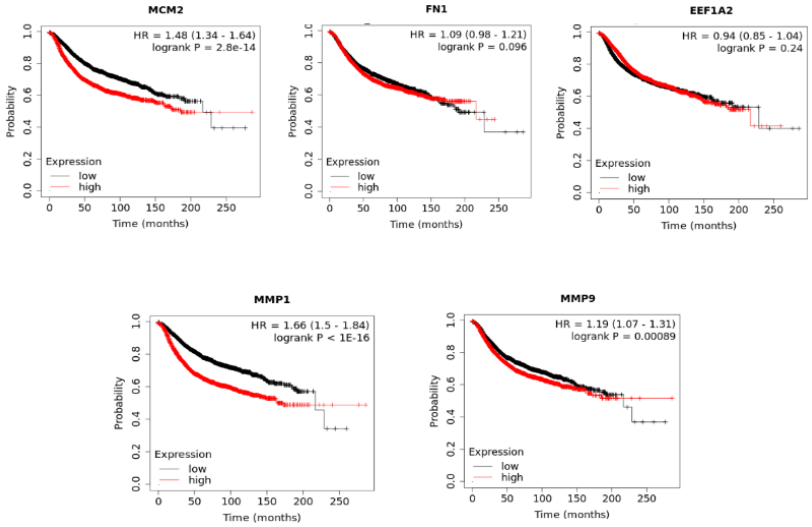

Figure 6: The effect of the selected genes on BC survival using the Kaplan-Meier plotter. Overexpression of *MCM2, MMP1* and *MMP9* decreases OS for BC. However, alterations in the expression of *FN1* and *EEF1A2* genes have no effect on OS for BC.
MCM2: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1

that modulates the expression of *MCM2, MMP1*, and *MMP9* genes by acting on miR-145-5p in MCF-7 cells.

DISCUSSION

Turmeric (Curcuma longa) is a famous Indian spice. Polyphenol curcumin is the primary constituent of turmeric and the one accountable for the majority of its biological effects (9). Curcumin has been utilized for an extensive period to address issues regarding the gastrointestinal tract, liver, respiratory system, and sinusitis; to promote wound healing; and to alleviate pain and burning sensations. Studies have demonstrated the pharmacological properties of curcumin, including its anti-oxidant, anti-inflammatory, cell death-inducing, and anticarcinogenic effects in various diseases (5). The potential of curcumin to alter miRNA expression and its target genes has created new opportunities for cancer-targeted treatment (39). The present study has investigated the effects of curcumin on the proliferation and migration of the MCF-7 human BC cell line and whether its anticancer effects are mediated by acting on miR-145-5p and its possible target genes. The findings have shown curcumin treatment and the overexpression of miR-145-5p to decrease the proliferation of MCF-7 cells as well their migration. Additionally, the relative expression level of the tumor suppressor miRNA miR-145-5p was significantly elevated in the curcumin-treated cells. Curcumin treatment reduced the relative expression of the putative miR-145-5p target genes *MCM2*, *MMP1*, *MMP9*, and *EEF1A2*. Furthermore, the overexpression of miR-145-5p decreased the expression levels of the *MCM2*, *MMP1*, and *MMP9* genes.

Previous studies have demonstrated the expression of miR-145-5p to be lower in BC tissues compared to normal breast tissues, with this being linked to large tumors, metastasis, and high Ki-67 expression levels (14, 15, 17, 40). By modifying the expression of different signaling proteins and molecular pathways, curcumin suppresses the proliferation of BC cells. Consequently, one of the best ways to treat cancer is to target oncogenes and tumor suppressor genes (41). Studies have shown curcumin to be able to successfully inhibit the proliferation of different cancerous cells by acting on miR-145-5p, such as in laryngeal squamous cell carcinoma and in human prostate cancer stem cells (18, 42). When considered together, no study has been conducted evaluating the regulatory effect of curcumin on miR-145-5p in BC. The current results suggest curcumin treatment and miR-145-5p overexpression to both markedly suppress MCF-7 cell proliferation and migration by modulating the expression of the *MCM2*, *MMP1*, and *MMP9* genes.

MCM2 is a promising proliferative marker in numerous different cancer types (e.g., thyroid, rectal, and BC). MCM2 is also a more effective proliferative and prognostic marker in BC than Ki-67 (39). Samad et al.'s study demonstrated *MCM2* gene expression levels to be higher in BC patients, suggesting *MCM2* as a useable biomarker for the prognosis of BC (43).

The genes studied herein have been shown to be correlated with many genes previously linked to BC (Table 4), with the flap structure-specific endonuclease 1 (*FEN1*) being one example of a gene correlated with the *MCM2* gene. *FEN1* overexpression has been shown to promote BC.

Table 4: The top 10 co-expression correlations of *MCM2, MMP1*, and *MMP9* in BC according to the Correlation AnalyzeR results

Studied gene	Correlated genes	Gene name	r	p
MCM2	*MCM5*	Minichromosome Maintenance Complex Component 5	0.861	4.235e-08
	FEN1	Flap Structure-Specific Endonuclease 1	0.859	4.35e-08
	MCM7	Minichromosome Maintenance Complex Component 7	0.848	5.016e-08
	MCM6	Minichromosome Maintenance Complex Component 6	0.845	5.207e-08
	CDCA5	Cell Division Cycle Associated 5	0.843	5.338e-08
	CDK2	Cyclin Dependent Kinase 2	0.837	5.741e-08
	RACGAP1	Rac Gtpase Activating Protein 1	0.835	5.879e-08
	MYBL2	MYB Proto-Oncogene Like 2	0.833	6.02e-08
	PRC1	Protein Regulator of Cytokinesis 1	0.832	6.091e-08
	MCM3	Minichromosome Maintenance Complex Component 3	0.829	6.307e-08
MMP1	*PRDM8*	PR/SET Domain 8	0.756	1.334e-07
	LAYN	Layilin	0.741	1.529e-07
	G0S2	G0/G1 Switch 2	0.739	1.557e-07
	PTX3	Pentraxin 3	0.734	1.627e-07
	COL13A1	Collagen Type XIII Alpha 1 Chain	0.732	1.656e-07
	ROBO4	Roundabout Guidance Receptor 4	0.718	1.872e-07
	PRSS3	Serine Protease 3	0.715	1.921e-07
	ANTXR2	ANTXR Cell Adhesion Molecule 2	0.712	1.971e-07
	HBEGF	Heparin Binding EGF-Like Growth Factor	0.711	1.987e-07
	SERPINE1	Serpin Family E Member 1	0.71	2.004e-07
MMP9	*IL10RA*	Interleukin 10 Receptor Subunit Alpha	0.667	2.863e-07
	C1QA	Complement C1q A Chain	0.664	2.934e-07
	PIK3R5	Phosphoinositide-3-Kinase Regulatory Subunit 5	0.649	3.309e-07
	CD86	CD86 Molecule	0.633	3.758e-07
	SPI1	Spi-1 Proto-Oncogene	0.625	4.003e-07
	CD2	CD2 Molecule	0.621	4.131e-07
	C1QB	Complement C1q B Chain	0.619	4.196e-07
	C1QC	Complement C1q C Chain	0.618	4.229e-07
	IGHG1	Immunoglobulin Heavy Constant Gamma 1	0.611	4.468e-07
	IGHG1	Immunoglobulin Heavy Constant Gamma 1	0.611	4.468e-07

r: Pearson correlation coefficient. The p values are presented by the scientific mathematical method. All p values indicate p<0.001.

Zou et al. showed a negative association between *FEN1* expression and cisplatin sensitivity in BC cells. Moreover, curcumin treatment (dose-dependent) downregulated the expression of *FEN1*. In addition, they showed an *in vitro* and *in vivo* combination of curcumin with cisplatin to increase the sensitivity to cisplatin, which was found to occur through the downregulation of *FEN1* in BC. Interestingly, their study concluded that curcumin sensitizes cells to cisplatin via the downregulation of *FEN1* (44). As a result, this opens up a new approach to treatment by increasing cancer cells' sensitivity to chemotherapy treatments.

MMP1 is associated with many physiological processes via the modification of the tumor microenvironment and the extracellular matrix (45). *MMP1* is upregulated in BC tissues and has been shown to be associated with invasion and metastasis (46). The current study has demonstrated curcumin's ability to alter the expression of oncogenic genes such as MMP1 by down-regulating their expression.

MMP9 is also a member of the MMP family that promotes the invasion and migration of BC cells. *MMP9* gene was found to be linked to the signal transducer and activator of transcription 3 (*STAT3*) gene activation, which is involved in cellular proliferation, apoptosis, tumorigenesis, and angiogenesis. Furthermore, the knockdown of *STAT3* suppressed *MMP9* expression and metastasis in BC cells (47). These findings suggest the inhibition of *MMP1* and *MMP9* expression by curcumin and miRNA miR-145-5p to be possible novel strategies for controlling BC.

The present study has investigated the prognostic significance of the five hub genes with regard to the OS of BC patients and revealed the overexpression of the *MCM2*, *MMP1*, and *MMP9* genes to decrease the OS of BC patients. However, no influence was found regarding alterations in the expression of the *FN1* and *EEF1A2* genes (Figure 6). A comprehensive bioinformatics analysis study assessing the significant value of MCMs in BC demonstrated the *MCM2-7* genes to be significantly overexpressed in BC, particularly in tumor subtypes that proliferate and spread quickly. This predicted a worse prognosis with shorter OS and relapse-free survival (RFS) for BC patients, providing insight into the prognostic value of MCMs regarding the various molecular subtypes of BC (48). Consequently, the current and other findings have suggested *MCM2* to be a possible good prognostic biomarker for BC and to perhaps be a potential therapeutic target. One study showed the Y-box binding protein-1 (*YB-1*) to mediate the invasion and metastasis of BC by acting on *MMP1* and beta-catenin (*CTNNB1*). That study's analysis of breast tumor samples from the Gene Expression-Based Outcome for Breast Cancer Online (GOBO) database showed a markedly reduced 10-year distant metastasis free survival to be predicted with the overexpression of the *YB-1*, *MMP1*, and *CTNNB1* genes (49). *MMP1* may be a good marker of the prognosis for BC metastases (49). Moreover, the elevated expression level of the *MMP9* gene in both blood and tumor tissue samples of 108 BC patients has indicated worse prognoses (50). Accordingly, MMPs are being extensively investigated as potential therapeutic targets for cancer progression.

