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ORIGINAL RESEARCH

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Does the Stoma Site Marking Affect Stoma Quality?

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Abstract

Objective

Ostomy structure is an indispensable part of colorectal surgical procedures. With stoma, surgeons hope to get rid of the morbidity of the anastomosis they made. Ostomies are the source of morbidity both during construction and closure. Problem-free ostomies both reduce healthcare costs and support patients' quality of life. In this study, we aimed to reveal the relationship between the marking performed by the wound, ostomy and continence nurse (WOC nurse) before surgery and the quality of the stoma.

Material and Method

150 patients who underwent surgery for malignant diagnosis in our oncology clinic and needed stoma construction were included in the study. The medical records and database of the patients kept by the WOC nurse were retrospectively reviewed. Demographic and clinicopathological data were recorded and grouped. Patients who underwent both emergency and elective procedures were included in the study. Statistical analyzes were performed within the 95% confidence interval. A p-value of less than 0.05 was considered statistically significant.

Results

A significant relationship was observed between the marking the stoma site and BMI with the complications (p=0.03, p=0,01). Patients with ileostomy were associated with significantly more peristomal skin complications (PSC) than patients with a colostomy (p=0.02). Again, in the different analyses performed, a significant difference was observed between stoma marking status and stoma diameters; large stoma diameters were associated with patients who were not consulted by the WOC nurse (p=0.001).

Conclusion

The findings showed that WOC nurse preoperative consultation was associated with fewer complications affecting stoma quality.

Keywords: Complication, stoma, stoma quality, WOC nurse

Introduction

Stoma construction is a common procedure in colorectal surgery as part of operations for malignant and benign gastrointestinal system diseases. These formations (ileostomy or colostomy) are an integral part of the surgical management of intra-abdominal pathologies in both emergency and elective patients. The underlying basic principle is that feces' flow is directed from the pathology site towards the anterior

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abdominal wall through the end or side intestinal ring. Thus, it is aimed to reduce the morbidity and mortality associated with gastrointestinal diseases through a stoma (1). Therefore, stoma formation is a simple but nontrivial attempt.

The stoma can be thought of as a new organ created to lead a healthy and normal life, rather than just a passage through which feces can be excreted. We should see the ostomy as an anastomosis of the intestinal system to the skin and apply the surgical technique principles here as in anastomoses (2). Despite advances in surgical techniques and stoma care products, stoma complications are still common (3). A complication rate of 21-70% has been reported in various studies. (4). Contact dermatitis and many other peristomal skin problems are the most common complications (5). Inappropriate stoma site, stoma mismanagement, and stoma complications, together with decreased quality of life (6), also cause a significant increase in health costs regardless of ostomy durations (7).

Early and regular follow-up and adequate stoma diameter and height are essential to prevent complications associated with a stoma. Cooperations of surgeons and the wound, ostomy and continence nurse (WOC nurse), providing feedback is extremely vital to improving stoma quality (8).

In this study, we aimed to investigate the relationship between the preoperative marking of the stoma site by the WOC nurse in our oncology clinic with stoma complications, stoma size, and stoma quality.

Material and Method

Following obtaining the local ethics committee approval of Ankara University Faculty of Medicine, the medical records of 156 patients diagnosed with malignant tumors who needed stoma creation at the Oncological Surgery Clinic between January 2016 and December 2020 were collected and retrospectively reviewed. Demographic and clinicopathological data were recorded. Patients who underwent both emergency and elective procedures were included in the study. Six patients were excluded due to missing data. Patients were grouped in terms of gender, age, BMI, diagnosis, urgency, stoma status (type, location, duration), complications, and preoperative marking of the stoma area by our WOC nurse. All data were presented as mean±standard deviation (SD), number, percentage, maximum and minimum values. Afterward, statistical analyzes were made. Parametric test assumptions were reviewed before differential analysis was performed. Normality was checked by the Kolmogorov Smirnov test, skewness, and kurtosis. In the case where the assumptions were provided, the difference analysis was performed using the one-way analysis of variance (ANOVA) and the Kruskal Wallis test when not. Paired comparisons were made using the Mann-Whitney U test. The relationship between categorical variables was analyzed using the chi-square test. Statistical analyzes were performed within the confidence interval of 95%. A p-value of less than 0.05 was considered statistically significant.

Our stoma marking method: Our WOC nurse evaluated patients scheduled for surgery for possible stoma formation one day before or on the day of surgery, according to the recommendations of the Wound Ostomy Continence Nurses Society and the American Society of Colon and Rectal Surgeons. Patients were allowed to lie down, sit, or stand, and markings were made with special rulers to avoid the possible stoma site coinciding with the belt line and any abnormal skin folds (Figure 1). Stoma diameters were recorded by measuring the skin edge and the intestinal mucosa junction line on the postoperative 10th day. Besides, stoma shape, complications, stoma localizations of the patients as the left-upper, left-lower, right-upper, and right-lower quadrants were recorded.

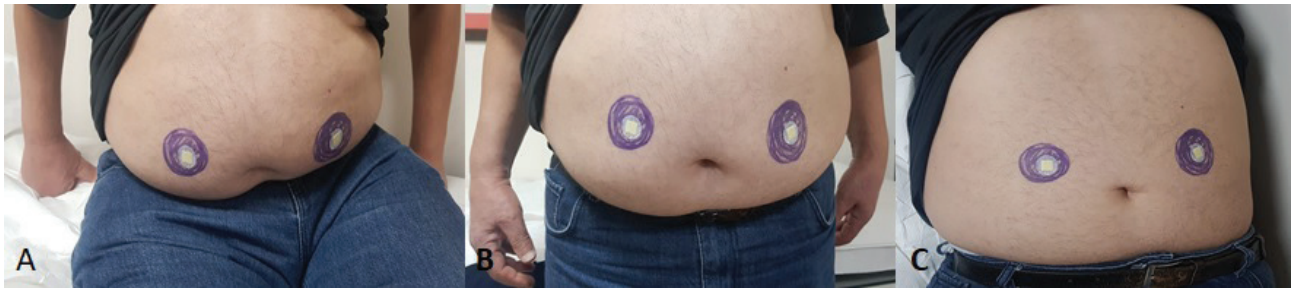

Figure 1
A.Stoma signs in sitting position, B.Stoma signs in standing position, C.Stoma signs in supine position

Results

Of the 150 malignant diagnosed patients included in the study, 53.3% (n=80) were male, and 46.7% (n=70) were female, and the mean age was 58.30±5.03 years (Table 1). BMI of 28 (18.6%) patients was over 25 (Table 2). Demographic and clinicopathological characteristics of the patients are summarized in Table 2. While the rate of patients marked by the WOC nurse before the operation was 51.3% (n=77),

Table 1 Age of patients and the diameter of the stoma

	Mean	SD	Max	Min
Age (years)	58.3	5.0	86	19
Stoma diameter (mm)	38.66	5.6	30	57

Table 2 Demographic and clinicopathological characteristics of the patients

Variables	Number (n)	Percentage (%)
Gender		
Male	80	53.3
Female	70	46.7
Diagnosis		
Rectal cancer	86	57.3
Colon cancer	26	17.3
Other malignancies	38	25.4
Surgery performed		
LAR[1]	59	39.3
APR [2]	14	9.3
Colon resection	77	51.4
Degree of urgency		
Urgent	31	20.7
Planned	119	79.3
Stoma type		
Ileostomy	78	52
Colostomy	72	48
Marking status		
Marked	77	51.3
Unmarked	73	48.7
Stoma site		
Lower left	59	39.3
Upper left	7	4.7
Lower right	76	50.7
Upper right	8	5.3

Table 2 continued Demographic and clinicopathological characteristics of the patients

Variables	Number (n)	Percentage (%)
Stoma duration		
Temporary	105	70
Permanent	45	30
BMI[3]		
>25	28	18.7
<25	122	81.3
Complication status		
No	98	65.3
Related to skin	29	19.3
Mucocutaneous separation	15	10
Retraction	8	5.4

1 Low anterior resection 2 Abdominoperineal resection 3 Body mass index

Table 3 Distribution of complications by stoma site marking condition and BMI

Parameters(N) (N=150)	Marked N=77		Unmarked N=73		BMI>25 N=28		BMI<25 N=122	
	N	%	N	%	N	%	N	%
No complication (98)	59	60	39	40	3	3	95	97
Skin lesions (19)	10	35	19	65	7	24	22	76
Mucocutaneous separation (15)	5	33	10	67	11	73	4	27
Retraction (8)	3	38	5	62	7	87	1	13

Table 4 Statistical significance between complications and variables

	Stoma Type	Stoma Site	Degree of Urgency	BMI*	Marking Status
Complication	p = 0.002	p = 0.52	p = 0.536	p = 0.01	p = 0.03

* Body mass index

the number of patients operated without marking was 48.7% (n=73). 79.3% (n=119) of the operations were planned, 20.7% (n=31) were performed with emergency procedures. The distribution of the operations performed and ostomy sites were summarized in Table 2. Complications were observed in 52 (34.6%) of the patients. The distribution of complications by stoma site marking status and BMI are presented in Table 3.

A significant relationship was observed in the analysis performed between the marking of the stoma site, BMI and complications (p=0.03, p=0.01) (Table 4). Significantly fewer complications were observed in patients for whom marking was performed and with a BMI of <25. This significant relationship was not observed between stoma type, stoma site, the case's urgency, and complications (p>.05). However, patients with ileostomy were associated with significantly more skin complications than patients with a colostomy (p=0.02). Again, in the different analyses performed, a significant change was observed between stoma marking status and stoma diameter (p=0.001). This change was also observed between patients who developed complications and stoma diameter (p = 0.015). Stoma diameters of patients who underwent marking and those without complications were significantly smaller than those without.

Discussion

Our study aimed to examine the relationship between stoma site marking performed by a WOC nurse before surgery and stoma quality. Stoma complications were less, and stoma diameters were smaller in patients for whom marking was performed. Stoma quality is closely related to the rarity of complications and small stoma diameters. Especially stoma adapter incompatibility causes skin irritation and increased peristomal skin problems with leakage (4). In our study, the diameter of the stoma with complications was more extensive than those without complications. Drawing attention to this relationship, Pilgrim et al. concluded a 10% increase in the risk of developing parastomal hernia with each mm diameter increase (9). In their meta-analysis, Hsu et al. found that in patients with stoma site marking, marking was associated with a reduction in stoma and peristomal complications in all types of the stoma (10). Although Bass et al. confirmed similar results, they also stated that stoma care training reduces the adverse outcomes (11). Preoperative stoma site marking has a highly protective role in reducing the complication rate and improving the quality of life of patients (12).

As a result, preoperative consultation and marking by a WOC nurse improves stoma quality and reduces postoperative morbidity (4).

Although a complication rate of 21-70% has been reported in various studies, Cottom et al. identified 34% of ostomies as problematic in a database-based study of 93 hospitals enrolling 3970 stoma patients; they reported different complications rates of 6-96%. Stoma height, stoma type, and gender of the patient were identified as significant risk factors, and they reported that BMI did not affect results, but there was a high probability relationship between urgency and a problematic stoma (13). In our study, the complication rate was 34.6%, and the most common complications we encountered were peristomal skin complications (PSC) at rates similar to the literature. Especially PSC was more common in the group with an ileostomy. Although we found a relationship between BMI and a problematic stoma in our study, we could not find the association of the emergency procedure performed to the patient in this relationship. Persson et al. reported PSC as the most common complication; although the PSC rate varied depending on the type of ileostomy, it was encountered as the most common in the ileostomy stoma group at a rate of 60-73% (Persson et al., 2010). Evidence suggests the development of bag leakage as a common problem for patients with a poor-quality stoma and the associated peristomal skin irritation that can adversely affect the quality of life (14).

Stoma creation is full of complications and should not be seen as a minor intervention. They significantly change the patients' lifestyles by creating severe complications that require urgent reoperation and minor problems that expose the patient to day and night distress (15). Stoma diameter decreases significantly during the first two weeks after hospital discharge, and the physical configuration of the stoma improves during this time (16). Most early complications are also observed during this period.

Studies continue to identify conditions to minimize stoma complications and associated morbidity and achieve a common consensus. Thanks to the formation of consensus (Delphi Consensus), which includes application guidelines on care and follow-up instructions to be applied to patients, it has become easier for WOC nurses to identify the factors that play an essential role in evaluating body and stoma profiles to determine the best pouching system (14).

Conclusion

The dramatic change in complications from the center to center indicates that surgical technique and stoma care are critical factors in stoma complications. WOC nurses must take a more active role in stoma treatment with surgeons, and preoperative consultations are essential in colorectal surgery clinics. Prospective, randomized controlled studies are needed to understand the more common risk factors better.

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

Ethical Approval
The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee (Date: 14.01.2021, Decision No: İ10-627-20) for studies involving humans.

Consent to Participate and Publish
Informed consent form was not required because of retrospective designed study.

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Availability of Data and Materials
Data available on request from the authors.

Authors Contributions
SİB: Conceptualization; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

ŞD: Conceptualization; Data curation; Formal analysis; Methodology; Validation; Visualization; Writing-original draft.

MAÇ: Data curation; Investigation; Methodology; Writing-original draft.

FA: Data curation; Investigation; Methodology; Validation; Visualization; Writing-original draft.

SD: Conceptualization; Validation; Visualization; Writing-original draft.

References

1. Pine J, Stevenson L, On J. Intestinal stomas. Surg (United Kingdom) 2020;38(1):51–7.
2. Aktokmakyan TV, Ercetin C, Yigitbas H. A Retrospective Analysis of Determinants Affecting Intestinal Stomas. Clin J Surgery 2020;3(1):2–4.
3. Koc U, Karaman K, Gomceli I. A Retrospective Analysis of Factors Affecting Early Stoma Complications. Ostomy Wound Manage 2017;63(1):28–32.
4. Shabbir J, Britton DC. Stoma Complications: A Literature Overview. Colorectal Dis 2010;12(10):958–64.
5. Steinhagen E, Colwell J, Cannon LM. Intestinal Stomas--Postoperative Stoma Care and Peristomal Skin Complications. Clin Colon Rectal Surg 2017;30(3):184–92.
6. Tsujinaka S, Tan K-Y, Miyakura Y, Fukano R, Oshima M, Konishi F, et al. Current Management of Intestinal Stomas and Their Complications. J Anus, Rectum Colon 2020;4(1):25–33.
7. Taneja C, Netsch D, Rolstad BS, Inglese G, Lamerato L, Oster G. Clinical and economic burden of peristomal skin complications in patients with recent ostomies. J Wound, Ostomy Cont Nurs 2017;44(4):350–7.
8. Persson E, Berndtsson I, Carlsson E, Hallén AM, Lindholm E. Stoma-related Complications and Stoma Size - a 2-Year follow up. Colorectal Dis 2010;12(10):971–6.
9. Pilgrim CHC, McIntyre R, Bailey M. Prospective Audit of Parastomal Hernia: Prevalence and Associated Comorbidities. Dis Colon Rectum 2010;53(1):71–6.
10. Hsu MY, Lin JP, Hsu HH, Lai HL, Wu YL. Preoperative Stoma Site Marking Decreases Stoma and Peristomal Complications. J Wound Ostomy Cont Nurs 2020;47(June):249–56.
11. Bass EM, Del Pino A, Tan A, Pearl RK, Orsay CP, Abcarian H. Does Preoperative Stoma Marking and Education by the Enterostomal Therapist Affect Outcome? Dis Colon Rectum 1997;40(4):440–2.
12. Arolfo S, Borgiotto C, Bosio G, Mistrangelo M, Allaix ME, Morino M. Preoperative Stoma Site Marking: A Simple Practice to Reduce Stoma-Related Complications. Tech Coloproctol 2018;22(9):683–7.
13. Cottam J, Richards K, Hasted A, Blackman A. Results of a Nationwide Prospective Audit of Stoma Complications within 3 Weeks of Surgery. Color Dis 2007;9(9):834–8.
14. Colwell JC, Bain KA, Hansen AS, Droste W, Vendelbo G, James-Reid S. International Consensus Results: Development of Practice Guidelines for Assessment of Peristomal Body and Stoma Profiles, Patient Engagement, and Patient Follow-up. J Wound, Ostomy Cont Nurs 2019;46(6):497–504.
15. Kwiatt M, Kawata M. Avoidance and Management of Stomal Complications. Clin Colon Rectal Surg 2013;26(2):112–21.
16. Lindholm E, Persson E, Carlsson E, Hallén AM, Fingren J, Berndtsson I. Ostomy-related Complications After Emergent Abdominal Surgery a 2-Year follow-up Study. J Wound, Ostomy Cont Nurs 2013;40(6):603–10.

ORIGINAL RESEARCH

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Periodontal Health and Salivary Thiol-Disulphide Homeostasis in Multiple Sclerosis Patients

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Abstract

Objective
Multiple sclerosis (MS) is a chronic autoimmune disease in which neuroinflammation and oxidative stress play important roles in its pathology. Thiol-disulphide homeostasis is considered a marker of oxidative stress and shown to be affected in several disorders including MS. The aim of this study was to compare salivary disulfide and thiol levels in MS patients with systemically healthy controls and to evaluate whether periodontal status had an effect on thiol-disulfide homeostasis in saliva.

Material and Method
This descriptive study included a total of 184 volunteers, 92 with MS and 92 systemically healthy volunteers. Each person underwent medical, neurological and oral examinations. In saliva samples, native thiol (NT), total thiol (TT), disulphide levels were measured. The ratios of NT/TT, disulphide/NT, D/TT were calculated and compared between the patient and control groups.

Results
There was not any difference in the periodontal parameters between the MS and healthy volunteers ($p>0.05$), however, the biomarkers of thiol-disulphide homeostasis in saliva were significantly different between the groups ($p<0.002$), except for TT. When grouped according to periodontal status, although salivary parameters did not differ in both the MS and control groups ($p>0.05$), MS patients showed decreased NT/TT and increased disulphide/NT ratios compared to the healthy volunteers ($p<0.05$).

Conclusion
Our results have shown that salivary thiol-disulphide balance was shifted to the oxidative side in MS patients.

Keywords: Disulphide, multiple sclerosis, periodontitis, saliva, thiol

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Introduction

Multiple sclerosis (MS) is a heterogeneous, multi-factorial, immune-mediated disease of the central nervous system. It is one of the most frequent neurological diseases in young adults. Though the precise etiology of the disease is unknown, a complex interplay between genetic, epigenetic factors and abnormal immune responses leads to inflammation, demyelination, axonal loss, and gliosis which are the pathological hallmarks of the disease (1). Besides inflammation oxidative stress is also suggested as a preponderant key driver in the pathogenesis (2, 3). In both, inital and chronic stages of MS reactive oxgen species (ROS) and nitrogen species plays a pivotal role. ROS contribute to the formation of many pathological changes such as loss of blood-brain barrier integrity, demyelination, oligodendrocyte death and axonal degeneration in the central nervous system (2). The presence of an imbalance between oxidants and antioxidants, with increased concentrations of ROS in cerebrospinal fluid and blood of MS patients have consistently been reported as one of the common features in persons with MS (2-6).

Periodontitis causing destruction of the periodontium and subsequent teeth loss is a common chronic disorder. Studies report evidence of the relationship between periodontal disease and systemic diseases which are diabetes and cardiovascular disease etc (7).

MS patients may confront with many symptoms during their disease course. Loss of dexterity in MS patients due to sensory, motor or coordination problems and mobility restriction due to increasing disability may affect their ability to perform routine self-oral care. It has been reported that people with MS may be at higher risk of periodontal disease and present with poorer oral hygiene (8). Inflammation and oxidative stress are implicated as the common shared pathogenetic factor in these associations and support the link between the two disease (9-11).

Oxidative stress refers to the proportional imbalance between oxidants and antioxidants, characterized with increased ROS generation and relative deficiency of antioxidants. Generation of ROS, an evolutionarily conserved process, plays an important role in the cell signaling and defense mechanisms. Under normal physiological conditions, low concentrations of ROS have a critical role in various cellular processes such as proliferation, differentiation, and apoptosis whereas higher concentrations leads to tissue damage by triggering many pathophysiological processes such as autophagy, apoptosis, and necrosis (12). The antioxidant system, maintain cellular redox homeostasis and cellular integrity by modulating gene expression and various signaling pathways. Recently, dynamic thiol-disulfide homeostasis stands out as a new oxidative stress indicator in protection from oxidative stress and related mechanisms (13, 14). It has been suggested that dysregulated thiol-disulfide homeostasis has an important role in many diseases in which chronic inflammation is involved in the pathogenesis, such as MS and periodontitis (15-17).

The study aim was determined as salivary disulphide and thiol levels in MS patients and compared to systemically healthy individuals and evaluated whether periodontal status had an effect on thiol-disulphide homeostasis in saliva.

Material and Method

This study was carried out in the Department of Periodontology, Faculty of Dentistry, and the Department of Neurology, Faculty of Medicine at Süleyman Demirel University, Isparta, Türkiye, after obtaining approval from the Clinical Research Ethics Committee (13.12.2018/234). The volunteers were informed in accordance with the Declaration of Helsinki (2002 revision), and then written informed consent was obtained.

MS patients satisfying the criteria for definite MS according to McDonald criteria (18), aged over 18 years, had no neurological attack and corticosteroid use in the last 3 months were included in the study. Exclusion criteria for all participants were as follows: significant cognitive impairment, presence of any comorbid disease (cardiovascular disease, hematological disease, thyroid dysfunction, diabetes, obesity, menopause), pregnancy, breast-feeding, the medication causing gingival enlargement, periodontal treatment in the last 6 months and use of antibiotics and anti-inflammatory drugs in the last 3 months and 1month respectively.

A total of 184 volunteers, including MS patients (n=92) and systemically healthy controls (n=92), were included in the study. Attention was paid to gender and age matching between the groups. Each participant answered a questionnaire regarding the socio-demographics and habits. All participants underwent general medical and neurological examinations. The severity of MS was assessed with the Expanded Disability Status Scale (EDSS) (19). Current medications of MS patients were recorded.

Periodontal records [Plaque index (PI) (20), Gingival index (GI) (21), percentage of bleeding on probing (%BOP) (22), probing depth (PD), clinical attachment level (CAL)] of each participant were obtained after intraoral and radiological examinations. Periodontal status of each individual was classified (23). Intra-examiner analysis showed an intraclass correlation coefficient of 0.96 for PD and 0.94 for CAL measurement. Intra-examiner weighted k(-1 mm) values ranged from 0.84 to 0.93 for PD and 0.84 to 0.92 for CAL, respectively.

Salivary Sampling and Analysis

Unstimulated total saliva samples were taken before the intraoral examination. The salivary flow rate (SFR) of each participant was calculated (24). Samples were stored at –80 °C until laboratory analysis.

Total thiol (TT), native thiol (NT) and disulphide levels in saliva were determined by spectrophotometric method described by Erel and Neselioglu, (25). In this method, the reduction of dynamic disulfide bonds to free thiol groups with sodium borohydride (NaBH4) and the reaction of all thiol groups with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) are evaluated. NT (-SH), TT(-SH+-S-S) and disulfide [(TT-NT)/2] levels were determined as µmol/L. Disulphide/total thiol (D/TT) [-S-S-/(-SH + -S-S-)], disulphide/native thiol (D/NT) (-S-S-/-SH), and native thiol/total thiol (NT/TT) [-SH/(-SH + -S-S-)] ratios were calculated.

Statistics

The data were evaluated using the SPSS v. 20.0 package program (IBM, Chicago, IL, USA). Whether the variables met the parametric assumptions for the normal distribution was evaluated using the Kolmogorov-Smirnov test. Independent samples t-test and analysis of variance was used to compare salivary parameters according to MS with control groups and periodontal status factors for normally distributed continuous variables. Periodontal and salivary parameters were compared between MS subgroups also analyzed with generalized linear models, with age taken as the covariate variable. Chi-square test was performed to evaluate the relationships between categorical data. The significance level was determined as p= 0.05.

Results

Socio-demographics of the study groups are shown in Table 1. The individuals with MS and healthy controls had similar features in terms of age, gender, education level, income, smoking and tooth brushing frequency (p>0.05). Majority of the individuals with MS had relapsing-remitting MS (RRMS) (89%), the remaining 11% had progressive MS (2 primary progressive and 8 secondary progressive). The individuals with progressive MS (PMS) were older (43.70±7.26 years versus 35.78±9.66 years), had longer disease duration (8.30±4.83 years versus 5.81±4.39 years) and more disabled (EDSS score 4.5±1.08 versus 2.81±1.18) in comparison to RRMS group. PMS cases smoked more heavily and had poor oral hygiene practices than RRMS cases (Table1).

The clinical periodontal findings did not revealed any significant differences between healthy volunteers and patients with MS (p>0.05) (Table 2). Likewise, the percentages of periodontal status of individuals in both groups were similar (Table 2). However, PD, CAL and PI were significantly elevated in the individuals with PMS in comparison to the individuals with RRMS and also there was no periodontally healthy and gingivitis person in PMS group (Table 2).

TT levels were not different between MS patients and healthy controls or between people with RRMS and PMS. However, there was a significantly elevated concentration of NT and decreased concentration of disulphide in healthy controls in comparison to MS group (Table 3). Parallel to this, healthy control group had lower D/NT and D/TT ratios and higher NT/TT ratio than MS patients group. A reverse pattern was observed between RRMS and PMS. RRMS cases had lower native thiol and higher disulphide concentrations and also higher D/NT and D/TT ratios than those in PMS cases. NT/TT ratio was not different between RRMS and PMS groups but was higher in controls than those groups.