This study has combined a bioinformatic analysis with *in vitro* experiments, including cell proliferation assays, scratch wound assays, and expression studies, to investigate the effect of curcumin in combating cancer through miR-145-5p and its target genes. One limitation of the study is that it only examined the expression of the selected genes at the mRNA level. It did not evaluate changes in protein levels to verify the relationship between miR-145-5p and its target genes. Additional research is required to analyze the collective influence of miR-145-5p and curcumin on cell proliferation and migration, as well as their effects on the expression of target genes.

CONCLUSION

Curcumin exerts a strong anticancer impact on BC MCF-7 cells by suppressing cellular growth. This effect is likely mediated by the upregulation of miR-145-5p and the downregulation of the *MCM2*, *MMP1* and *MMP9* genes. The upregulation of *MCM2*, *MMP1* and *MMP9* genes is negatively associated with the OS in BC. Obviously, different studies should validate the findings of the *in silico* and *in vitro* studies at the protein levels. These will be valuable for evaluating the potential therapeutic role and clinical utility of curcumin and the miR-145-5p mimic prior to clinical trials and therapeutic usage when considering a tumor's pathological results. Additionally, due to the study's bioinformatic analysis providing information about the potential the target genes of miR-145-5p have regarding OS, further studies are recommended on patient-derived BC samples based on their clinical, biological and pathological characteristics for a better understanding of the impact these genes have on OS.

Ethics Committee Approval: No ethics committee approval was need for this study, since the study was conducted on human breast cancer cell line and public available datasets.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.A., M.K.; Data Acquisition- A.A., M.K., İ.S., S.E.; Data Analysis/Interpretation- A.A., M.K., İ.S., S.E., Ş.Ö.; Drafting Manuscript- A.A., M.K., İ.S., S.E., F.A.; Critical Revision of Manuscript- A.A., M.K., Ş.P., K.Ç., Ş.Ö.; Final Approval and Accountability- A.A., M.K., İ.S., S.E., F.A., Ş.P., K.Ç., Ş.Ö.

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

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INFECTION EVENTS IN MULTIPLE MYELOMA DURING THE EARLY PERIOD OF AUTOLOGOUS STEM CELL TRANSPLANTATION

MULTİPL MİYELOMDA OTOLOG KÖK HÜCRE TRANSPLANTASYONUNUN ERKEN DÖNEMİNDE ENFEKSİYON OLAYLARI

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ABSTRACT

Objective: Multiple myeloma (MM) patients have a high risk of developing infections. In this study, we documented the infection events in patients with MM who underwent autologous haematopoietic stem cell transplantation (AHSCT).

Material and Method: Patients who received an induction regimen and underwent AHSCT were enrolled in the study. Routine antimicrobial prophylaxis was not given. Infection treatment was performed in accordance with the febrile neutropenia guidelines.

Result: Between May 2007 and November 2016, 150 patients with MM underwent AHSCT. The median age was 51.7±7.2 years, and the male-to-female ratio was 84/66. Nearly all patients developed fever. The median time from the HSCT day to the first fever episode was 7.4±2.8 days. Pneumonia and oropharyngeal candidiasis were frequently associated with fever. Blood and urine culture positivity was 18.6% and 20%, respectively. The neutropenia duration was not associated with culture positivity but proved to be longer in patients who had received two types of induction regimen (8.4±3.7 vs. 7.4±2.3 days, p=0.056). The mortality rate in the first 100 days was 0.6%, which was similar to the results of other experienced centers.

Conclusion: Our study encompassed the period when the induction regimen included combinations of chemotherapy, and novel agents were used after chemotherapy refractoriness or for improvement in response quality. In relation to this, most

ÖZET

Amaç: Multipl miyelom (MM) hastalarında enfeksiyon gelişme riski yüksektir. Bu çalışmada, otolog hematopoetik kök hücre nakli (OHKHN) yapılan MM hastalarında enfeksiyon olayları derlenmiştir.

Gereç ve Yöntem: İndüksiyon rejimi ardından OHKHN uygulanan MM hastaları çalışmaya dahil edilmiştir. Rutin antimikrobiyal profilaksi uygulanmamıştır. Enfeksiyon tedavileri febril nötropeni kılavuzlarına paralel yapılmıştır.

Bulgular: Mayıs 2007 ve Kasım 2016 tarihleri arasında toplam 150 MM hastasına OHKHN uygulandı. Ortanca yaş 51,7±7,2 yıl ve erkek/kadın oranı 84/66 idi. Hastaların neredeyse tamamında ateş gelişti. HKHN gününden ilk ateş atağına kadar geçen süre ortanca 7,4±2,8 gündü. Ateşle en sık ilişkili enfeksiyonlar pnömoni ve orofarengeal kandidiyazis idi. Kan ve idrar kültürü pozitifliği sırasıyla %18,6 ve %20 olarak bulundu. Nötropeni süresi kültür pozitifliği ile ilişkili değildi, ancak iki tip indüksiyon rejimi alan hastalarda nötropeninin daha uzun sürdüğü görüldü (8,4±3,7 vs, 7,4±2,3 gün, p=0,056). İlk 100 günde mortalite oranı %0,6 olup diğer deneyimli merkezlerin sonuçlarına benzerdi.

Sonuç: Çalışmamız, indüksiyon rejiminin kemoterapi kombinasyonları ile yapıldığı ve yeni ajanların kemoterapi refrakterliğinden sonra veya yanıt kalitesinde iyileşme için kullanıldığı dönemi kapsamaktadır. Bununla bağlantılı olarak, hastaların çoğu ikinci

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patients received second-line induction therapy. The infection events were manageable, and patients showed acceptable outcomes and a very low early mortality rate.

Keywords: Multiple myeloma, autologous haematopoietic stem cell transplantation, infection, febrile neutropenia

basamak indüksiyon almıştır. Enfeksiyon olayları yönetilebilir nitelikteydi; hastalar kabul edilebilir sonuçlar ve çok düşük erken ölüm oranı gösterdi.

Anahtar Kelimeler: Multipl miyelom, otolog hematopoietik kök hücre nakli, enfeksiyon, febril nötropeni

INTRODUCTION

Multiple myeloma (MM) patients are at increased risk of infection. MM itself, its disease pathophysiology, and treatment contribute to significant cellular and humoral immunity dysfunction. Along with the patient's comorbidities including physical and social compromise, infection in MM was associated with an individual variable rate (1-7).

In the last two decades, MM treatment has evolved from chemotherapy to pathology-targeted therapies. First, drugs that intervene in the microenvironment interaction, increase cellular immunity, and contributing to tumour cell apoptosis are released in the medical area. Thalidomide and its analogues with proteasome inhibitors were the first novel players in MM treatment. The treatment evolution schedules the MM treatment in fragments as induction, consolidation, and maintenance. All these innovations contributed to significantly improved survival, but relapse was inevitable. The second wave of MM treatment came with immunotherapy, first with monoclonal antibody followed by bispecific antibodies, and last with chimeric antigen receptor (CAR)-T cell application, which took MM treatment to the top (8, 9). MM's treatment story created an area where to mention minimal residual disease negative life and cure. Unfortunately, access to this treatment evolution in some low-income countries was a little behind.

We aimed to document the infection rates in patients with MM by reflecting on the MM treatment story in our country, which may assume a role as historical data. Across the AHSCT, the induction, mobilisation regimens, and conditioning were assessed. The duration of neutropenia and the relationship between neutropenia duration and other parameters were evaluated.

MATERIALS and METHODS

We retrospectively assessed 150 patients diagnosed with MM who underwent AHSCT. An institutional electronic database and the European Society of Blood and Marrow Transplantation (EBMT) data collection forms were used for data collection. All of the patients' treatment costs were covered by social insurance, and the treatment protocol was based on the health authority indications and national reimbursement criteria. Transplant eligibility was assessed by the HSCT team of the institution following the EBMT guidelines (10).

The patient's induction regimen is based on drugs licenced by the Ministry of Health and is covered by the Social Security and General Health Insurance. Induction regimens mainly comprised combined chemotherapy (VAD regimen, vincristine (V), doxorubicin (A), and high-dose dexamethasone (D)). Proteasome inhibitors with or without an immunomodulatory drug (IMID) were added to the induction therapy when the disease response was insufficient before AHSCT. Treatment was continued until the completion of AHSCT.

The mobilisation regimen was based on the patient's MM response depth, either with chemotherapy-based regimens, such as cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF), cyclophosphamide+etoposide plus G-CSF, or G-CSF alone. The target CD34-positive cell number was $\geq 2x10^6$/kg.

The conditioning regimen was melphalan administered at a single dose of 200 mg/m^2 (140 mg/m^2 in patients with kidney failure). Reverse isolation rules were applied. Antimicrobial prophylaxis was not routine and was used only as secondary therapy according to the patient's medical history. All patients with HBsAg and/or anti-HBc IgG positivity received antiviral prophylaxis.