Oxidative stress parameters did not differed according to periodontal status neither in control nor in MS group. However, comparison exactly according to the periodontal status revealed higher NT/TT and lower D/NT ratios in healthy control group than MS patient group, in spite of similar NT, TT and disulphide concentrations (Table 4).

Discussion

Inflammation and oxidative stress are implicated as the common shared pathogenetic mechanism in the relationship between periodontal disease and systemic diseases. Dynamic thiol-disulphide homeostasis stands out as a novel oxidative stress indicator with its crucial role in antioxidant protection, detoxification, signal transduction, apoptosis, enzymatic activation, and regulation of cellular signaling mechanisms (13,14). Dysregulated thiol-disulphide homeostasis

Table 1 Sociodemographics

Groups / Parameters	Control (92) n(%)	MS (92) n(%)	p*	RRMS (82) n (%)	PMS (10) n (%)	p**
Gender [Female (%)]	60 (65.2)	60 (65.2)	1.000	57(69.5)	3(30)	**0.013**
Age (yrs)	36.8±11.5[a]	34.16±8.8	0.111	35.78±9.66[a]	43.70±7.26[b]	0.083
Education level Primary	28 (30.4)	29 (31.5)		24(29.3)	5 (50)	0.727
High school	20 (21.7)	22 (23.9)	0.896	20 (24.4)	2 (20)	
University	44 (47.8)	41 (44.6)		38 (46.3)	3 (30)	
Income (TL/month) < 2000	13 (14.1)[a]	17 (18.5)		14 (17.1)[a]	3 (30)[b]	0.686
2000-5000	71 (77.2)[a]	69 (75.0)	0.655	63 (76.8)[a]	6 (60)[a]	
> 5000	8 (8.7)[a]	6 (6.5)		5 (6.1)[a]	1 (10)[b]	
Smoking (cigarette/day) None	72 (78.3)[a]	64 (69.6)		61 (74.3)[a]	3 (30)[b]	**0.009**
<10	2 (2.2)[a]	3 (3.3)	0.405	3 (3.7)[a]	0 (0)[a]	
10-20	18 (19.6)[a]	25(27.2)		18 (22.0)[a]	7 (70)[b]	
Tooth brushing frequency < 1/day	13 (14.1)	17 (18.5)		10 (12.2)	7 (70)	**0.000**
1/day	36 (39.1)	36 (39.1)	0.695	33 (40.2)	3 (30)	
≥ 2/day	43 (46.7)	39 (42.4)		39 (47.6)	0 (0)	
EDSS		2.99±1.28		2.81±1.18	4.5±1.08	**0.000**
MS duration (yrs)	-	6.08±4.48		5.81±4.39	8.30±4.83	0.096
Drugs	-			INF: 27 (32.9) GA: 18 (22.0) TRF: 5 (6.1) DMF:16 (19.5) FNG:14(17.1) NTZ: 1(1.2) OCR: 1(1.2)	FAM:3(30) OCR:7(70)	

Sociodemographics are presented as n (%), except age, EDSS, MS duration (mean±standard deviation). MS: Multiple sclerosis, PMS: Progressive MS, RRMS: Relapse-remitting MS, DMF: dimethyl fumarate, FAM: fampridine, FNG: fingolimod; INF: interferon beta1a/b; GA: glatiramer acetate, NTZ: natalizumab; OCR: ocrelizumab; TRF: teriflunomide.

* Comparison between the MS and control groups, ** Comparison among MS subgroups and control (The PMS group consisted of 8 secondary progressive and 2 primary progressive MS cases). Bold denotes significance at $p<0.05$. Letters indicate differences among the MS subgroups and control.

Table 2 Periodontal data and the distribution of individuals according to their periodontal status

Groups	Control	MS	p*	RRMS	PMS	p**
Parameters						
PD	2.80±0.84[b]	2.76±0.93	0.780	2.67±0.85[b]	3.58±1.18[a]	**0.008**
CAL	2.83±2.23[b]	2.78±2.41	0.883	2.51±2.22[b]	5.03±2.81[a]	**0.050**
PI	1.39±0.31[b]	1.39±0.35	0.922	1.36±0.32[b]	1.66±0.47[a]	0.064
GI	1.30±0.24	1.28±0.26	0.706	1.27±0.25	1.42±0.33	0.386
BOP%	20.19±16.07	19.21±18.43	0.701	18.08±17.80	28.50±21.76	0.391
Teeth number	25.96±2.97	26.10±2.55	0.729	26.24±2.32	24.90±3.93	0.919
Periodontal status [n (%)]						
Healthy	26 (28.3)	28 (30.4)	0.428	28(34.1)	-	**0.033**
Gingivitis	4 (4.3%)	9 (9.8%)		9(11.0)	-	
P-S1	16 (17.4%)	18 (19.6%)		15(18.3)	3 (30)	
P-S2	28 (30.4%)	19 (20.7%)		17(20.7)	2(20)	
P-S3	18 (19.6%)	18 (19.6%)		13(15.9)	5(50)	

PD: Probing depth, CAL: Clinical attachment level, PI: Plaque index, GI: Gingival index, BOP %: Percentage of bleeding on probing, P-S1,2,3: Periodontitis - Stage 1,2,3, Bold denotes statistically significance at p<0.05. * Comparison between MS and control groups, ** Comparison among MS subgroups and control. Letters indicate differences among the MS subgroups and control.

Table 3 Salivary parameters in MS and control groups

Groups Parameters	Control	MS	p*	RRMS	PMS	p**
SFR (mL/min)	0.26±0.06	0.27±0.05	0.377	0.27±0.05	0.22±0.06	0.219
TT (µmol/L)	12.56 ±2.03	12.52±2.80	0.908	12.51±2.79	12.57±3.02	0.991
NT (µmol/L)	9.74 ±2.04 [a]	8.59±2.08	0.000	8.58 ±2.15 [b]	8.71 ±1.46 [ab]	0.001
Disulphide (µmol/L)	1.41 ±0.86 [b]	1.96±1.44	0.002	1.97 ±1.46 [a]	1.93 ±1.31 [ab]	0.008
NT/TT	0.78 ±0.12 [a]	0.71±0.17	0.001	0.71 ±0.17 [b]	0.71 ±0.14 [b]	0.004
Disulphide /NT	0.16 ±0.12 [b]	0.27±0.32	0.002	0.28 ±0.34 [a]	0.23 ±0.16 [ab]	0.006
Disulphide /TT	0.11 ±0.06 [b]	0.15±0.09	0.001	0.15 ±0.09 [a]	0.14 ±0.07 [ab]	0.004

SFR: Saliva flow rate, TT: Total thiol, NT: Native thiol. * Comparison between MS and control groups, ** Comparison among MS subgroups and control. Bold denotes statistical significance at p<0.05. Letters indicate differences among the MS subgroups and control.

has been suggested to have a pivotal role in the pathogenesis of many diseases, especially diseases characterized by chronic inflammation including MS and periodontitis (15-17).

In this study, we determined salivary disulphide and thiol levels in MS patients and compared to systemically healthy controls and evaluated whether periodontal status had an effect on thiol-disulphide homeostasis in saliva. Previous studies have reported conflicting results on periodontal health in MS (8,26,27). We did not found any difference between MS patients and healthy controls in terms of periodontal parameters.

Table 4 The salivary parameters according to the periodontal status.

Groups:		MS		Control		
Parameters	**Periodontal status**	**Mean±SD**	**p***	**Mean±SD**	**p***	**p****
TT(µmol/L)	Healthy	13,22±2,67	0.267	12,79±2,29	0,450	0,528
	Gingivitis	12,09±3,27		10,89±1,14		0,283
	P-S1	13,03±3,43		12,93±1,38		0,137
	P-S2	12,19±2,60		12,40±2,32		**0,025**
	P-S3	11,49±2,09		12,53±1,73		0,194
NT(µmol/L)	Healthy	9,43±2,21	0,070	10,08±2,19	0,279	0,179
	Gingivitis	8,90±2,10		8,90±1,40		0,194
	P-S1	8,03±1,89		10,02±2,16		0,496
	P-S2	7,86±2,20		9,12±2,02		1,000
	P-S3	8,46±1,56		10,17±1,76		0,423
Disulphide (µmol/L)	Healthy	1,89±1,65	0,260	1,35±0,80	0,359	0,662
	Gingivitis	1,59±1,35		0,99±0,70		0,510
	P-S1	2,50±1,44		1,46±0,85		0,474
	P-S2	2,16±1,52		1,64±0,95		0,510
	P-S3	1,51±0,84		1,18±0,83		0,914
NT/TT	Healthy	0,74±0,18	0,142	0,79±0,11	0,284	**0,009**
	Gingivitis	0,76±0,16		0,82±0,11		0,767
	P-S1	0,64±0,15		0,77±0,14		**0,050**
	P-S2	0,66±0,20		0,74±0,12		0,157
	P-S3	0,74±0,12		0,82±0,12		**0,022**
Disulphide /NT	Healthy	0,27±0,47	0,547	0,15±0,10	0,348	0,102
	Gingivitis	0,19±0,19		0,12±0,09		**0,033**
	P-S1	0,33±0,22		0,17±0,15		0,102
	P-S2	0,34±0,32		0,19±0,12		0,113
	P-S3	0,19±0,12		0,13±0,11		**0,004**
Disulphide /TT	Healthy	0,13±0,09	0,142	0,10±0,06	0,284	0,241
	Gingivitis	0,12±0,08		0,09±0,06		0,067
	P-S1	0,18±0,07		0,11±0,07		0,079
	P-S2	0,17±0,10		0,13±0,06		0,120
	P-S3	0,13±0,06		0,09±0,06		0,079

TT: Total thiol, NT: Native thiol, P-S1,2,3: Periodontitis - Stage 1,2,3, p*: Within group differences, p**: Between groups differences, Bold denotes significance at p<0.05.

MS often occurs in young adults and affects women more than men. The MS group consisted of young adults and mostly female individuals (27-29). In this study, the sociodemographic features were not different between MS patients and systemic healthy controls, however, the individuals with PMS were older and mostly male, smoked more, had longer disease duration and more disabled in comparison to RRMS group. Also, they had worse EDDS scores and periodontal parameters than the patients with RRMS (28,29). The individuals with PMS had more disability, smoked more cigarettes and brushed their teeth less frequently, which may contribute to deterioration of periodontal health. Smoking is a risk factor for the development and progression of both periodontal disease and MS; in MS the risk increases with duration and intensity and also more robust in males (1,27).

Karim et al (30) have reported higher salivary thiol levels in periodontally healthy controls compared to gingivitis and periodontitis patients and found a negative relation between the severity of periodontitis and thiol level. Tayman et al. (17) have detected lower plasma NT and TT and higher disulphide levels in periodontitis patients than periodontally healthy controls. Unlike the results of these studies, when individuals were compared within the group according to their periodontal status, thiols and disulphide levels in saliva did not differ in either the control group or the MS group. The majority of individuals in the study had localized periodontal disease which may have led to the lack of differences in thiol homeostasis among the periodontal disease subgroups.

Demiröğen et al (31) have reported lower plasma NT and TT levels in patients with SPMS than the naïve MS and healthy controls but disulfide, D/NT, D/TT, NT/TT ratios were similar between the study groups. Arslan et al (32) have found that serum TT and NT levels in relapsed patients were significantly lower than those in remission. In this study, salivary TT levels were not different either between MS patients and healthy controls or between patients with RRMS and PMS. However, there were significantly decreased levels of the NT, NT/TT ratios and increased levels of the disulphide, D/NT, D/TT ratios in MS group in comparison to healthy controls. These significant differences were actually in the comparisons between the RRMS group and the control group data, except NT/TT. These findings may be due to an immune milieu shifted into a more inflammatory one in RRMS (2-6).

That lower NT/TT and higher D/NT ratios were observed in MS patients than systemically healthy controls when compared by periodontal status was though that thiol-disulphide balance in saliva could shifted to the oxidative side in MS patients. In this cross-sectional study, age and sex were matched between MS and systemically healthy volunteers. However, there was not naïve MS patient and the percentage of PMS was relatively few in the study group which may be thought as limitations of the study. Long-term studies with naïve MS patients undergoing periodic periodontal examination may shed light on the shared pathophysiological mechanisms between MS and periodontal disease. Immunomodulatory drugs the MS patients are on may cause immune deviations towards an anti-inflammatory phenotype in targeted brain tissue and also in peripheral immune system (33). In spite anti-inflammatory effect of immunomodulator drugs, the presence of more significant oxidative stress in RRMS supports further the underlying inflammation in the pathogenesis of the disease.

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

Ethical Approval
This study was conducted after the approval of the Clinical Research Ethics Committee of the Faculty of Medicine (approval no: 13.12.2018/234). The volunteers participating in the study were informed in accordance with the Declaration of Helsinki (2002 revision).