Routine antimicrobial prophylaxis was not given. For patients with a history of previous invasive aspergillosis, expected prolonged neutropenia of >2 weeks, or prolonged neutropenia before transplantation, antifungal prophylaxis was applied.

CMV monitoring was not routinely performed. In cases with clinical suspicions such as unexplained and/or unresponsive fever, CMV assessment was performed using CMV PCR. CMV reactivation was defined as the detection of plasma CMV DNA ≥ 500 IU/mL in plasma.

The post-transplant management protocol was based on supportive therapy, mainly febrile neutropenia treatment and transfusion. All patients received G-CSF to accelerate haematopoietic recovery from the 5th HSCT day until engraftment.

Neutropenic fever was defined as a temperature of ≥38.3°C orally or ≥38.0°C over 1 h with an absolute neutrophil count (ANC) of ≤500/ µL, or ≤1000/ µL and expected to fall ≤500/ µL over the next 48 h. This management

was in accordance with the recommendations of the National Comprehensive Cancer Network (NCCN) guideline 2023 (11).

Microbiological evaluation of neutropenic fever included obtaining at least two sets of blood cultures from the catheter lumen and peripheral vein, urine cultures, and other secretions or body fluids in case of clinical suspicion. High-resolution chest computed tomography was performed in patients with fever persisting for >72 hours according to the European Society for Medical Oncology (ESMO) Practise guidelines for the management of febrile neutropenia (12). Thorax CT was skipped when the patient's clinical performance was not suitable for imaging.

All patients with neutropenic fever received empirical intravenous broad-spectrum antipseudomonal antibiotics; in case of pneumonia, diarrhoea, and/or central venous catheter infection, vancomycin was added to the treatment. When an infection agent was documented in culture, the antimicrobials were changed according to the clinical course and antibiotic susceptibility test results. All patients were re-evaluated 48–72 hours after the initiation of antibiotics. In patients with refractory fever, the antipseudomonal antibiotic was changed to carbapenem, and/or vancomycin was added, if not started at the beginning of the fever. Although no clear distinction has yet been made, they were categorised to classify information on the causative agents and resistance patterns produced and to guide antibiotic use strategies at intermediate stages. According to this categorisation, multidrug-resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), and pandrug resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories (13). At the 96th hour after refractory fever, invasive fungal infection (IFI) was evaluated using blood galactomannan analysis and thorax computed tomography (CT). In general, empiric antifungals with caspofungin or liposomal amphotericin B were added to the antibacterial treatment. In cases of proven or probable aspergillosis, voriconazole was added to anti-microbial therapy according to the clinical protocol. Ganciclovir was used to treat CMV-DNA viremia.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were defined as numbers and percentages for categorical variables and mean, standard deviation, minimum, and maximum values for numeric variables. Student's t-test was used to compare normally distributed parameters, and the Mann–Whitney U test was used for other tests. The qualitative variables were compared using Pearson's Chi-square test. The statistical significance level was defined as $p<0.05$.

The study was approved by İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 21/07/2023, No: 15).

RESULTS

The study included patients with MM who underwent AHSCT between May 2007 and November 2016.

The median age of the patients was 51.7±7.2 years (range between 30 and 69 years). The male-to-female ratio was 84 (56%) to 66 (44%). In addition to MM, hypertension (22.6%), diabetes mellitus (15.3%), chronic hepatitis B (6%), coronary artery disease (3.3%), tuberculosis history (1.3%), and chronic obstructive pulmonary disease (0.7%) were noted in the medical records.

VAD was the most frequently used induction regimen related to the social insurance system financial support limitation for other regimens. Table 1 lists the induction regimens used. The total number of induction courses was 8.2±3.4 (between 2-23). The time interval to AHSCT proved to be 179.7±158.7 days from diagnosis.

Table 1: Induction regimen types as first- and second-line regimens

Chemotherapy	n	%
First-line		
VAD	109	73.2
High-dose dexamethasone	15	10.1
VD	16	10.7
CyBORD	5	3.3
TD	4	2.7
Second-line		
VD	68	45.6
CyBORD	25	16.8
Cyclophosphamide, dexamethasone	10	6.7
TD	14	9.4
DCEP	5	3.4
Rd	6	4.0
CEP	1	0.7

VAD: vincristine, adriamycin, dexamethasone, VD: bortezomib, dexamethasone, CyBORD: bortezomib, dexamethasone, cyclophosphamide, TD: thalidomide, dexamethasone, DCEP: dexamethasone, cyclophosphamide, etoposide, cisplatin, Rd: lenalidomide, dexamethasone, CEP: cyclophosphamide, etoposide, cisplatin

G-CSF alone was the most used (59.3%) mobilisation regimen. Chemotherapy-based regimens were equally distributed. G-CSF plus cyclophosphamide was administered to 32 patients (21.3%) and cyclophosphamide plus etoposide to 29 (19.3%). The mean infused CD34-positive cell dose was $6.2x10^6$/kg (range 1.35-$27.2x10^6$). Neutrophil engraftment occurred on 13±3.7 days (between 6-33 days) and platelet engraftment on 14±4.7 days (6-31 days). The duration of neutropenia was longer in patients who received two two-line induction regimens compared with only one line (8.4±3.7 vs. 7.4±2.3 days, p=0.056).

All patients experienced mucositis at different degrees. Diarrhoea developed in 50 (33.3%) patients in the early post-transplant period.

All patients developed fever except 21 (14.0%). The median time from AHSCT day to fever development was 7.4±2.8 days. There was no significant difference between infused CD34-positive cell count and febrile neutropenia (p=0.34) episode. The relationship between neutropenia duration and neutropenic fever was not statistically significant (p=0.74).

The infection source could not be identified in 72 (48%) patients. The most frequently occurring infection was pneumonia (12.0%). Oropharyngeal candidiasis is the second most frequent (10.6%) infection-related clinical diagnosis. Rare infections included skin and soft-tissue infections (5.3%), herpetic stomatitis (4%), and neutropenic enterocolitis (2.6%). One patient (0.66%) with diarrhoea was diagnosed with CMV colitis based on histological examination of tissues biopsied on colonoscopy. Nineteen (12.6%) patients received antifungal therapy due to clinical diagnosis of possible IFI.

Imaging studies were performed on 30 patients. Fourteen (46.6%) patients presented with pulmonary infiltrates with pleural effusion on thorax CT. One patient with severe abdominal pain had intestinal wall thickening on abdominal CT suspecting inflammation and broad-spectrum antimicrobial agents were initiated with a preliminary diagnosis of neutropenic enterocolitis (typhlitis).

A total of 28 patients (21.7%) of 129 who developed neutropenic fever had blood culture positivity (Table 2). When the Gram-negative bacilli isolated from the patients were analysed, ESBL positivity was detected in 75% of the isolates, XDR positivity in 9.5%, and resistance to all tested antimicrobials, PDR, in only one clinical isolate (4.76%). Urine culture positivity was detected in 30 patients (23%) (Table 3). The most commonly isolated microorganisms were coagulase-negative *staphylococci* in blood culture and *Escherichia coli* in urine culture. Among catheter tip cultures, *Staphylococcus* infections were the most frequently encountered agents in 17 patients, followed by methicillin-sensitive *Staphylococcus aureus* positivity in 2 patients, and *Pseudomonas* positivity in 2 patients. *Candida* spp. were isolated from catheter tip cultures of two patients. In two patients with uncontrollable fever, *despite algorithmic management, surveillance culture revealed* vancomycin-resistant *Enterococcus* (VRE), and linezolid was replaced with vancomycin. There was no relationship between neutropenia duration and culture positivity.

Table 2: Results of blood culture during neutropenic fever

Blood culture	n	%
None	122	81.3
MSCoNS	9	6.0
MRCoNS	8	5.3
MSSA	3	2.0
Escherichia coli	4	2.7
Enterococci sp.	1	7.0
Klebsiella pneumoniae	3	2.0

MSCoNS: Methicillin Susceptible Coagulase-negative staphylococci, MRCoNS: Methicillin Resistant Coagulase-negative staphylococci, MSSA: Methicillin-sensitive *Staphylococcus aureus*

Table 3: Results of urine culture during neutropenic fever

Urine culture	n	%
None	120	79.3
Escherichia coli	10	6.7
Enterococcus spp.	6	4.0
Klebsiella pneumoniae	5	3.3
Candida spp.	3	2.0
MSSA	1	0.7
Stenotrophomonas maltophilia	1	0.7
Proteus sp.	1	0.7
Pseudomonas sp.	1	0.7
Acinetobacter sp.	1	0.7
Morganella sp.	1	0.7

MSSA: Methicillin susceptible *Staphylococcus aureus.*

Within 100 days of AHSCT, only one patient (0.6%) required intensive care unit care and died of sepsis.

There was no statistically significant difference between the number of pre-transplant treatment courses and the timing of AHSCT with antimicrobial lines (p=0.34 and p=0.44, respectively).

DISCUSSION

MM, with its disease evolution and pathophysiology, forms a major research area that contributes to the development of new drugs. Every progress in treatment is associated with some adverse effects. Infection is the main adverse effect of MM treatment, but the disease itself causes an immunosuppressive environment.

Our study included a cross-section of MM treatment history from combination chemotherapy to novel drugs. We documented infection events during the early period of AHSCT in patients with MM, which encompassed 4 weeks after transplantation.