Consent to Participate and Publish (If applicable)
Written informed consent was obtained from the volunteers participating in the study.

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Availability of Data and Materials
Authors can confirm that all relevant data are included in the article.

Authors Contributions
FYK: Conceptualization; Project administration; Methodology; Data curation; Validation; Visualization; Supervision; Writing-original draft; Writing-review and editing.

SD: Conceptualization; Project administration; Methodology; Data curation; Validation; Visualization; Supervision; Writing-original draft; Writing-review and editing.

ÇV: Project administration; Formal analysis; Investigation; Validation; Writing-original draft.

Table 4 The salivary parameters according to the periodontal status.

Groups:		MS		Control		
Parameters	**Periodontal status**	**Mean±SD**	**p***	**Mean±SD**	**p***	**p****
TT(µmol/L)	Healthy	13,22±2,67	0.267	12,79±2,29	0,450	0,528
	Gingivitis	12,09±3,27		10,89±1,14		0,283
	P-S1	13,03±3,43		12,93±1,38		0,137
	P-S2	12,19±2,60		12,40±2,32		**0,025**
	P-S3	11,49±2,09		12,53±1,73		0,194
NT(µmol/L)	Healthy	9,43±2,21	0,070	10,08±2,19	0,279	0,179
	Gingivitis	8,90±2,10		8,90±1,40		0,194
	P-S1	8,03±1,89		10,02±2,16		0,496
	P-S2	7,86±2,20		9,12±2,02		1,000
	P-S3	8,46±1,56		10,17±1,76		0,423
Disulphide (µmol/L)	Healthy	1,89±1,65	0,260	1,35±0,80	0,359	0,662
	Gingivitis	1,59±1,35		0,99±0,70		0,510
	P-S1	2,50±1,44		1,46±0,85		0,474
	P-S2	2,16±1,52		1,64±0,95		0,510
	P-S3	1,51±0,84		1,18±0,83		0,914
NT/TT	Healthy	0,74±0,18	0,142	0,79±0,11	0,284	**0,009**
	Gingivitis	0,76±0,16		0,82±0,11		0,767
	P-S1	0,64±0,15		0,77±0,14		**0,050**
	P-S2	0,66±0,20		0,74±0,12		0,157
	P-S3	0,74±0,12		0,82±0,12		**0,022**
Disulphide /NT	Healthy	0,27±0,47	0,547	0,15±0,10	0,348	0,102
	Gingivitis	0,19±0,19		0,12±0,09		**0,033**
	P-S1	0,33±0,22		0,17±0,15		0,102
	P-S2	0,34±0,32		0,19±0,12		0,113
	P-S3	0,19±0,12		0,13±0,11		**0,004**
Disulphide /TT	Healthy	0,13±0,09	0,142	0,10±0,06	0,284	0,241
	Gingivitis	0,12±0,08		0,09±0,06		0,067
	P-S1	0,18±0,07		0,11±0,07		0,079
	P-S2	0,17±0,10		0,13±0,06		0,120
	P-S3	0,13±0,06		0,09±0,06		0,079

TT: Total thiol, NT: Native thiol, P-S1,2,3: Periodontitis - Stage 1,2,3, p*: Within group differences, p**: Between groups differences, Bold denotes significance at p<0.05.

MDÜ: Project administration; Formal analysis; Investigation; Validation; Writing-original draft.

MC: Formal analysis; Writing-original draft.

HO: Formal analysis; Writing-original draft.

References

1. Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple Sclerosis. Lancet 2018;391(10130): 1622-1636. doi:10.1016/ S0140-6736(18)30481-1
2. van Horssen J, Witte ME, Schreibelt G, de Vries HE. Radical Changes in Multiple Sclerosis Pathogenesis. Biochim Biophys Acta 2011;1812(2):141-150. doi: 10.1016/j.bbadis.2010.06.011
3. Karlík M, Valkovič P, Hančinová V, Krížová L, Tóthová Ľ, Celec P. Markers of Oxidative Stress in Plasma and Saliva in Patients with Multiple Sclerosis. Clin Biochem 2015;48(1-2):24-28. doi: 10.1016/j.clinbiochem.2014.09.023
4. Minohara M, Matsuoka T, Li W, Osoegawa M, Ishizu T, Ohyagi Y, Kira J. Upregulation of Myeloperoxidase in Patients with Opticospinal Multiple Sclerosis: Positive Correlation with Disease Severity. J Neuroimmunol 2006;178(1-2):156-160. doi: 10.1016/j.jneuroim.2006.05.026
5. Acar A, Cevik MU, Evliyaoglu O, Uzar E, Tamam Y, Arıkanoglu A, Yavuz Y, Varol S, Onder H, Taşdemir N. Evaluation of Serum Oxidant/Antioxidant Balance in Multiple Sclerosis. Acta Neurol Belg 2012;112(3):275-80. doi: 10.1007/s13760-012-0059-4
6. Zhang S-Y, Gui L-N, Liu Y-Y, Shi S, Cheng Y. Oxidative Stress Marker Aberrations in Multiple Sclerosis: A Meta-Analysis Study. Front. Neurosci 2020;14:823. doi: 10.3389/fnins.2020.00823
7. Albandar JM, Susin C, Hughes FJ. Manifestations of Systemic Diseases and Conditions that Affect the Periodontal Attachment Apparatus: Case Definitions and Diagnostic Considerations. J Clin Periodontol 2018;45(20):171-189. doi: 10.1111/jcpe.12947
8. Manchery N, Henry JD, Nangle MR. A Systematic Review of Oral Health in people with Multiple Sclerosis. Community Dent Oral Epidemiol 2020;48(2):89-100. doi: 10.1111/cdoe.12512
9. D'Aiuto F, Nibali L, Parkar M, Patel K, Suvan J, Donos N. Oxidative Stress, Systemic Inflammation, and Severe Periodontitis. J Dent Res 2010;89(11):1241–1246. doi: 10.1177/0022034510375830
10. Wang J, Schipper HM, Velly AM, Mohit S, Gornitsky M. Salivary Biomarkers of Oxidative Stress: A Critical Review. Free Radic Biol Med 2015;85:95-104. doi: 10.1016/j.freeradbiomed.2015.04.005
11. Al-Ansari A. Is There an Association Between Multiple Sclerosis and Oral Health? Evid Based Dent 2021;22(1):44-45. doi: 10.1038/s41432-021-0159-1
12. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem 2017;44(2):532-553. doi: 10.1159/000485089
13. Erel Ö, Erdoğan S. Thiol-disulfide Homeostasis: An Integrated Approach with Biochemical and Clinical Aspects. Turk J Med Sci 2020;50(SI-2):1728-1738. doi: 10.3906/sag-2003-64
14. Biswas S, Chida AS, Rahman I. Redox Modifications of Protein-Thiols: Emerging Roles in Cell Signaling. Biochem Pharmacol 2006;71:551–564. doi: 10.1016/j.bcp.2005.10.044
15. Vural G, Gümüşyayla Ş, Deniz O, Neşelioğlu S, Erel Ö. Relationship Between Thiol-Disulphide Homeostasis and Visual Evoked Potentials in Patients with Multiple Sclerosis. Neurol Sci 2019;40(22):385–391. doi:10.1007/s10072-018-3660-3
16. Ozben S, Kucuksayan E, Koseoglu M, Erel O, Neselioglu S, Ozben T. Plasma Thiol/disulphide Homeostasis Changes in Patients with Relapsing-Remitting Multiple Sclerosis. Int J Clin Pract 2021;75(7):14241. doi: 10.1111/ijcp.14241
17. Tayman MA, Bal C, Nural C, Günhan M. Evaluation of Dynamic Thiol/Disulfide Homeostasis in Patients with Periodontitis. Meandros Med Dent J 2021;22:41-49. doi: 10.4274/meandros.galenos.2020.14622
18. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J et al. Diagnosis of Multiple Sclerosis: 2017 Revisions of the McDonald Criteria. Lancet Neurol 2017;17(2):162–173. doi: 10.1016/S1474-4422(17)30470-2
19. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An Expanded Disability Status Scale (EDSS). Neurology 1983;33(11):1444-1452. doi: 10.1212/wnl.33.11.1444
20. Silness J, Loe H. Periodontal Disease in Pregnancy. II. Correlation Between Oral Hygiene and Periodontal Condition. Acta Odontol Scand 1964;22:121-135. doi: 10.3109/00016356408993968
21. Loe H, Silness J. Periodontal Disease in Pregnancy. I. PrevalenceaAnd Severity. Acta Odontol Scand 1963;21:533-51. doi: 10.3109/00016356309011240
22. Ainamo J, Bay I. Problems and Proposals for Recording Gingivitis and Plaque. Int Dent J 1975;25(4):229-35. PMID: 1058834
23. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus Report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018;45(20):162-170. doi: 10.1111/jcpe.12946
24. Gümüş P, Nizam N, Lappin DF, Buduneli N. Saliva and Serum Levels of B-cell Activating Factors and Tumor Necrosis Factor-α in Patients with Periodontitis. J Periodontol 2014;85(2):270–280. doi: 10.1902/jop.2013.130117
25. Erel O, Neselioglu S. A Novel and Automated Assay for Thiol/Disulphide Homeostasis. Clin Biochem 2014;47(18):326-332. doi: 10.1016/j.clinbiochem.2014.09.026
26. Sheu JJ, Lin HC. Association Between Multiple Sclerosis and Chronic Periodontitis: A Population-Based Pilot Study. Eur J Neurol 2013;20:1053-1059. doi: 10.1111/ene.12103
27. Gustavsen MW, Celius EG, Moen SM, Bjølgerud A, Berg-Hansen P, et al. No Association Between Multiple Sclerosis and Periodontitis After Adjusting for Smoking Habits. Eur J Neurol 2015;22:588-590. doi: 10.1111/ene.12520
28. Hatipoglu H, Canbaz SK, Gungor MH, Ozden H. Expanded Disability Status Scale-Based Disability and Dental-Periodontal Conditions in Patients with Multiple Sclerosis. Med Princ and Pract 2016;25(1):49-55. doi: 10.1159/000440980
29. Dulamea AO, Boscaiu V, Sava MM. Disability Status and Dental Pathology in Multiple Sclerosis Patients. Mult Scler Relat Disord 2015;4(6):567-571. doi: 10.1016/j.msard.2015.09.001
30. Karim S, Pratibha PK, Kamath S, Bhat GS, Kamath U, et al. Superoxide Dismutase Enzyme and Thiol Antioxidants in Gingival Crevicular Fluid and Saliva. Dent Res J (Isfahan) 2012;9(3):266-272. PMID: 23087730
31. Demirdöğen BC, Kılıç OO, Yılmaz AA, Mungan S, Neşelioğlu S, Erel Ö. Neurocognitive Impairment in Multiple Sclerosis and its Association with Thiol-Disulfide Homeostasis and Ischemia-Modified Albumin. J Neurosci Res 2023;101(4):508-523. doi: 10.1002/jnr.25163
32. Arslan B, Arslan GA, Tuncer A, Karabudak R, Dinçel AS. Evaluation of Thiol Homeostasis in Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorders. Front. Neurol 2021;12:716195. doi: 10.3389/fneur.2021.716195
33. Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. Am J Med 2020;133(12):1380-1390. doi: 10.1016/j.amjmed.2020.05.049

ORIGINAL RESEARCH

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Diagnostic Efficacy of Natriuretic Peptide: NT-ProBNP in Hemodialysis Patients with Left Ventricular Failure

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Abstract

Objective

In the general population, plasma concentrations of natriuretic peptides such as brain natriuretic peptide (BNP), are useful markers to predict left ventricular hypertrophy and left ventricular (LV) systolic dysfunction. Left ventricular hypertrophy in dialysis patients is exceedingly frequent and predicts mortality in these patients. LV systolic dysfunction is also frequent due to high coronary artery disease (CAD) prevalence in this population. The present study aimed to evaluate the clinical diagnostic potential of N-terminal pro-brain natriuretic peptide (NT-proBNP); as an indicator for left ventricular (LV) systolic dysfunction and left ventricular mass (LVM), in chronic hemodialysis patients.

Material and Method

76 patients with end-stage renal disease (54 males and 22 females, mean age 60.51±13.96 years) who had been on regular hemodialysis treatment twice or three times a week were enrolled in this study. Patients were divided into two groups based on left ventricular ejection fraction (LVEF). Left ventricular systolic dysfunction was defined as LVEF ≤40%. Basic biochemical parameters, NT-proBNP, and echocardiographic parameters including left ventricular mass (LVM) and left ventricular mass index (LVMI) were measured.

Results

Mean concentration of serum NT-proBNP was 8333 (208-35000) pg/ml, and this parameter was not significantly different between the two groups (13136 (361-35000) pg/ml vs. 6617 (20-33805) pg/ml, p:0.16). Multivariate analysis results of logarithmic transformed NT-proBNP showed correlation only with RVEF. The left ventricular end-systolic diameter was significantly lower in the normal LV systolic function group (35.4±8.2 vs. 31.3±7.1 mm, p:0.04).

Conclusion

Our findings suggest that NT-proBNP is inadequate to determine LV systolic dysfunction in chronic hemodialysis patients.

Keywords: Hemodialysis, left ventricular systolic dysfunction, NT-proBNP

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Introduction

Across the whole population, plasma concentrations of natriuretic peptides like BNP are beneficial in predicting LV dysfunction and LVH. BNP is more often produced by ventricular myocytes (1). BNP release is increased by ventricular dysfunction or LVH (1-3). BNP is first produced as inactive pro-hormone and splits up into two fragments. The first is c-BNP, which is an active fragment, and the second is the NT-proBNP. Both can be measured in plasma or serum (4-6).

Chronic kidney disease (CKD) is a common clinical condition in which ANP and BNP are usually elevated (7). Only a few clinical studies have demonstrated the clinical diagnostic potential of NT-proBNP, particularly in CKD patients with LV dysfunction and LVH who require hemodialysis. (7-9). In addition, LVH is extremely common in dialysis patients and predicts mortality in these patients (10).

Because of this history, we hypothesized in the present study that NT-proBNP is a beneficial serum biomarker predicting LV dysfunction and LVM in CKD patients on hemodialysis (HD) treatment.

Material and Method

This study included 76 CKD patients (54 males and 22 females; mean age 60.51±13.96 years) receiving regular hemodialysis treatment two/three times a week. All participants had admitted to the Suleyman Demirel University Dialysis Center; Isparta/Turkey, between March 2023 and April 2023.

The patients were divided into two groups according to their LV ejection fraction (LVEF). LV systolic dysfunction was defined as LVEF ≤40%. Patients participating in this study had no rhythm disturbances during the follow-up period. Our study exclusion criteria were; patients on peritoneal dialysis treatment, acute decompensated heart failure (NYHA Class III-IV), chronic liver disease, chronic obstructive airway disease (COPD), mild or severe valvular heart dysfunction, and patients who refused to give study constent.

All study populations were anuric (24-hour urine volume ≤ 200 mL/day) and they all were on standard bicarbonate dialysis treatment (Na 138 mmol/L, HCO3 35mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) or by high-flux HD with cuprophan or other semisynthetic membranes (dialysis filters surface area: 1.1 to 1.7 m2) two or three times weekly. Thirty-three patients were habitual smokers (43.4%).

The participants were given detailed information about our research. Then, an informed consent form was signed by the participants. The study was prepared per the Declaration of Helsinki and was approved by the ethics committee for clinical research at Suleyman Demirel University Medical Faculty (Date: 06/03/2023, No: 39). All studies were performed during a day without dialysis.

Echocardiography

2-D echocardiography examinations were done by a single cardiologist. The clinician was blinded to all clinical data of patients. While echocardiography was performed, participants were lying in the left decubitus position. GE-VingMed System 5 echocardiography machine (GE-VingMed Sound AB, Horten, Norway) with a 2.5 MHz FPA probe was used during the examination.

Fluid status or volume overload was not evaluated at enrollment. However, for all patients, TTE was performed on an interdialytic day in the evaluation phase for standardization. LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), thickness of interventricular septum (IVS), left ventricular posterior wall (LVPW) and LV mass (LVM) were calculated according to the guidelines of the American Society of Echocardiography (11-12).

LV systolic dysfunction was defined as LVEF of ≤40%. LVM was measured by the Devereux Formula and indexed to height2.7 to calculate the LVM index (LVMI) (13). LVM was indexed by height rather than body surface area to minimize possible disruption from extracellular volume overload. LVH was stated if LVMI was higher than 47 g/m2.7 in women and 50 g/m2.7 in men. LVEF was achieved from apical two and four-chamber views using a modified biplane Simpson's method (14).

Biochemical Analysis

At enrollment, a 5-ml blood sample was collected into a plastic tube containing potassium EDTA (1 mg/ml blood). Within five hours, the samples were centrifuged at 1,000 relative centrifugal force for 15 minutes at 4 °C then plasma samples were protected in plastic bottles at -70 °C to assess NT-proBNP, albumin, hemoglobin, and C-reactive protein (CRP).

Serum NT-proBNP was measured with radio-immunoassay kits (Roche Diagnostics GmbH, D-68298 Mannheim) by electrochemiluminescence immunoassay on the Immulite 2000 analyzer

(Siemens, USA) and in a range of 5 to 35,000 pg/ml. If NT-proBNP sample concentrations are above the higher measuring range, will be taken as 35,000 pg/ml. Measurements of CRP quantified by micro ELISA (Enzyme-Linked Immunosorbent Assay) on Immulite 2000 analyzer (Siemens, USA).

Statistical Analysis

Statistical analysis was performed by using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± standard deviation or as median. Student's t-test and x2 test were used to compare continuous and discontinuous variables between the two groups, respectively.

Regression analysis was used to measure the correlation of other patient characteristics with NT-proBNP and it was log-transformed for skewed distribution before entering the linear regression model. The correlation between serum NT-proBNP and echocardiographic parameters was compared between groups. p-value < 0.05 was considered to be statistically significant.

Results

Patient's Characteristics

The basic demographic characteristics of all study patients are detailed in Table 1. Fifty-five patients (72%) were treated with erythropoietin, and fifty-six patients (74%) were taking angiotensin-converting enzyme (ACE) inhibitors monotherapy, angiotensin II type I (AT1) antagonists, and calcium channel blockers. Twenty patients (26%) were on combination therapy with various compositions of these drugs.

The clinical characteristics of the two groups are presented in Table 2. The mean age was 62.3±9.9 years in patients with LV systolic dysfunction and 59.9±15.1 years in patients with normal LV systolic function group; this finding was not statistically significant. The mean systolic blood pressure was 136 ± 24 mmHg, and the mean diastolic blood pressure was 87 ± 10 mmHg in the total patient population. There was no significant difference in systolic and diastolic blood pressure between groups (139 ± 20 mmHg vs. 135 ± 25 mmHg, p=0.54, and 90 ± 11 mmHg vs. 87 ± 10

Table 1 Demographic, anthropometric and hemodynamic characteristics of all study population

Characteristic	Values , n= 76
Age (Year)	60.5±13.9
Men / Women	54/22
BMI (kg/m²)	26±5
Duration of dialysis treatment (Months)	67±39
Hypertansion (%)	28 (%37)
Diabetes Mellitus (%)	26 (%34)
Hyperlipidemia (%)	24 (%31)
Systolic blood pressure (mmHg)	136±24
Diastolic blood pressure (mmHg)	87±10
Heart rate (b.p.m)	78±10
Myocardial infarction (%)	19 (%25)
Stroke (%)	15 (%20)
Peripheral vascular disease (%)	8 (%10)
Drug therapy	
Erythropoietin	55 (%72)
ACE inhibitors	24 (%32)
Calcium channel blockers	17 (%22)
β blockers	21 (%28)
AT1 antagonist or α blockers	16 (%21)
Double or triple therapy	20 (%26)

Unless specified otherwise, data are means ± SD. Percentages may not add up to 100 due to rounding off of decimal places.

Table 2 Demographic, anthropometric and hemodynamic characteristics of two study groups

Characteristic	EF≤40, n= 20	EF>40, n=56	P value
Age (Year)	62.3±9.9	59.9±15.1	0.50
Men / Women	16/4	38/18	0.39
BMI (kg/m²)	25±5	26±5	0.31
Duration of dialysis treatment* (Months)	56 (14-108)	71 (16-231)	0.48
Hypertansion (%)	6 (%30)	22 (%39)	0.59
Diabetes Mellitus (%)	6 (%30)	20 (%36)	0.78
Hyperlipidemia (%)	10 (%50)	14 (%25)	0.05
Systolic blood pressure (mmHg)	139±20	135±25	0.54
Diastolic blood pressure (mmHg)	90±11	87±10	0.60
Heart rate (b.p.m)	79±11	75±10	0.23
Myocardial infarction (%)	15 (%75)	4 (%7)	<0.01
Stroke (%)	4 (%20)	11 (%20)	0.99
Peripheral vascular disease (%)	5 (%25)	3(%5)	0.02
Drug therapy Erythropoietin	14 (%70)	41 (%73)	0.77
ACE inhibitors	11 (%55)	13 (%23)	0.01
Calcium channel blockers	4 (%20)	13 (%23)	0.99
β blockers	8 (%40)	13 (%23)	0.16
AT1 antagonist or α blockers	7 (%35)	9 (%16)	0.10
Double or triple therapy	7 (%35)	13 (%23)	0.37

Data are expressed as mean ±SD or as median *(interquartile range), as appropriate.

Table 3 Main laboratory findings of patients

Characteristic	Total, n= 76	EF<40, n= 20	EF>40, n=56	P value
NT-pro BNP (pg/ml) *	8333 (208-35000)	13136 (361-35000)	6617 (20-33805)	0.16
Log-transformed NT-pro BNP	3.61±0.57	3.79±0.60	3.55±0.54	0.10
Serum albumin (g/dL)	3.8±0.4	3.7±0.4	3.8±0.4	0.72
Serum C-reactive protein (mg/L)*	1.96 (0.02-20.4)	1.68 (0.28-4.10)	2.07 (0.02-20.44)	0.46
Serum cholesterol (mg/dl)	175±34	191±38	169±34	0.04
Serum phosphate (mg/dl)	4.7±1.0	4.5±0.8	4.8±1.0	0.24
Serum calcium (mg/dl)	9.1±1.0	9.4±0.9	9.0±1.0	0.05
Serum iPTH (pg/ml)*	473 (16-2500)	454 (101-1051)	480 (16-2500)	0.75

Data are expressed as mean ±SD or as median *(interquartile range), as appropriate.

Table 4 Echocardiographic findings of patients

Characteristic	EF≤40, n= 20	EF>40, n=56	P value
LVEF (%)	34.9±5.8	58.5±5.2	<0.01
LVEDD (mm)	48.1±7.0	48.5±6.2	0.84
LVESD (mm)	35.4±8.2	31.3±7.1	0.04
LVPW (mm)	14.2±2.4	14.0±2.4	0.65
IVS (mm)	13.6±2.4	12.8±2.3	0.20
LVMI (g/height$^{2.7}$)	272.6±51.9	267.0±82.7	0.77
LVM (g)	73.7±14.0	74.0±24.6	0.95
LA (mm)	35±7.1	31±5.2	0.04
TR jet velosity (m/s)	3.1±0.9	2.8±0.7	0.7
PAPs (mmHg)	38±7.6	31±6.9	0.06

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, LVPW: Left ventricular posterior wall thickness, IVS: Interventricular septum thickness, LVMI: Left ventricular mass index, LVM: Left ventricular mass, LA: Left atrium diameter, TR: Tricuspid regurgitation, PAPs: estimated pulmonary artery peak systolic pressure

mmHg, p=0.60). The history of myocardial infarction and antihypertensive therapy with ACEi were more common in the LV systolic dysfunction group; and these differences were statistically significant (p<0.01 and p=0.01, respectively).

Laboratory Findings

Table 3 summarizes the main laboratory findings of two study populations. There was no significant difference in mean serum levels of the following parameters groups, respectively; hemoglobin (11±1.1 g/dL vs. 11±1.2 g/dL; p:0.72), serum albumin (3.7±0.4 g/dL vs 3.8±0.4 g/dL, p:0.72), serum phosphate concentration (4.5±0.8 mmol/L vs. 4.8±1.0 mmol/L, p:0.24), serum iPTH concentration (454 (101-1051) pg/ml vs. 480 (16-2500) pg/ml, p:0.75) and serum C-reactive protein (1.68 (0.28-4.10) mg/L vs. 2.07 (0.02-20.44) mg/L, p:0.46). Serum total cholesterol (191±38 mg/dl vs. 169±34 mg/dl, p:0.04) and serum calcium (9.4±0.9 mmol/L vs. 9.0±1.0 mmol/L, p:0.05) levels were significantly higher in the LV systolic dysfunction group.

The mean concentration of serum NT-pro-BNP was 8333 (208-35000) pg/ml, and this parameter was not significantly different between the two groups (13136 (361-35000) pg/ml vs. 6617 (20-33805) pg/ml, p:0.16). NT-pro-BNP is not a significant predictor of a history of MI, peripheral vascular disease, ACEi usage, total cholesterol concentrations, LVEF, or LV end-systolic diameter (LVESD) in either univariate and the multivariable Cox regression models. In multivariate analysis, log-transformed NT-pro-BNP correlated only with RVEF (β = 0.390, P = 0.001).

The echocardiographic findings of the patients are presented in Table 4. The ejection fraction (EF) was significantly lower in the LV dysfunction group than the normal LV systolic function group (34.9±5.8% vs. 58.5±5.2%, respectively, p<0.01). There were no statistically significant differences between the two groups concerning LVEDD, IVS, LVPW, LVM, and LVMI. However, LVESD was significantly lower in the normal LV systolic function group than in the LV systolic dysfunction group (35.4±8.2 vs. 31.3±7.1 mm, p:0.04).