We found that all patients developed fever, except 21 (14.0%). The infection source could not be identified in 72 (48%) patients. A study from Poland reported infectious complications after AHSCT, including lymphoma, acute myeloid leukaemia, and MM patients. The infectious complication rate was 92.3% during neutropenia after AHSCT. The clinically documented infection ratio was 9.3%, and fever of unknown origin was 51.7% (14). Another study from Türkiye reported that febrile neutropenia developed in 92% of autologous stem cell transplant recipients (15). This result is also consistent with the review written by Nesher L and Rolston K in Principles and Practise of Transplant Infectious Disease (16). They reported that approximately 40-50% of febrile neutropenic patients have neither clinical evidence of infection nor positive microbiological documentation of infection, which are episodes of unexplained fever.

In our study, only one patient (0.6%) required intensive care unit care and died of sepsis within 100 days of AHSCT. A study from Taiwan reported that the early mortality rate defined as death within 60 days after diagnosis was 12.6%. They reported that infection was the cause in nearly two-thirds of those early deaths (17). Another study from the USA revealed an overall early mortality rate, defined as death within 6 months after MM diagnosis, of 8.3%. Advanced-stage disease, poor ECOG performance, and older patients (aged ≥ 70 years) were found to be predictors of early mortality (18). The low death rate in our study may be related to the restricted definition of transplant candidates.

We experienced that nearly half of the febrile neutropenic patients had no clinical or laboratory evidence of infection. Nearly half of the febrile patients (48%) did not have any clinical symptoms or signs leading to an obvious infection diagnosis. Pneumonia occurred in 12% of patients. The most frequently isolated pathogen was Gram-positive cocci. Urinary tract infection was also not at a low rate. The rate of urinary system infection by *E. coli* was 20%. CMV infection and IFI were not major complications in our cohort. Consistent with our study, a study from Pakistan documented the incidence and main characteristics of infections in patients with MM treated at their centers over 10 years. They found that the lung was the most common site of infection, followed by the genitourinary system, and *E. coli* was the most common organism (19). They also reported that infection was the main cause of death at a rate of 6.3%. The same experience was reported in Germany, which conducted a large retrospective analysis of 479 patients with MM. The study showed that the rate of infections was stable over time and was mainly associated with high disease burden, relapsed disease, and treatment with high-dose chemotherapy (20). A meta-analysis conducted in China showed that patients with MM treated with IMIDs are at high risk of serious infection (21). A systematic review encompassing the frontline, maintenance, and relapsed/refractory settings of MM treatment within randomised clinical trials reported that the significant risk factors were severe infection, pneumonia, and neutropenia (22).

A retrospective data evaluation from China revealed that newly diagnosed patients with MM were highly susceptible to viruses, including mainly Epstein–Barr virus (EBV) and hepatitis B virus (HBV) (23). Our institution's antiviral prophylaxis protocol against HBV was routine according to the criteria defined in the method part of the study. In our study, only one CMV organ disease had intestinal involvement. Therefore, CMV infection was not a significant problem with historical induction regimens. In the study conducted in Italy, covering 327 AHSCT (n=201 MM, n=126 lymphoma), 11% required specific antiviral treatment for symptomatic CMV reactivation (n=32) or end-organ disease (n=4), which increased transplant-related mortality (24).

For antibacterial prophylaxis, levofloxacin is recommended during the first 3 months, particularly in patients at intermediate and high risk for early infection (25). Our institutional protocol excluded antibacterial prophylaxis.

A multicenter study in Melbourne focusing on IFI in MM patients. Based on the clinical and microbiology records review, the IFI rate was low in patients with MM treated with novel drugs, including monoclonal antibodies (26). Similarly, IFI was not a major complication in our cohort.

Our study is retrospective. The emphasis is that in our study population, the total number of treatment courses given for remission induction was high, and the time from diagnosis to HSCT was long. The explanation for this is institution-related. The institution transplant unit's working intensity resulted in an obligatory waiting list. On the other hand, the infection events were manageable, and patients showed acceptable outcomes and a very low early mortality rate. Radiological imaging was performed on a few patients who developed neutropenic fever. These are the limitations of our study. On the other

hand, our results provide historical data and reflect infection events in Türkiye when immunotherapy, either daratumumab, elotuzumab, or isatuximab, has not yet been reimbursed, and combined chemotherapy was allowed as frontline therapy.

Data availability statement: Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request and all authors have access the all data.

Ethics Committee Approval: The study was approved by İstanbul üniversity, İstanbul Faculty of Medicine Clinical Research local Ethics Committee (Date: 21/07/2023, No: 15).

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

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REVIEW / DERLEME
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LYMPHEDEMA AND PERIPHERAL LYMPHOSCINTIGRAPHY

LENFÖDEM VE PERİFERİK LENFOSİNTİGRAFİ

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ABSTRACT

Peripheral lymphedema occurs because of mechanical or functional obstruction of the lymphatic system. Accurate diagnosis is important for the appropriate management of patients with lymphedema. Lymphoscintigraphy is a useful technique among the first-choice methods to detect lymphedema. Factors affecting the outcome of the test and different test protocols can make it difficult to interpret. The aim of this review is to provide a literature-based overview of the aetiology and diagnostic methods of extremity lymphedema and to summarise the current knowledge on lymphoscintigraphy protocols and interpretation.

Keywords: Peripheral lymphedema, lymphoscintigraphy, scintigraphy protocols

ÖZET

Periferik ekstremite lenfödemi lenfatik sistemin mekanik veya fonksiyonel obstrüksiyonu sonucunda oluşur. Lenfödemli hastaların uygun şekilde yönetilebilmesi için doğru tanı konulması önemlidir. Lenödemin saptanması için ilk tercih edilecek yöntemler arasında lenfosintigrafi önemli bir yer tutar. Testin sonucunu etkileyen faktörler, farklı test protokollerinin yorumlanmasını zorlaştırabilir. Bu derlemenin amacı, alt ekstremite lenfödeminin etiyolojisi ve tanı yöntemleri hakkında literatüre dayalı bir bakış sağlamak ve lenfosintigrafi protokolleri ve yorumlaması hakkındaki güncel bilgileri özetlemektir.

Anahtar Kelimeler: Periferik lenfödem, lenfosintigrafi, sintigrafi protokolleri

INTRODUCTION

Powerful diagnostic methods are needed for the definitive diagnosis and appropriate treatment of peripheral lymphedema, which is manifested by the deterioration of lymphatic drainage in the extremities due to various aetiologies. Peripheral lymphoscintigraphy, which has been used for many years in imaging lymphatic ducts and nodes, is a useful method for both differential diagnosis and clarification of the aetiology of lymphedema. In this review, a brief history of lymphoscintigraphy, anatomy and physiology of peripheral lymphatic ducts, aetiology of lymphedema, and other imaging methods will be discussed. Radiopharmaceuticals used, application techniques, interpretation, advantages and disadvantages, and clinical indications of lymphoscintigraphy will also be mentioned.

History

Walker was the first to report the activity observed in regional lymph nodes after radioactive Gold-198 colloid injection (1). In a study conducted by Sherman and Ter-Pogossian in 1953, subcutaneous radioactive colloid gold was injected into the right and left sides of the anterior abdominal wall of rabbits, and it was shown that the colloid quickly drained into the regional lymph nodes (2) . Threefoot and his colleagues used this method to demonstrate lymphaticovenous and lymphaticolymphatic communication in humans in 1963 (3). Radioactive gold-198 colloid was not used in later years because of its high beta radiation, but lymphoscintigraphy has taken its place as a valuable method in the diagnosis of lymphedema with the development of new and various radiopharmaceuticals. Peripheral lymphoscintigraphy is most commonly performed to investigate extremity lymphedema.

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Anatomy and physiology

The lymphatic system comprises two parts in the extremities: superficial and deep. While the superficial (epifascial) system collects the lymphatic flow of the skin and subcutaneous tissues, the deep (subfascial) system drains lymph from muscle, bone tissues, and deep blood vessels. This lymphatic drainage is directed to the pelvic lymph nodes in the lower extremities and the axillary lymph nodes in the upper extremities (4, 5).

In contrast to the closed systemic circulation, the lymphatic system is open. It transports macromolecules and immune and excess interstitial fluids in tissues through lymphatic channels and delivers them to cells and systemic circulation through the subclavian vein. The lymphatic vascular structure initially consists of subunits called lymphangion. These structures form lymphatic microcapillaries, starting with blunt ends under the epidermis in the extremities and in the tunica adventitia of the vessels. By regular contraction, lymph reaches the systemic circulation. Initially, there is no basement membrane in this structure. It comprises lymphatic endothelial cells with spaces between them. These cells have filamentous extensions that attach to the surrounding tissues. Endothelial cells are in relationship with the extracellular matrix and move away from each other and come closer with the oncotic and hydrostatic pressure difference, taking fluid and other molecules into this microcapillary structure and providing drainage. The lymph fluid formed in this manner moves passively. Meanwhile, the lymphatic microcapillaries grow a little more and become a conducting vessel or channel structure with a basement membrane and peripheral smooth muscle. Because of the one-way-opening valves, lymph fluid does not flow backwards but is pumped to the next vascular segment. Any mechanical or other cause that disrupts this function causes accumulation of lymph fluid and lymphedema (6, 7).

LYMPHEDEMA

Lymphedema may occur because of local or systemic reasons (8, 9). Systemic diseases such as congestive heart failure, renal failure, hypoalbuminemia, and nephropathies that cause protein loss can induce lymphedema. Local causes include primary and secondary lymphedema, lipedema, deep vein thrombosis, chronic venous diseases, postoperative complications, and cellulitis.

Lymphedema can be divided into two groups; primary and secondary.

Primary lymphedema occurs congenitally in the lymphatic system. There are three different types depending on the age of emergence. Primary congenital lymphedema usually occurs under 1-2 years of age. It may be sporadic or familial. It may be a symptom of some genetic diseases, such as Milroy disease. Primary lymphedema precocial is the most common type. It usually occurs between the ages of 1 and 35. It is more common in women and occurs most commonly during puberty. It usually becomes evident when the compensated lymphatic structure is decompensated after a minor trauma. Primary lymphedema tarda occurs after the age of 35 (9).