Discussion

Previous studies showed that BNP and NT-proBNP are mean diagnosting parameters in ventricular dysfunction (15-17). They also predict prognosis in heart failure (HF) (18-21). These parameters are also useful in excluding HF in the general population. They are highly sensitive and specific (over 85%) and increase the diagnostic precision of heart failure from 73% to 84% (15, 22, 23). In addition, the measurement of the plasma concentrations of cardiac natriuretic peptides, such as NT-proBNP, in patients with CKD proved to be beneficial for the diagnosis

of LVH and LV dysfunction. LVH and LV dysfunction are recently recognized as the strongest predictors of cardiovascular and total mortality in the dialysis population (24, 25). Volume overload and higher LV filling pressures are the main factors of LVH in both healthy subjects and renal insufficiency patients (26). Since elevated ventricular mass and pressure load independently increase natriuretic peptide synthesis, the plasma values of these peptides are strongly correlated with cardiac mass and function (27-32).

BNP is a peptide hormone that is secreted in response to muscular relaxation in the left ventricle and is converted to two biologically active forms: c-BNP and N terminal proBNP. N-terminal proBNP has a prolonged half-life and is more biologically stable than c-BNP, and it has greater clinical significance (1).

In our study, we demonstrated that NT-pro-BNP level was numerically higher in the LV systolic dysfunction group than in the normal LV systolic function group, however, it did not reach to statistical significance level (13136, 6617; P = 0.16). Log-transformed NT-pro-BNP concentration was also similar between the two study groups. In addition, LVM (g) and LVMI (g/m 2.7) were also similar between the two groups. There is a significant interaction between systolic dysfunction and total cholesterol levels, and it was higher in the systolic dysfunction group.

Many recent studies have been conducted on correlations between BNP level, cardiac function, and patient prognosis. Kim et al. (33) and Goto et al. (34) published that serum BNP concentration was inversely proportional to LV ejection fraction. Nitta et al. (35) showed that the mean serum BNP concentration in hemodialysis patients was higher than in normal healthy individuals, and there was an inverse correlation between serum BNP concentration and LV function.

There are few studies evaluating the diagnostic utility of NT-proBNP, in hemodialysis patients with LV dysfunction (2-4). In a recent study, Wang et al. (8) also reported that NT-proBNP levels were significantly related to acute decompensated heart failure, in chronic peritoneal dialysis patients with end-stage renal disease as in our study. This study also indicated that cardiovascular congestion was strongly related to LVMI, however, we did not find any significant interaction between LVMI and NT-proBNP concentration. Another study suggests that a serum NT-proBNP cut-off value of>/=7200 ng/L discriminates CKD stage 5 patients without LV dysfunction (LVD) from those with LVD (36). In our study, we also evaluated NT-proBNP values but did not find a correlation between LV functions. This may be related to the stringent exclusion criteria we used on echocardiography. To the best of our knowledge, our study is one of the first-line studies to evaluate the diagnostic utility of NT-proBNP, in hemodialysis patients with LV dysfunction.

Several limitations of this study should be considered. First, the number of patients was small, because it was conducted at a single hemodialysis center. This decreases the statistical power of the study. However after exclusion criteria were performed, limited patients were eligible and underwent the analysis. Second, we only evaluated the diagnostic utility of NT-proBNP in hemodialysis patients, but we don't know how this parameter changes in peritoneal dialysis or in chronic kidney disease patients who do not receive dialysis treatment. Third, fluid status or volume overload was not evaluated at enrollment. This may have allowed us to evaluate the higher NT-proBNP concentration in baseline volume overload or cardiovascular obstruction.

NT-proBNP is an important indicator of LV systolic dysfunction in the general population. However, our findings suggest that NT-proBNP levels are inadequate to determine LV systolic dysfunction in patients with chronic kidney disease on hemodialysis treatment.

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

Ethical Approval
In this study, we undertake that all the rules required to be followed within the scope of the ''Higher Education Institutions Scientific Research and Publication Ethics Directive'' are complied with, and that none of the actions stated under the heading ''Actions Against Scientific Research and Publication Ethics'' are not carried out.The study was prepared by the Declaration of Helsinki and was approved by the ethics committee for clinical research at Suleyman Demirel University Medical Faculty (Date: 06/03/2023, No: 39). The study was conducted in accordance with the principles set forth in the Declaration of Helsinki.

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all participants included in the study.

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Availability of Data and Materials
Data are available on request due to privacy or other restrictions.

Authors Contributions
ŞT: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

BAU: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-original draft.

References

1. Yasue H, Yoshimura M, Sumida H, Kituta K, et al. Localization and Mechanism of B-Type Natriuretic Peptide in Comparison with Those of A-Type Natriuretic Peptide in Normal Subjects and Patients with Heart Failure. Circulation 1994;90(1):195-203.
2. Mukoyama M, Nakao K, Hosoda K, Suga S, et al. Brain Natriuretic Peptide as a Novel Cardiac Hormone in Humans. Evidence for an Exquisite Dual Natriuretic Peptide System, Atrial Natriuretic Peptide, and Brain Natriuretic Peptide. J Clin Invest 1991;87(4):1402-12.
3. Kohno M, Horio T, Yokokaw K, Murakawa K, et al. Brain Natriuretic Peptide as a Cardiac Hormone in Essential Hypertension. Am J Med 1992;92(1):29-34.
4. Hunt PJ, Yandle TG, Nicholls MG, Richards AM, Espiner EA. The Amino-Terminal Portion of Brain Natriuretic Peptide (Pro-BNP) Circulates in Human Plasma. Biochem Biophys Res Commun 1995;214(3):1175-83.
5. Pemberton CJ, Johnson ML, Yandle TG, Espiner EA. Deconvulotion Analysis of Cardiac Natriuretic Peptides During Acute Volume Overload. Hypertension 2000;36(3):355-9.
6. Yandle TG, Richards AM, Gilbert A, Fisher S, Holmes S, Espiner EA. Assay of Brain Natriuretic Peptide (BNP) in Human Plasma: Evidence for High Molecular Weight BNP as a Major Plasma Component in Heart Failure. J Clin Endocrinol Metab 1993;76(4):832-8.
7. Mallamaci F, Zoccali C, Tripepi G, et al. Diagnostic Potential of Cardiac Natriuretic Peptides in Dialysis Patients. Kidney Int 2001;59(4):1559-66.
8. Wang AY, Lam CW, Yu CM, Chan IH, et al. N-Terminal Pro-Brain Natriuretic Peptide: An İndependent Risk Predictor of Cardiovascular Congestion, Mortality, and Adverse Cardiovascular Outcomes in Chronic Peritoneal Dialysis Patients. J Am Soc Nephrol 2007;18(1):321-30.
9. Zoccali C, Mallamaci F, Benedetto FA, Tripepi G, et al. Cardiac Natriuretic Peptides are Related to Left Ventricular Mass and Function and Predict Mortality in Dialysis Patients. J Am Soc Nephrol 2001;12(7):1508-15.
10. Foley RN, Parfrey PS, Harnett JD, Kent GM, et al. The Prognostic İmportance of Left Ventricular Geometry in Uremic Cardiomyopathy. J Am Soc Nephrol, 1995;5(12):2024-31.
11. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations Regarding Quantitation in M-Mode Echocardiography: Results of a Survey of Echocardiographic Measurements. Circulation 1978;58(6):1072-83.
12. Schiller NB, Shah PM, Crawford M, DeMaria A, et al. Recommendations for Quantitation of the Left Ventricle by Two-Dimensional Echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1989;2(5):358-67.
13. de Simone G, Devereux RB, Roman MJ, Alderman MH, Laragh JH. Relation of Obesity and Gender to Left Ventricular Hypertrophy in Normotensive and Hypertensive Adults. Hypertension 1994;23(5):600-6.
14. Otterstad JE, Froeland G, St John Sutton M, Holme I. Accuracy and Reproducibility of Biplane Two-Dimensional Echocardiographic Measurements of Left Ventricular Dimensions and Function. Eur Heart J 1997;18(3):507-13.
15. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, et al., Rapid Measurement of B-Type Natriuretic Peptide in the Emergency Diagnosis of Heart Failure. N Engl J Med, 2002;347(3):161-7.
16. Mueller C, Scholar A, Laule-Kilian K, Martina B, et al. Use of B-Type Natriuretic Peptide in the Evaluation and Management of Acute Dyspnea. N Engl J Med 2004;350(7):647-54.
17. Davis M, Espiner M, Richards G, Billing J, Town I, et al. Plasma Brain Natriuretic Peptide in Assessment of Acute Dyspnoea. Lancet 1994;343(8895):440-4.
18. Berger R, Huelsman M, Strecker K, Bojic A, et al. B-Type Natriuretic Peptide Predicts Sudden Death in Patients with Chronic Heart Failure. Circulation 2002;105(20):2392-7.
19. Cheng V, Kazanagra R, Garcia A, Lenert L, Krishnaswamy P, Gardetto N, et al. A rapid Bedside Test for B-Type Peptide Predicts Treatment Outcomes in Patients Admitted for Decompensated Heart Failure: A Pilot Study. J Am Coll Cardiol 2001;37(2):386-91.
20. Koglin J, Pehlivanlı S, Schwaiblmair M, Voseger M, et al. Role of Brain Natriuretic Peptide in Risk Stratification of Patients with Congestive Heart Failure. J Am Coll Cardiol 2001;38(2):1934-41.
21. Yu CM, Sanderson JE. Plasma Brain Natriuretic Peptide--An İndependent Predictor of Cardiovascular Mortality in Acute Heart Failure. Eur J Heart Fail 1999;1(1):59-65.
22. Morrison LK, Harrison A, Krishnaswamy P, Kazanegra R, Clopton P, Maisel A. Utility of a Rapid B-Natriuretic Peptide Assay in Differentiating Congestive Heart Failure from Lung Disease in Patients Presenting with Dyspnea. J Am Coll Cardiol 2002;39(2):202-9.
23. Cowie MR, Struthers AD, Wood DA, Coats AJ, et al. Value of Natriuretic Peptides in Assessment of Patients with Possible New Heart Failure in Primary Care. Lancet 1997;350(9088):1349-53.
24. Parfrey PS, Harnett JD, Barre PE. The Natural History of Myocardial Disease in Dialysis Patients. J Am Soc Nephrol 1991;2(1):2-12.
25. Parfrey PS, Foley RN, Harnett JD, Kent GM, Murray DC, Barre PE. Outcome and Risk Factors for Left Ventricular Disorders in Chronic Uraemia. Nephrol Dial Transplant 1996;11(7):1277-85.
26. London GM, Guerin AP, Marchais SJ. Pathophysiology of Left Ventricular Hypertrophy in Dialysis Patients. Blood Purif 1994;12(4-5):277-83.
27. Kohno M, Horio T, Yokokawa K, Yasunari K, et al. Brain Natriuretic Peptide as a Marker for Hypertensive Left Ventricular Hypertrophy: Changes During 1-Year Antihypertensive Therapy with Angiotensin-Converting Enzyme Inhibitor. Am J Med 1995;98(3):257-65.
28. Yamomoto K, Burnett JCJr, Jougasaki M, Nishimura RA, et al. Superiority of Brain Natriuretic Peptide as a Hormonal Marker of Ventricular Systolic and Diastolic Dysfunction and Ventricular Hypertrophy. Hypertension 1996;28(6):988-94.
29. Nishikimi T, Yoshihara F, Morimoto A, Ishikawa K, Ishimitsu T, et al. Relationship Between Left Ventricular Geometry and Natriuretic Peptide Levels in Essential Hypertension. Hypertension 1996;28(1):22-30.
30. Muschol MW, Schunkert H, Muders F, et al. Neurohormonal Activity and Left Ventricular Geometry in Patients with Essential Arterial Hypertension. Am Heart J 1998;135(1):58-66.

31. Schirmer H, Omland T. Circulating N-Terminal Pro-Atrial Natriuretic Peptide is an Independent Predictor of Left Ventricular Hypertrophy in the General Population. The Tromso Study. Eur Heart J 1999;20(10):755-63.
32. Daniels LB, Maisel AS. Natriuretic Peptides. J Am Coll Cardiol 2007;50(25):2357-68.
33. Kim WCW, Kim WY, Lim KS, Park YK. B-Natriuretic Peptide (BNP) Assay for Diagnosis of Congestive Heart Failure. J Korean Soc Emerg Med 2003;14:624-9.
34. Goto T, Takase H, Toriyama T, Sugiure T, et al. Increased Circulating Levels of Natriuretic Peptides Predict Future Cardiac Event in Patients with Chronic Hemodialysis. Nephron 2002;92(3):610-5.
35. Nitta K, Kawashima A, Yumura W, Naruse M, et al. Plasma Concentration of Brain Natriuretic Peptide as an İndicator of Cardiac Ventricular Function in Patients on Hemodialysis. Am J Nephrol 1998;18(5):411-5.
36. David S, Kümpers P, Seidler V, Biertz F, et al. Diagnostic Value of N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) for Left Ventricular Dysfunction in Patients with Chronic Kidney Disease Stage 5 on Haemodialysis. Nephrol Dial Transplant 2008;23(4):1370-7

ORIGINAL RESEARCH

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Retrospective Evaluation of Coronary Artery Fistulas with CT Angiography

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Abstract

Objective
Coronary artery fistula is the termination of the coronary artery branch in the cardiac chamber or pulmonary vein. In this study, we aimed to evaluate the type, origin, termination, and accompanying anomalies, if any, of coronary artery fistulas in patients who underwent coronary CTA in our clinic.