Secondary lymphedema is lymphedema that occurs later in the lymphatic system because of infection, cancer, trauma, and other reasons. Secondary lymphedema is most common in developing countries due to filariasis infection and in developed countries in patients who undergo axillary or inguinal lymph surgery and radiotherapy due to breast and pelvic cancers (4). Again, surgeries performed in the inguinal region and any type of lymph node excision may cause secondary lymphedema. Lymphedema occurs within 1 to 3 years in approximately 20-30% of breast cancer patients who undergo axillary surgery (10, 11).

Lymphedema is generally a slowly progressing disease. At first, the oedema is soft and leaves a pit when pressed with a finger, but over time it hardens and does not leave a pit. As the process progresses, skin thickening (hyperkeratosis) and coarsening (papillamotosis) develop. Disruption of skin integrity causes recurrent infections, cellulitis, and lymphangitis. Lymphatic drainage deteriorates further and enters a vicious cycle if left untreated (9). Therefore, early diagnosis and a correct treatment approach are important. In treatment, conservative or surgical approaches are applied depending on the stage of the disease. The conservative approach, namely, decongestant lymphatic therapy, includes methods such as skin hygiene, massage, manual lymphatic drainage, extremity compression, and exercise. Surgical methods such as subcutaneous tissue resection, lymphovenous microanastomoses between lymphatic vessels and the venous system, and vascularised lymph node transfer are used (12, 13).

IMAGING METHODS

Lymphangiography

Before lymphoscintigraphy, lymphangiography was used to show lymphatic pathways and investigate lymphedema. Kinmoth introduced traditional lymphangiography (14). In this method, following the intradermal injection of blue dye, the lymph channels are stained blue and become visible through an incision made in the foot skin. After the fat-soluble iodine contrast agent is injected into the visible lymph channels with a thin cannula, serial imaging is taken with X-ray or computed tomography at the 60., 90., minutes and 24. h. It is a quite invasive method. Because it requires incision and cannulation, it is susceptible to infection and local inflammation. It is a technically difficult procedure. It has not been a widely used method because of reasons such as being painful, the procedure taking time, allergy to contrast material or embolisation (6, 7, 9, 15, 16).

Ultrasonography

Ultrasonography (USG) and Doppler USG provide information about the lymph nodes but do not show the lymphatic channels and vascular structure. In the evaluation of lymphedema in the extremities, USG helps to exclude venous aetiologies by showing the presence of obstruction or reflux in the venous system (6). With USG, volumetric features, such as thickening of the dermis, thickening of the subcutaneous tissue, and changes in muscle mass, as well as structural features, such as changes in the echogenicity of the dermis and subcutaneous tissues, can be evaluated in the extremities with lymphedema (17).

Computed tomography

In lymphedema, computed tomography (CT) like USG, shows skin and subcutaneous tissue thickening, increased fat density, and anatomical changes around the muscle. In tomography, there is often a honeycomb appearance in the subcutaneous tissue, but it is not a specific finding because it is also seen in cellulitis and generalised oedema. In addition, as an anatomical imaging modality, it also provides information about the morphology and number of lymph nodes (18). Because it cannot differentiate between lymphedema and oedema, it plays a supportive role in lymphedema diagnostics.

Magnetic resonance imaging

Anatomical evaluation of the lymphatic system can be made with standard magnetic resonance imaging (MRI) images, and information about deep lymphatic vessels can be obtained. MRI provides the extent of oedema and adipose tissue deposition (19). Non-contrast MRI is non-invasive but has not been widely used because of image degradation. Using contrast-enhanced MR Lymphangiography, information is obtained about the anatomical and functional status of the lymphatic vascular structure in the extremities. In lymphedema, volume changes in the extremities, as in USG and CT, show structural changes in the skin and subcutaneous tissue. Its advantages are that it is radiation-free, shows the entire extremity, and allows 3D reconstruction (19, 20). However, prolonged use reduces patient comfort, has low sensitivity in distinguishing lymphatic-venous vessels, has no standard protocol or reporting, and gadolinium contrast material allergy may occur.

Indocianine green lymphography

This is a simple, sensitive, and precise method that does not involve radiation and is used in the diagnosis of lymphedema and in the planning of lymphaticovenous anastomosis treatment. For the first time, in 2008, Unno et al. used near infrared fluorescence (NIRF) imaging with Indocyanine green to make the lymph channels visible in the diagnosis of lymphedema in 2008 (21). When an indocyanine green substance is injected into the vein, lymphatic channels become visible and their location and functions can be determined. Usually injected perioperatively, the vessels are visible with good resolution. It can demonstrate superficial lymphatic flow and areas of congestion with high resolution (19). Its disadvantage is that it is insufficient to show lymphatics deeper than 1- 2 cm. The risk of allergy to contrast medium should also be considered. Indocyanine green is also used with near-infrared fluorescence imaging. Near-infrared fluorescence imaging with indocyanine green is a minimally invasive, reliable, and reproducible method that has emerged in recent years. It is performed especially for evaluation in terms of plastic surgery before lymphovascular anastomosis. However, it is not yet widely available in every clinic. There is limited experience and availability (19). However, it is one of the most promising methods in the future.

PERIPHERAL LYMPHOSCINTIGRAPHY

Radiopharmaceuticals

Unfortunately, there is no ideal radiopharmaceutical for lymphoscintigraphy today. Gold-198 colloid, which has historical importance, could not be used much because of its high beta radiation. The most standard radiopharmaceuticals used today are nanocolloids labelled Technetium 99m (Tc99m). However, colloids marked with Tc99m do not have a worldwide standard because they have different diameters. For example, because nanocolloid is not licenced in the United States, sulphur colloid, which was formerly used in liver scintigraphy, is filtered through 0.1 micrometer philtres to obtain colloids smaller than 100 nm. In Europe, Rhenium sulphide colloid and albumin nanocolloids marked with Tc99m are mostly used. Once radioactive colloids are injected into the interstitial tissue, they are absorbed slowly, with most of the activity remaining at the injected site. Progression occurs slowly in the lymph channels (4).

Unlike colloids, non-colloidal substances such as human serum albumin, dextran, and human immunoglobulin labelled with Tc99m have also been used for lymphoscintigraphy. When injected, they are rapidly absorbed and lymph vessels become immediately visible. For this reason, they are preferred for quantitative analyses, but different criteria may be required when interpreting due to differences in absorption mechanisms (22-24).

Today, the most commonly used colloids in lymphoscintigraphy are Tc99m nanocolloids with a diameter of less than 100 nanomicrons, which are also used in our clinic.

Imaging protocol

There is no single globally accepted methodology for peripheral lymphoscintigraphy. As with radiopharmaceuticals, the injection method and protocols may vary slightly from country to country and from department to department. No special patient preparation is required, such as fasting or stopping medications before scanning.

Injection: Injection is made intradermally or subdermally into the space between the first and second finger roots of both normal and oedematous extremities. After intradermal injection, lymphatic channels appear immediately; however, with subdermal injection, they appear more slowly. In lymphedema, the results of the test using subdermal injection are more reliable than those using intradermal injection (25, 26). In our department, 200-300 microCurie of Tc99m-nanocolloid is injected subdermally in approximately 0.5 ml volume. The nanocolloid has a slight burning effect at the injection site. The diffusion of the radioactive material can be increased by gently massaging the area after injection. In addition, after early dynamic imaging, the patient can be mobilised and encouraged to walk, and if it is upper extremity oedema, lymph flow can be stimulated with a light exercise for the arm muscles. Then, delayed images can be obtained.

Instrumentation: Although imaging varies depending on the facilities in the Nuclear Medicine Department, a double-headed SPECT-CT (Single Photon Emission Computerised Tomography) hybrid gamma camera with high-resolution parallel hole collimators is used in our department. For Tc99m gamma rays, a 140 keV photopeak and 20% window are set in the camera.

Positioning: Depending on the location of the lymphedema, the patient is placed in the supine position so that the injection sites on both lower extremities or both arms and the parts of the extremities that can enter the camera field.

Imaging: There is no complete consensus on imaging protocols. In some clinics, they start with dynamic imaging, whereas in some departments, imaging starts by scanning the extremities. In scanning mode, the extremities are scanned immediately after injection, with the camera moving 10 cm/min. In dynamic imaging, after the injection sites are placed at the bottom of the camera field, 20 images of 10 s are taken, then the camera is moved to the knee or elbow area and another 20 images of 10 s are obtained, and finally it is shifted to the pelvic or axillary region, continuing and ending the dynamic images in the same way. A 300-s static acquisition of the lymph nodes in the axillary or pelvic area is taken immediately after the scan mode or dynamic images are finished and at 1, 2, and 3 h and later if necessary. If the regional lymph nodes are not visible, images can be taken after the patient is mobilised and walked for a while. SPECT-CT study may also be recommended if lymphedema occurs, especially in the pelvis, abdomen, and thoracic regions (27). Late images are particularly useful in demonstrating dermal reflux, and the visualisation of liver uptake indicates that the radiopharmaceutical has reached the hepatic circulation (28). Late imaging is also essential for assessing dermal backflow or post-traumatic stagnation. If available in the clinic, a Cobalt-57 flood phantom source is placed between the patient and the gamma camera during imaging to determine the patient's body contours.

Greater sensitivity and better three-dimensional spatial resolution can be achieved by acquiring single-photon emission computed tomography (SPECT) by combining LS with single-photon emission computed tomography. SPECT/CT study is recommended when lymphedema concerns the pelvic-abdominal-thoracic districts (29). Yoon et al. developed a hybrid SPECT/CT classification using dermal backflow of SPECT and honeycomb pattern of CT and compared it with lymphoscintigraphic staging and clinical severity (30). In this study, the addition of SPECT/CT to planar scintigraphy showed a 15.4% modification rate in lymphoscintigraphic staging (30).

Normal distribution and Interpretation

Qualitative and visual assessment

In people with normal lymphatic anatomy and function, the radiopharmaceutical flows symmetrically through the lymphatic channels (usually 3-5 vessels in the calf, 1-2 vessels in the thigh) and drains simultaneously into the regional lymph nodes in about 30-60 minutes (Figure 1) (31). When qualitatively interpreting peripheral lymphoscintigraphy, the symmetry of the two extremities is examined (4,9). The number and time of appearance of lymphatic drainage channels and vessels in both extremities is another criterion. The time of appearance of regional lymph nodes and whether there is a delay or not are noted (Figure 2). After these findings, the secondary findings are evaluated. Whether there is a sudden interruption in the continuation of lymphatic vessels or channels, collateral vessel development, and the presence of backflow in the skin were evaluated (Figure 3). Lymph nodes in the popliteal or epitrochlear region are usually

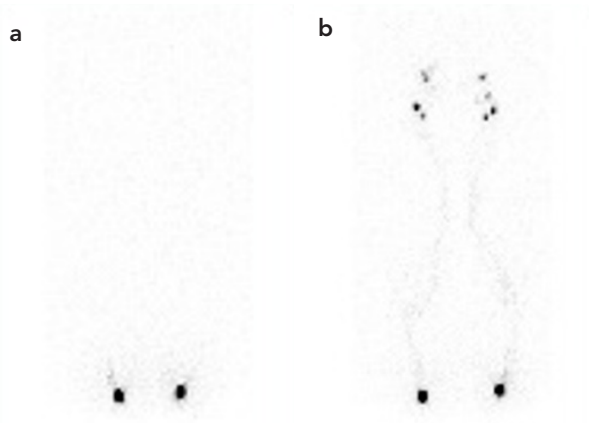

Figure 1: Lower extremity lymphoscintigraphy; normal findings. a) Immediate post-injection image: Injection sites on both feet. b) Static image at 1 h. Bilateral symmetrical and similar numbers of ilioinguinal lymph nodes are visualised.

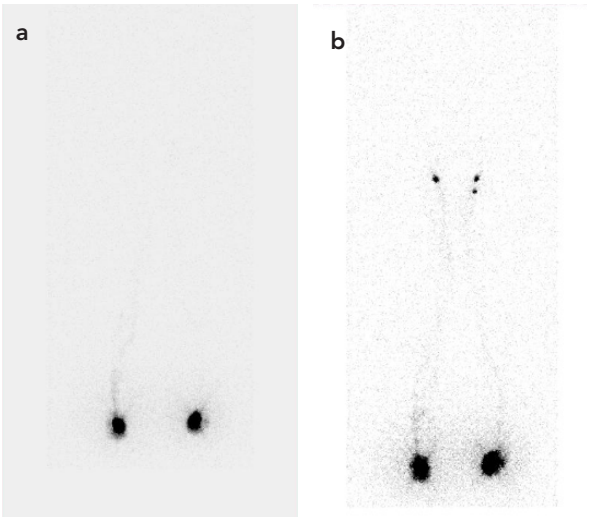

Figure 2: Lower extremity lymphoscintigraphy; a patient with bilateral lower extremity oedema. a) Immediate post-injection image: Injection sites on both feet. b) Delayed static whole-body image at 2. hour; there is delayed visualisation and decreased number of pelvic lymph nodes bilaterally but more prominent on the right side.

not seen, but when they are seen, it indicates that the lymph flow passes through the deep lymphatic system (4, 9). A semi-quantitative scoring system has also been developed using these qualitative evaluation criteria (26). Although there are no definitive findings distinguishing primary and secondary lymphedema, delay or interruption in the transport of the radiopharmaceutical is more common in primary lymphedema. Regional lymph nodes may be reduced in number or not seen at all. In advanced cases, reflux is observed on the skin. In secondary lymphedema, prominence and enlargement of lymphatic channels, delay in activity transport, presence of collateral channels, and skin reflux and lymphatic leakage are more common in late images (26, 31) (Table 1).

Quantitative evaluation

Quantitative evaluation may increase the sensitivity of the test compared with qualitative visual evaluation. However, there is no full consensus on this issue. Various researchers have used different measurement criteria with different radiopharmaceuticals and protocols. Different quantitative criteria, such as the clearance rate and time of the substance from the injection site, the remaining activity rate at the injection site, the time of the substance to reach the lymph node from the injection site (transport time or index), and the retention rate in the lymph node, etc., have been applied (32-34). In a study by Weissleder et al., 70% of 238 patients were diagnosed with lymphedema by visual evaluation, whereas this rate increased to 100% with quantitative analysis. It has been stated that quantitative evaluation increases the sensitivity in detecting new-onset lymphedema (35). However, there is no standard method for quantitative evaluation as in visual evaluation.

Table 1: Criteria for qualitative interpretation of lymphoscintigraphy

Interpretation of lymphoscintigraphy
1. Primary findings:
Symmetry
Appearance time of lymph vessels and nodes
Number of lymph nodes
2. Secondary findings:
Sudden interrruption of the lymphatic channels
Presence of collateral lymphatic vessels
Backflow activity in the skin

Advantages and disadvantages of lymphoscintigraphy

Lymphoscintigraphy, as a nuclear medicine method, has low resolution compared with other modalities and cannot show anatomical contours. Lymph nodes and large vessels appear with low resolution and may not indicate low-level lymph leakage in small lymphatic channels (6). Anatomical imaging can be improved with SPECT-CT hybrid imaging, and additional information can be obtained, especially regarding the typical honeycomb appearance due to lymphedema (27). Another disadvantage is the lack of a standard in terms of both the radiopharmaceutical used and the application of the test, that is, the injection site, volume, and acquisition protocol (6). This does not allow obtaining standard results in the meta-analytic evaluation of studies in which the test is used. However, lymphoscintigraphy is a non-invasive, easily applicable method for the diagnosis of lymphedema, and is accessible for clinical follow-up and pre/post-treatment evaluations.

The visual evaluation of lymphoscintigraphy in patients with lymphedema, Alavi et al. found that the test was 73% sensitive and 100% specific (36). The low sensitivity was due to three false negative cases, and pathology could not be detected in these cases because early imaging was not performed. In late images, the findings were evaluated as normal.

Hassanein et al. qualitatively evaluated lymphoscintigaphy studies performed in 227 patients with lymphedema and showed that the test was 96% sensitive and 100% specific (37). It was observed that the seven cases with false negative results were due to reasons such as misdiagnosis, new-onset disease, and not having enough delayed images. They reported that lymphoscintigraphy is successful in revealing the diagnosis in patients with borderline clinical findings, confirming people with normal lymphatic function, and indicating the severity of the disease (37).

Clinical Indications

1- Lymphoscintigraphy is an appropriate test for the evaluation of primary lymphedema or limb oedema of unclear aetiology. Lymphoscintigraphy can also be appropriate

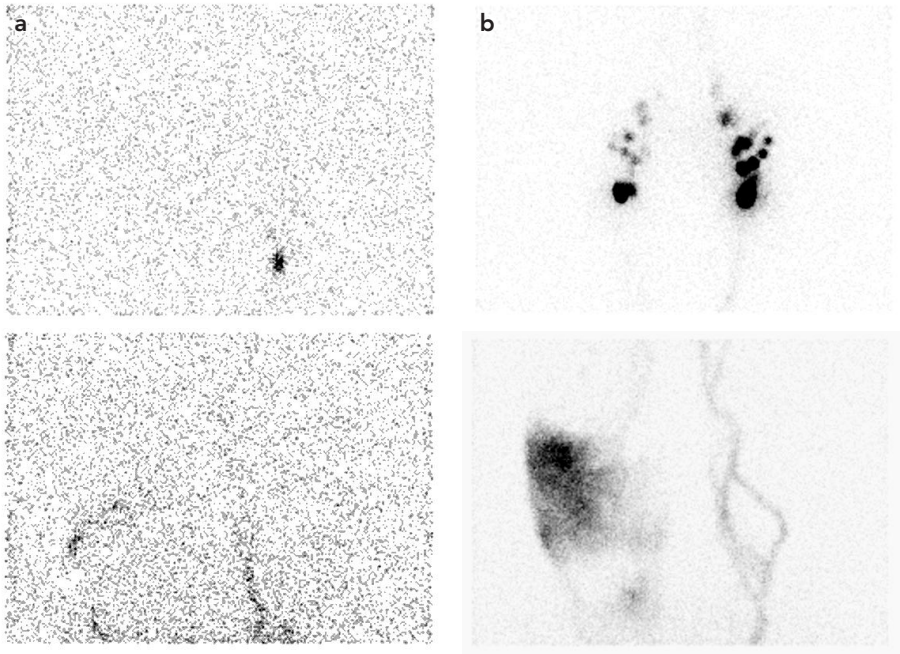

Figure 3: Lower extremity lymphoscintigraphy; a patient with bilateral lower extremity oedema. a) Early static images: Lower: Just above the injection sites and knee region, lymphatic channels are visualised asymmetrically; Upper: No inguinal lymph nodes are seen on the right and only one lymph node is seen on the left. b) Delayed static images at 4 h; lower: knee region, increased subcutaneous activity indicates dermal backflow on the right; upper: pelvic region, decreased number of lymph nodes on the right side indicates the presence of an obstruction on the right side of the extremities.

for patients with suspicion of secondary lymphedema, particularly if the clinical history or examination is not definitive for the diagnosis of lymphedema (38). It plays a role in the differential diagnosis of nonlymphomatous oedema of the extremities. Lymphoscintigraphy is usually normal in nonlymphomatous oedema. Lipedema (dense fat storage under the skin), deep vein thrombosis, and oedema developing after femoropopliteal bypass should be considered in the differential diagnosis. In these cases, lymphoscintigraphy will give normal results (9).