Material and Method
Coronary CTA examinations were performed on a 128-slice CT scanner. Images were evaluated using MPR, MIP and 3D VR reconstructions on the workstation. CTA image interpretation was performed independently by two radiologists with 15 and 2 years of experience in coronary CTA. In case of disagreement, a third radiologist was consulted.

Results
Coronary artery fistulas were found in 8 female and 6 male patients aged between 10 and 71 years, with a mean age of 39.07 years. Of the 15 fistula, 6 were coronacameral fistula, 7 were coronopulmonary fistula, 1 was between the left circumflex artery and the conal branch of the right coronary artery, and 1 was between the pulmonary trunk and the descending aorta. One patient was treated with coil placement in the interventional radiology department, while three cases were treated surgically. The other cases were followed up by the relevant clinics.

Conclusion
Coronary CT angiography provides three-dimensional images of the origin, course and termination of the fistula and is an important tool in guiding the patient's treatment plan.

Keywords: Coronary angiography, coronary artery fistulas, coronary CT angiography

Introduction

Normally, coronary arteries terminate within the capillary bed. Coronary artery fistula is the termination of coronary artery branch in a low-pressure vascular bed such as the cardiac chamber or pulmonary vein. It is seen in 0.1-0.5% of the population (1). They are mostly asymptomatic but can cause several life-threatening complications because of the defect in the nutrition of myocardium (2). In adults, some patients

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may remain asymptomatic for their entire lives, if the fistula is not hemodynamically significant. The variety of symptoms that occur depend on the origin, the place of termination, the length and the size of the fistula and the volume of blood stolen. More than 90% of them are congenital, although a few acquired cases may occur following chest trauma such as gunshot wounds or invasive cardiac procedure such as coronary artery bypass grafting, cardiac catheter angiography (3).

Coronary catheterization and coronary computerized tomography angiography (CTA) are the gold standards in diagnosing coronary artery fistulas. Coronary CTA is noninvasive and also able to detect coronary artery fistulas at a higher rate as compared to standard invasive coronary catheterization with angiography (4). CTA is an important tool in guiding the management of cases by showing the course, origin and termination of the fistula and associated vascular formations in 3 planes with volume-rendered images, as well as other associated anomalies.

Coronary artery fistulas can be grouped into two broad categories. The first one is coronary-cameral fistulas which are defined as abnormal connections between coronary arteries and any of the heart chambers. The second one is coronary arteriovenous malformations which are abnormal connections that occur between coronary arteries and parts of the systemic or pulmonary circulatory vessels. The most common fistula originates from the right coronary artery (RCA) and ends in the right ventricle (60%) (2).

Studies have shown that these aberrant coronary arteries, which form the malformation, have thicker tunica intima and media with tightly packed smooth muscle cells (5).

In symptomatic cases that cause hemodynamic problems, the aim of treatment is to embolize the artery feeding the fistula, either by surgery or by catheter angiography. It has been reported that precise access of coronary artery fistulas detected by 3D images in coronary CT angiography (CTA) will be more accurate (6).

In this study, we aimed to evaluate the type, origin, termination and accompanying anomalies, if any, of coronary artery fistulas in patients who underwent coronary CTA in our clinic.

Material and Method

We retrospectively reviewed 932 patients who underwent coronary CT angiography for various indications. Review of coronary CTA reports between March 2016 and January 2023 revealed fourteen patients diagnosed with coronary artery fistula.

Coronary CTA examination of the patients was performed using a 128-detector array CT scanner (Somatom Definition AS, Siemens Healthcare). In adults, after imaging with 3 mm slice thickness for coronary artery calcium scoring, ECG-gated (Electrocardiography-gated) CTA images with 0.5 mm slice thickness were obtained by administering an average of 70-80 ml of contrast material (at a rate of 5-6 ml/s) through antecubital vein followed by 50 ml of saline solution. A low dose (80 kVp) CTA protocol was used for children and the amount of contrast material was adjusted according to body weight. Bolus tracking technique was used to determine scan delay.

Reconstructions were routinely performed in 40% and 75% phases of the R-R interval period. Images were evaluated on the workstation using MPR (Multiplanar Reformation), curved MPR, MIP (Maximum Intensity Projection) and VR (Volume Rendered) 3D postprocessing.

Interpretation of CTA images of the patients was performed independently by two radiologists with 15 years and 2 years of experience in coronary CTA. In case of disagreement on interpretation, consensus was reached with a third radiologist. Coronary artery fistulas, type, origin, ending and accompanying anomalies, if any, were reshaped on the workstation and interpreted on volume rendered images (Table 1).

This study was performed retrospectively and Ethics Committee approval was obtained with a letter dated 28.03.2023.

Results

Coronary artery fistula was diagnosed in 8 female and 6 male patients, aged between 12 and 71 and the median age was 49.5 years. Four of the cases were under the age of 18, 2 were girls and 2 were boys, and the median age was 12 years. There were ten cases in the adult group, of which 6 were female and 4 were male, and the median age was 54.5 years.

Of 14 patients, 13 had a single fistula and 1 had a double fistula, with a total of 15 fistulas.

Of the 15 fistulas, 6 were coronacameral fistula, 7 were corona-pulmonary fistula, 1 was corona coronary fistula between the left circumflex artery (LCX) and the conal branch of right coronary artery and 1 was

Table 1 Characteristics and treatment methods of coronary artery fistulas in our case group

CASE	AGE-GENDER	TYPE OF FISTULA	ORIGIN OF FISTULA	DIAMATER OF FISTULA	DRAINAGE OF FISTULA	COEXIST ANOMALIES	MANAGEMENT
1	10/Male	Corona-pulmonary	LAD	(2mm) in fistulized vessels associated with the LAD and pulmoner trunk	Pulmoner trunk	-	Follow -up
2	12/Female	Corona-camaral	LCX	4mm (LCX)	RV apex	-	Follow -up
		Corona -coronary	LCX	-4mm (LCX) -5mm (Conal artery)	RCA – conal artery		
3	12/Female	Corona-camaral	LAD	-5.5 mm (LAD)	RV	-	Follow -up
4	15/Male	Corona-pulmonary	LAD-Diagonal-2	4.7 mm (Diagonal branch)	Pulmoner trunk	Hypertrophied bronchial arteries in the mediastinum	Surgical treatment
5	20/Male	Corona-camaral	LAD-Septal	4.5mm (LAD)	RV	Pektus excavatus deformity	Coil embolization
6	23/Female	Corona-camaral	RCA	(2mm) in fistulized vessels associated with the RCA and RA	RA	-Dilatation of the left ventricle -Signal and enhancement changes in chronic ischemic pattern in RCA trace in cardiac MRI -FMF disease -ASD closure operation history	Follow -up
7	48/Male	Pulmonary-Desenden aorta	Pulmoner trunk	(3.5mm) in fistulized vessels associated with the aorta and pulmonary trunk	Desenden aorta		Follow -up
8	51/Female	Corona -coronary	LAD	7.5mm (LAD)	Pulmoner trunk	Soft plaque with severe -moderate stenosis proximal to Diagonal-1	Surgical treatment
9	54/Female	Corona-camaral	RCA-PDA	5mm (RCA)	LV	Calcific plaque with moderate stenosis proximal to Diagonal -1	Follow -up
10	55/Male	Corona-camaral	LCX	-8mm (LM) -16MM (LCX)	LA		Surgical treatment
11	57/Female	Corona-pulmonary	RCA	5mm (RCA)	Pulmoner trunk	-Enlargement of the pulmonary trunk -Superficial bridging in LAD	Follow -up
12	59/Male	Corona-pulmonary	LAD	(3 mm) in fistulized vessels associated with the LAD and pulmonary trunk	Pulmoner trunk	-Consistent with ischemia in the left ventricular inferolateral wall in scintigraphic evaluation	Follow -up
13	60/Female	Corona-pulmonary	RCA	(3 mm) in fistulized vessels associated with the RCA and pulmonary trunk	Pulmoner trunk	-	Follow -up
14	71/Male	Corona-pulmonary	LAD	4mm (LAD)	Pulmoner trunk	-Moderate stenosis secondary to mixed plaque in the LCX artery and secondary to diffuse calcified plaques in the coronary arteries 2068 agaston score was measured.	Follow -up

(RV: Right ventricle, LV: Left ventricle, LA: Left Atrium, RA: Right Atrium, LAD: Left Anterior Descending Artery, LCX: Left Circumflex Artery, RCA: Right Coronary Artery, FMF: Familial Mediterranean Fever, ASD: atrial septal defect)

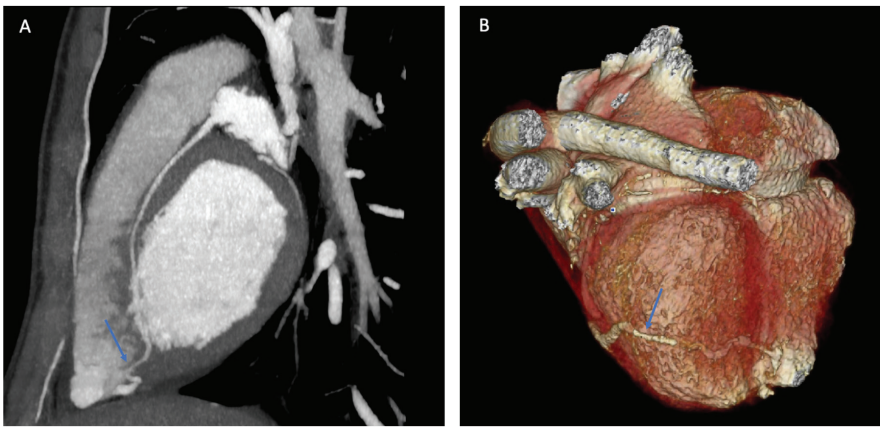

Figure 1
(A) Curved planar reformatted CT image and (B) three dimensions (3D) volume rendered (VR) CT image shows coronacamaral fistula between right ventricle apex and left anterior descending artery.

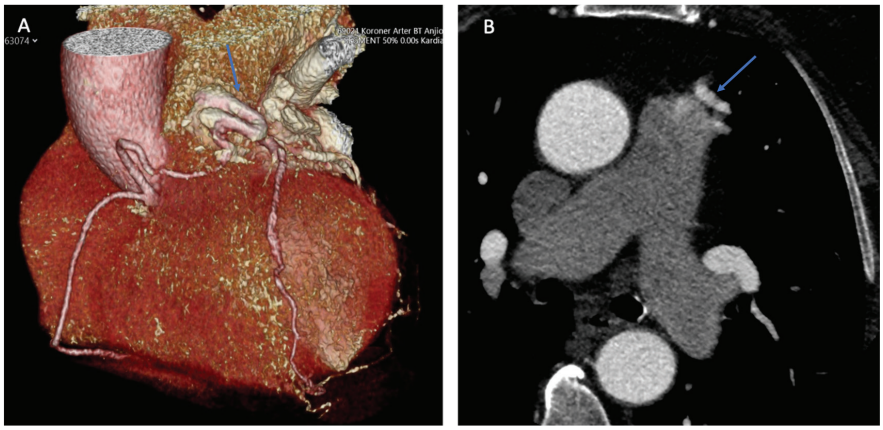

Figure 2
(A) Three dimensions (3D) volume rendered CT image shows corona pulmonary fistula between right descending artery and pulmonary trunk, (B) axial plan contrast-enhanced CT image shows the turbulent flow of the fistulous vessel.