2- Lymphoscintigraphy can be performed to determine the severity and degree of the disease. In a study evaluated using the scoring criteria developed by Papalardo and Cheng, it was shown that lymphoscintigraphic findings were compatible with the clinical findings of lymphedema (26).

3- It is performed to evaluate the presence of lymphatic drainage after axillary surgery. Lymphatic drainage of the axilla after surgery in breast cancer has been evaluated in various studies. In a study of 313 patients who underwent axillary lymph node dissection, axillary lymph nodes were not seen in 35.8% of the patients, whereas lymphatic drainage was observed in the others (39). Szuba et al. showed that axillary lymph nodes were visible with lymphoscintigraphy in 86.7% of patients who had breast cancer and underwent axillary surgery (40). These studies show that lymphatic drainage is restored after surgery in most cases.

Table 2: Indications for lymphoscintigraphy

Indications
1. Evaluation of primary lymphedema or limb oedema of unclear aetiology.
2. Evaluation of secondary lymphedema, particularly if the clinical history or examination is not definitive for the diagnosis of lymphedema .
3. Differential diagnosis of nonlymphatic oedema of the extremities (lipedema (dense fat storage under the skin), deep vein thrombosis, and oedema developing after femoropopliteal bypass should, etc.)
4. Understanding the severity and degree lymphedema.
5. To evaluate the presence of lymphatic drainage after axillary surgery (i.e.in breast cancer).
6. To confirm lymphatic dysfunction before lymphatic surgery.
7. To demonstrate the effectiveness of the treatment.

4- Lymphoscintigraphy can be helpful in confirming lymphatic dysfunction before lymphatic surgery (38).

5- Lymphoscintigraphy is used to show the effectiveness of the treatment in patients receiving lymphedema treatment. For example, in patients who have undergone lymph node or vein transfer for treatment, the function of the new transplant and the status of lymphatic drainage were investigated. Complications at the donor site where the lymph node was removed were examined. Lymphoscintigraphy reveals the prognostic value of treatment.

Studies have shown that lymphoscintigraphy findings are compatible with clinical findings (41).

However, although guidelines recommend lymphoscintigraphy, in a recent study of 57,000 patients, only 2.5% of patients underwent lymphoscintigraphy. Most patients undergoing lymphoscintigraphy are diagnosed with melanoma or breast cancer (42).

In addition, according to the expert opinion consensus report published in 2022 consisting of The American Venous Forum, American Vein and Lymphatic Society, and the Society for Vascular Medicine in the diagnosis and treatment of lymphedema, no consensus was reached regarding routine clinical practice use of radionuclide lymphoscintigraphy as a mandatory diagnostic tool in lymphedema (43).

CONCLUSION

Lymphoscintigraphy is a very sensitive and specific method for the diagnosis, treatment, and monitoring of lymphedema when it is easily accessible. It is easy to apply, reliable, has a low radiation dose, and does not depend on the operator when performed in experienced clinics. Although its low resolution limits anatomical evaluation, it provides information about lymphatic drainage and function. The lack of a single standard application method is a disadvantage. Expert representatives from 11 professional societies, as part of an autonomous work group, researched and developed appropriate use criteria (AUC) for lymphoscintigraphy in sentinel lymph node mapping and lymphedema (38).

Further studies with a larger number of patients are required on this subject. Although lymphoscintigraphy is a widely used method, contrast-enhanced MR Lymphangiography and Indocyanine Green Near Infrared Lymphangiography are also used more and more frequently in recent years and are promising methods in the future.

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CASE REPORT / OLGU SUNUMU
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A DERMOID CYST IN THE CECAL COLON MIMICKING AN OVARIAN CYST: A RARE CASE REPORT

ÇEKAL KOLONDA OVER KİSTİNİ TAKLİT EDEN DERMOİD KİST: NADİR BİR OLGU SUNUMU

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ABSTRACT

Dermoid cysts are benign tumors and occur very rarely in the cecum. To date, the literature shows very few cases to have been reported of a dermoid cyst in the cecum, one of which was laparoscopic. Even though dermoid cysts have ultrasonographic characteristic appearances, other pathologies in the pelvis can mimic this appearance. A 35-year-old female patient who had had a dilation and curettage a week prior was examined with a complaint of abdominal pain. A semisolid mass lesion with a size of 105x77 mm originating from the right lateral of the uterus was detected by transvaginal ultrasonography. During the operation, the frozen section of the mass was shown to not be malignant. The mass was removed by ileocecal resection. The surgery was terminated after ileocolonic anastomosis. The mass was examined histomorphologically and was reported as a dermoid cyst in the cecal colon. Cecal dermoid cysts should be considered for a differential diagnosis of pelvic cysts. A more detailed preoperative radiological imaging could have contributed significantly to the diagnosis. Surgical resection of a dermoid cyst in the cecal colon is a curative treatment. Due to the rarity of such cases, conduct a prospective study is quite difficult, which is what makes this study important.

Keywords: Dermoid cyst, colon, tumor

ÖZET

Dermoid kistler iyi huylu tümörlerdir ve çekumda çok nadir görülürler. Literatürde bugüne kadar biri laparoskopik olmak üzere çok az sayıda çekumda dermoid kist olgusu rapor edilmiştir. Dermoid kistler ultrasonografik karakteristik görünümlere sahip olsa da pelvisteki diğer patolojiler de bu görünümü taklit edebilirler. Karın ağrısı nedeni ile başvuran 35 yaşındaki kadın hasta muayene edildi. Hastaya bir hafta önce küretaj yapılmıştı. Transvajinal ultrasonografide uterusun sağ lateralinden kaynaklanan 105x77 mm boyutlarında kitle lezyonu tespit edildi. Operasyon sırasında kitlenin frozen incelemesinde malign olmadığı görüldü. Kitle ileoçekal rezeksiyonla çıkarıldı. İleokolonik anastomoz yapıldıktan sonra ameliyat sonlandırıldı. Kitle histomorfolojik olarak incelendi ve çekal kolonda dermoid kist olarak rapor edildi. Pelvik kistlerin ayırıcı tanısında çekal dermoid kistler de düşünülmelidir. Ameliyat öncesi radyolojik görüntülemenin daha detaylı yapılmasının tanıya önemli katkı sağlayacağı düşünüldü. Çekal kolondaki dermoid kistin cerrahi olarak rezeksiyonu küratif bir tedavidir. Vaka sayısı çok az olduğundan prospektif bir çalışma yapmak oldukça zordur. Bu bakımdan bu çalışma önemlidir.

Anahtar Kelimeler: Dermoid kist, kolon, tümör

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INTRODUCTION

Dermoid cysts are benign tumors that involve all three germinal layers and have a malignancy risk of 1-2% (1). Benign dermoid cysts of the cecum are very rarely seen (2). The literature shows dermoid cyst cases in the cecum to very rarely have been reported, with one of these being laparoscopic. This type of case is rarely seen distally on the abdominal wall in the peritoneum, omentum, or cecum (3). Even though dermoid cysts have ultrasonographic characteristic appearances, other pathologies in the pelvis can mimic this appearance (4). The current case report presents a patient who was thought to have a cystic lesion originating from the right ovary but who underwent an ileocecal resection due to having a dermoid cyst.

CASE PRESENTATION

A 35-year-old female patient who had three previous caesarean sections and dilation and curettage one week prior at the gynecology and obstetrics clinic presented with abdominal pain, abdominal swelling, and constipation complaints after the curettage. The patient had no comorbidities. On physical examination, tenderness and pain were present in the right lower quadrant of the abdomen. She had no muscular defense or rebound tenderness in her abdomen.

A semisolid heterogeneous mass lesion with a size of 105x77 mm and originating from the right lateral of the uterus was detected by transvaginal ultrasonography (Figure 1). The laboratory tests showed her β-hCG (56 U/L) level to be high, the tumor marker values to be normal, and leukocytes to be 11.100/μL. The patient presented only with pain in the right lower quadrant of the abdomen in her physical examination and was operated on by gynecologists with prediagnoses of uterine myoma and cyst originating from the right ovary.

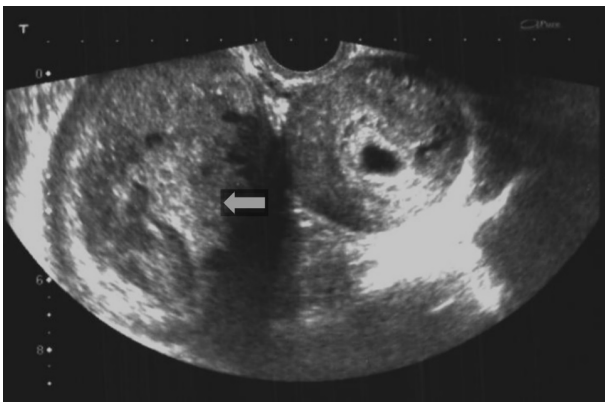

Figure 1: Appearance of the mass on the transvaginal ultrasound.

During the operation, the mass was seen to originate from the cecum, and the patient was consulted to general surgery. The size of the mass, which contained solid and cystic components, was approximately 10x7x7 cm. During the operation, a frozen section of the mass was seen to not be malignant. The mass was removed by ileocecal resection. The surgery was terminated after ileocolonic anastomosis (Figure 2).

Upon macroscopic examination of the tissue, the solid yellow cystic formation with thick walls located at the base of the cecum was not associated with mucosa, was 10 cm at its widest diameter, and contained white paste-like material. The microscopic examination revealed a keratinous material in the lumen of the cystic structure that had been laid with squamous epithelium (Figure 3).

A giant cell reaction in the form of a serosal foreign body secondary to the cystic lesion was found in the colon samples (Figure 4). The mass was examined histomorphologically and was reported as a dermoid cyst in the cecal colon.

Regression was observed in the patient's complaints during the postoperative period, and she was discharged on the 7th postoperative day. No recurrence was observed during the patient's follow-up one year later.

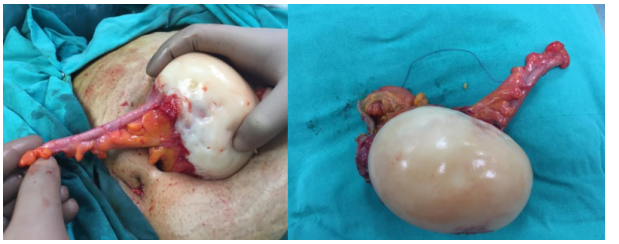

Figure 2: Dermoid cyst of the cecum.

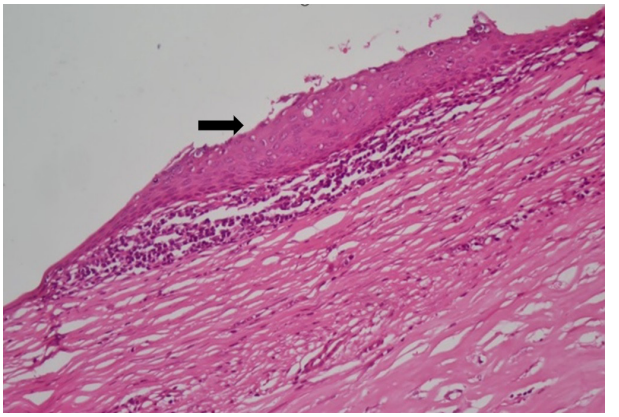

Figure 3: Squamous epithelium covering the lumen of the cyst (asterisk) (x20 H&E).

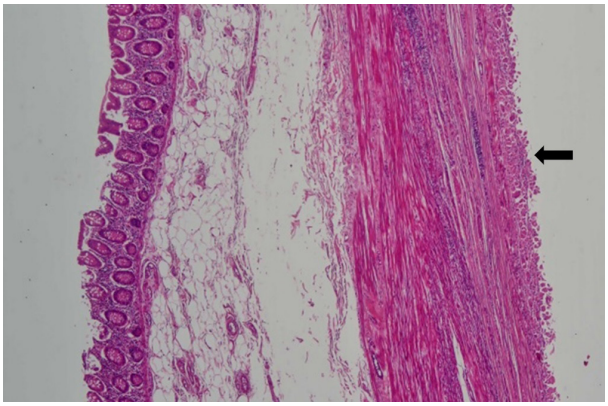

Figure 4: A foreign-body giant cell reaction that developed on the serosal surface of the colon. Serosal surface is covered by lots of giant cells (asteriks) (x40 H&E).

embryogenesis (5). In this present case, the patient had a history that included three abdominal operations.

Although the cause of extragonadal cysts is not known exactly, dermoid cysts seen on the midline (e.g., the mediastinum, anterior side of the neck, and central nervous system) frequently occur in the omentum in the abdomen (6, 7). Further related studies will help to better understand the subject (3). The cases with full texts in the literature are shown below Table 1.

Dermoid cysts in the cecal colon are frequently seen in the middle adult age group. However, the literature does show that they can also be seen congenitally in the pediatric age group.

Pathological examination is essential for the diagnosis of these dermoid cysts. Pre-surgery cross-sectional imaging is required for these cysts, which can cause abdominal pain and intestinal obstruction. Therefore, total excision is sufficient for these benign cysts. Unnecessary resection and lymph node dissection should be avoided.

Table 1: Reported cecal dermoid cyst cases in the literature (including the present case)

Case	Sex	Age	Symptom	Tumor size	History of surgery	Surgery	Year	Reference
1	F	1	Abdominal mass	8 cm	No	Terminal ileum and cecum resection	1971	Kay (8)
2	F	53	Melena	4 cm	Hysterectomy/ Appendectomy	Laparotomy- Right hemicolectomy	1977	Mossey (9)
3	F	34	Colic pain in the lower abdomen	10 cm	3 Caesarean sections	Laparotomy- Right hemicolectomy	1996	Wilkinson (2)
4	F	39	Abdominal distension and pain	20 cm	No	Laparotomy- Right hemicolectomy	2000	Mellado (10)
5	M	34	Lower abdominal pain	7.5 cm	No	Laparotomy- Cystectomy and appendectomy	2001	Nirenberg (11)
6	M	30	Right lower abdominal pain	8 cm	No	Laparotomy- Right hemi-cholectomy	2002	Schuetz (12)
7	F	41	Weight and mass	10 cm	No	Laparotomy- Right Mikulicz colostomy	2016	Nahidi (13)
8	M	2	Abdominal distension	6 cm	Anorectal malformation surgery	Laparotomy- Right hemi cholectomy	2019	Destro (14)
9	F	35	Right lower abdominal pain	10 cm	No	Laparoscopic cystectomy	2020	Mishra (3)
10	F	37	Right lower abdominal pain and distension	7 cm	Three Caesarean sections	Terminal ileum and cecum resection	2020	The present case

DISCUSSION

Dermoid cysts, also known as mature teratoma cysts, are classified as congenital or acquired. Acquired dermoid cysts may develop due to previous operations or trauma. They can also develop congenitally through ectoderm implantation during

CONCLUSION

In this present case, the preoperative ultrasonography conducted by gynecologists was observed to have been insufficient at detecting the origin of the mass, and additional imaging methods may be needed in such cases. Although cecal dermoid cysts are rarely seen, they should be considered for the differential diagnosis of pelvic cysts. Surgical resection of a dermoid cyst in the cecal colon is a curative treatment. Due to this type of case occurring very rarely, conducting a prospective study is very difficult, which is why this study is important.

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

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- N.B., E.B., Ö.E.; Data Acquisition- N.B., E.B., İ.B.; Data Analysis/Interpretation- N.B., E.B., İ.B., Ö.E.; Drafting Manuscript- N.B., E.B., İ.B.; Critical Revision of Manuscript- N.B., İ.B., Ö.E.; Final Approval and Accountability- N.B., E.B., İ.B., Ö.E.

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LETTER TO THE EDITOR / EDİTÖRE MEKTUP
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COMMENT ON "A PARTIAL TRISOMY 9 CASE WITH DICENTRIC CHROMOSOME DUE TO THE ADJACENT-2 SEGREGATION OF MATERNAL RECIPROCAL TRANSLOCATION"

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Dear Editor,

We have read the article entitled "A partial trisomy 9 case with dicentric chromosome due to the adjacent-2 segregation of maternal reciprocal translocation" by Urtekin et al. published in the Journal of Istanbul Faculty of Medicine (1). This paper is exceptional because the offspring of adjacent-2 segregation being born alive is a rarity. Although the authors have been careful with the details of each aspect, we would like to comment on certain possible theoretical imbalances, especially those caused by tertiary segregations. The authors state that adjacent-2 segregation is one of the few cases in which the infant is born alive with dysmorphic features as it results in trisomy/monosomy of chromosome 22 with partial deletion/duplication of chromosome 9. Moreover, in 3:1 segregation, there are two possibilities of disomy 22 with duplication and deletion of chromosome 9 derived from the tertiary monosomy/trisomy segregation pattern and that most gestational products will be monosomic or double trisomic for chromosomes 9 and 22 (interchange monosomy/trisomy products).

As the authors have correctly indicated, in this translocation, adjacent-1 segregation ironically results in gametes with greater imbalance than adjacent-2 segregation. Nonetheless, in tertiary 3:1 segregations, the only possibility for disomy of chromosome 22 is caused by gametes with 24 chromosomes retaining der(9). Such gametes will additionally have a disomy of 9q22.31-pter, whereas those with 24 chromosomes and der (22) will have a disomy of 9q22.31-qter only. The gametes with 22 chromosomes retaining der(22), namely -9, will have virtually complete nullisomy for chromosome 22 and nullisomy for 9q22.31-pter, and those retaining der(9), that is -22, will be nullisomic for 9q22.31-qter only. In such cases, the hypothetical offspring of these gametes will not conform to the expected imbalance in tertiary 3:1 segregation. However, the interchange aneuploidies would be the same as those for any other reciprocal translocation (trisomy or monosomy for each chromosome) and not monosomy or double trisomy, as suggested by the authors.

Furthermore, although it is appropriate to inform the family about reproductive options to avoid the recurrence of unbalanced offspring, the fact that this couple may also have phenotypically normal offspring, either with a normal karyotype or chromosomally balanced (like the mother) owing to an alternate segregation, should be noted. Finally, if the maternal grandparents are alive, a karyotype can be requested, and if one of them is a carrier of the translocation, karyotyping of other members can be recommended if they have it.

We believe that these comments do not discredit the study; rather, they complement it.

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1. Urtekin E, Kayhan G, Kazancioglu E, Yirmibes Karaoguz M. A partial trisomy 9 case with dicentric chromosome due to the adjacent-2 segregation of maternal reciprocal translocation. J Ist Faculty Med 2022;85(2):279-84. doi:10.26650/IUITFD.1038997 [CrossRef]