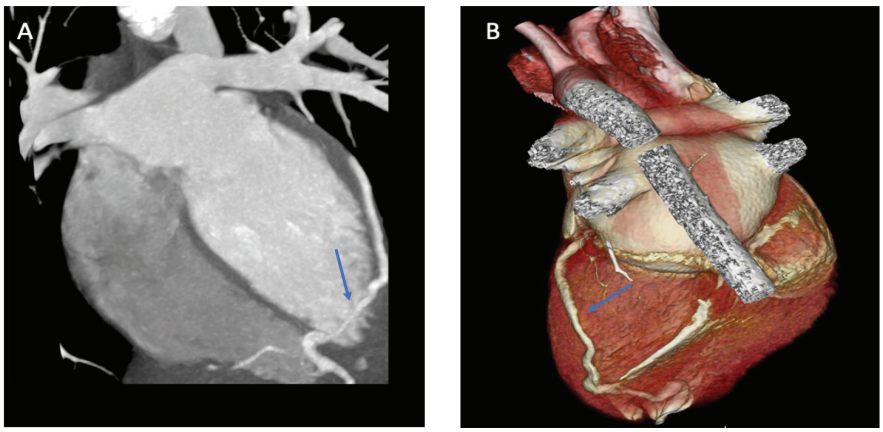

Figure 3
(A) MIP image and (B) Three dimensions (3D) volume rendered CT image shows the fistula between left circumflex artery and right ventricle apex.

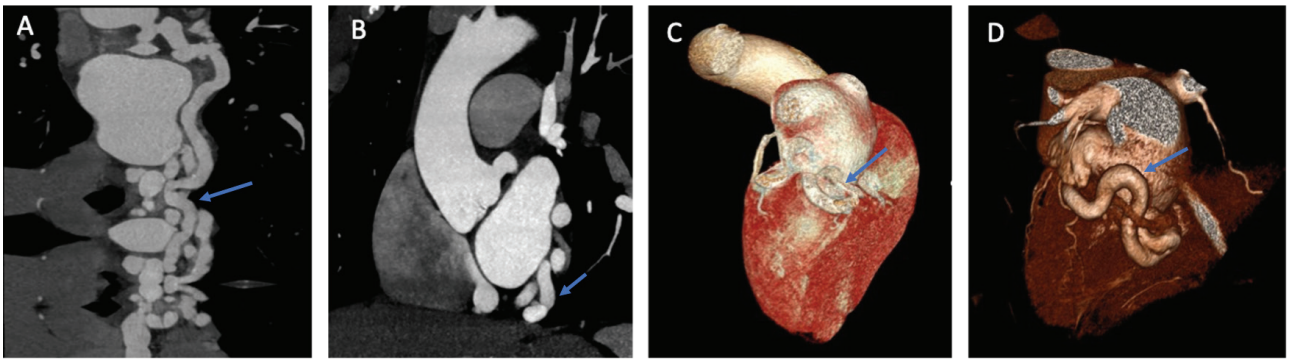

Figure 4
(A-B) MIP image, (C-D) 3D VR CT image shows images show marked increase in LCX artery diameter and tortuosity.

between the pulmonary trunk and the descending aorta (Figure 1-4).

Among the coronacameral fistulas, 3 of them ended in the right ventricle, 1 in the right atrium ,1 in the left atrium and 1 in the left ventricle. Of the coronacameral fistulas ending in the right ventricle, 2 originated from the left anterior descending artery (LAD) Left Anterior Descending Artery) and 1 from the LCX artery.

Of the 7 fistulas ending in the pulmonary trunk, 4 were originating from LAD, 2 from RCA, and 1 from second diagonal branch of LAD.

The diameter of the tortuous vascular structures developing secondary to the fistula varied between 2 mm and 16 mm.

The patient who had a coronacameral fistula between the LAD and the right ventricle was treated by placing a coil in the interventional radiology department without complications. Surgical operation was performed in the cases with a corona-pulmonary fistula between LAD diagonal branch-pulmonary trunk and LAD-pulmonary trunk, and in the case with coronacameral fistula between LCX artery and right ventricle, and the procedures were terminated without complications. Other cases were followed up by the relevant clinics.

In 2 cases, ischemic changes were accompanied by arterial tracing in the ventricular wall, one of which was verified by cardiac MRI and the other by scintigraphy. In addition to ischemic changes, left ventricular enlargement was found on cardiac MRI, and there was a history of Familial Mediterranean Fever (FMF) and atrial septal defect (ASD) closure operation in the patient's history (Table 1).

In 3 cases, there were soft and calcific plaque formations that caused moderate stenosis in other arterial structures unrelated to the fistula. Dilatation of the pulmonary trunk and superficial bridging in the LAD were observed in 1 patient. The patient who was treated with coil embolization had accompanying pectus excavatum deformity. In 1 other case, there were accompanying hypertrophic bronchial arterial structures in the mediastinum.

Discussion

The mean age of the cases in our group was 49.5 years and 6 were male and 8 were female. The most common fistula type was coronary to pulmonary trunk, and the width of the tortiosis vascular structures secondary to the fistula ranged from 2mm to 16mm.
In our case group, invasive procedures were performed in 4 cases in total. The dilatation level in the diameters of the arteries participating in the fistula was remarkable in the operated cases (LM 8mm, LCX 16mm, Diagonal artery reached 4.7mm in diameter). Although the LAD diameter (4.5 mm) was similar to the cases in the follow-up group, it was treated with coil embolization due to ischemic changes in the accompanying ECG. Other cases were included in the follow-up group considering the absence of findings such as ECG changes, heart failure, and arrhythmia. In those the diameter of the arteries related to the fistula was 3-4 mm on average and reaching a maximum of 5 mm.

While the incidence of coronary artery fistula is between 0.5-1% in the literature, this rate was slightly higher in our study and was 1.5%. We thought that this was related to the fact that the hospital where the study was performed was accepted as a reference

hospital in the region and problematic cases were frequently referred.

According to our case series experience, in cases of coronary fistula, while the patient's symptoms determined the surgical decision, the diameter of the artery involved in the fistula was also very useful in determining the treatment procedure.

There was no obvious gender trend in the literature in coronary artery fistula disease, and in our study, female were 57% and male were 43%. While this rate was 54% male and 46% female in Zhou K.'s study, it was 27% male and 63% female in Serap Baş' study (7,8).

Coronary cameral fistula is the most common type of coronary artery fistula in children, accounting for 75.0 % – 100.0 % of cases [10]. Coronary cameral fistula in children most commonly originates from the right coronary artery, followed by the left coronary artery (9). In our study, 50% of fistulas in our pediatric age group were coronacamaral type and associated with the right ventricle (one from LCX, the other from LAD). The other half had Coronary-Pulmonary Artery Fistula (one from LAD, one from diagonal branch of LAD).

In previous studies, the most common drainage sites in coronary artery fistula cases were right ventricle, right atrium, pulmonary artery, coronary sinus, left atrium, left ventricle, and superior vena cava (SVC) (10). Our study showed that 53.3% of the cases drained into the pulmonary trunk, which is similar to the study of Serap Baş (52.9%), while the rate of coronopulmonary fistula was 28% in the study of Zhou K. et al. (7,8). In our study, pulmonary trunk drainage was followed by right ventricle (20%), right atrium (6.6%), left ventricle (6.6%), left atrium (6.6%), coronary artery (6.6%).

It is difficult to cannulate all fistula-derived arteries during invasive coronary angiography, and it can be problematic to follow complex fistula tracts and optimally depict their anatomical relationships on two-dimensional fluoroscopic images. Coronary CT angiography is superior to catheter angiography in revealing the fistula tract and its relationships, as it allows 3D evaluation (11).

In our series of 14 cases, surgical operation was performed in 3 cases and transcatheter closure (TCC) in 1 case, while the others were followed up with medical treatment. Considering the fact that CAFs (Coronary Artery Fistula) are mostly asymptomatic, the first-line treatment is mainly medical therapy with follow-up over time (12). Surgical treatment is generally preferred in the presence of the need for bypass graft or surgical valve repair/replacement, distally located and highly tortuous CAFs, angina symptoms with single and large diameter fistulae, and volume overload (13). The biggest advantage of TCC over surgery is that no cardiopulmonary bypass or median sternotomy is performed, bleeding, ischaemia, infection, arrhythmia, cosmetic concerns are less and recovery time is faster (14).

Conclusion

CAFs can have complex anatomy; Therefore, CT angiographic evaluation, which allows three-dimensional imaging during the pre-treatment examination, provides very important data. While the treatment procedure is usually medical therapy, it may vary between invasive options depending on the patient's symptoms and risk factors in the light of CT angiography data.

Limitations of the Study

We had some limitations, the most important of which was that we did not know the current status of the patients under follow-up and the number of our cases was not sufficient to perform a statistical study. Also since conventional angiography was not performed on every patient in our case group, we did not have the chance to compare catheter angiography and CT angiography data.

Conflict of Interest Statement

The authors declare that they have no competing interest.

Ethical Approval

This study was carried out retrospectively and Ethics Committee approval was obtained from Ege University with a letter dated 28.03.2023- E.1201728. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki.

Consent to Participate and Publish

Written informed consent was obtained from the participants of this study.

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Availability of Data and Materials

Data available on request from the authors.

Authors Contributions

ZAO: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

SB: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing.

AC: Investigation; Validation; Writing-original draft.

NC: Formal analysis; Investigation; Visualization; Writing-original draft.

References

1. Boudoulas KD, Boudoulas H. Coronary Artery Fistulas. Cardiology 2017;136(2):90-92.
2. Verdini D, Vargas D, Kuo A, Ghoshhajra B, Kim P, Murillo H, et al. Coronary-Pulmonary Artery Fistulas: A Systematic Review. J Thorac Imaging 2016;31(6):380-390.
3. Lee CM, Song SY, Jeon SC, Park CK, Choi YW, Lee Y. Characteristics of Coronary Artery to Pulmonary Artery Fistula on Coronary Computed Tomography Angiography. J Comput Assist Tomogr 2016;40(3):398-401.
4. Lim JJ, Jung JI, Lee BY, Lee HG. Prevalence and Types of Coronary Artery Fistulas Detected with Coronary CT Angiography. AJR Am J Roentgenol 2014;203(3): W237-43.
5. Sakata N, Minematsu N, Morishige N, Tashiro T, Imanaga Y. Histopathologic Characteristics of a Coronary-pulmonary Artery Fistula with a Coronary Artery Aneurysm. Ann Vasc Dis 2011;4(1):43-6.
6. Saboo SS, Juan YH, Khandelwal A, et al. MDCT of Con-Genital Coronary Artery Fistulas. AJR Am J Roentgenol 2014;203(3):244-52.
7. Zhou K, Kong L, Wang Y, et al. Coronary Artery Fistula in Adults: Evaluation with Dual-Source CT Coronary Angiography. Br J Radiol 2015;88(1049):20140754.
8. Baş S. Coronary Artery Fistulas in Adults: Evaluation with Coronary CT Angiography. Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi 2021;8(2):104-108.
9. Li N, Zhao P, Wu D, Liang C. Coronary Artery Fistulas Detected with Coronary CT Angiography: A Pictorial Review of 73 Cases. Br J Radiol 2020;93:20190523
10. Zenooz NA, Habibi R, Mammen L, Finn JP, Gil- keson RC. Coronary Artery Fistulas: CT Findings. RadioGraphics 2009;29:781–789.
11. Yun G, Nam TH, Chun EJ. Coronary Artery Fistulas: Pathophysiology, Imaging Findings, and Management. Radiographics 2018;38(3):688-703.
12. Navid A. Zenooz, Reza Habibi, Leena Mammen, J. Paul Finn, and Robert C. Gilkeson. Coronary Artery Fistulas: CT Findings, RadioGraphics 2009;29:3,781-789.
13. Buccheri D, Chirco PR, Geraci S, Caramanno G, Cortese B. Coronary Artery Fistula Anatomy, Diagnosis and Management Strategies. Heart Lung Circ 2018;27(8):940-951.
14. Christmann M, Hoop R, Dave H, et al. Closure of Coronary Artery Fistula in Childhood Treatment Techniques and Long-Term Follow-Up. Clin Res Cardiol 2017;106:211–218.

ORIGINAL RESEARCH

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Evaluation of PD-1 and TIM-3 Expression Levels of CD8+ T Cells in Renal Transplant Patients

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Abstract

Objective

After kidney transplantation, CD8+ T cells can infiltrate the kidney and cause necrosis, tubulitis, and even transplant rejection. For this reason, control of the T cell response is very important, and T cell immunoglobulin and mucin domain 3 (TIM-3) and programmed death 1 (PD-1) molecules play a role in regulating the T cell response. It is thought that the levels of TIM-3 and PD-1 expressions may be guiding in determining the clinical course after transplantation. This study aimed to determine the relationship between the mRNA levels of PD-1 and TIM-3 genes in peripheral blood samples taken from kidney transplant patients and the clinical conditions of the patients.

Material and Method

60 peripheral blood samples were collected from 30 kidney transplant patients, both pre-transplantation (pre-tx) and post-transplantation (post-tx). CD8+ T cells were separated from other lymphocytes by magnetic cell separation system (MACS) and their purity was determined by flow cytometry. Then, RNA was isolated and after cDNA conversion, the expressions of PD-1 and TIM-3 genes were determined by real-time polymerase chain reaction.

Results

While it was determined that the TIM-3 gene expression level increased in patients with acute tubular necrosis, antibody-mediated rejection and cell-mediated rejection findings ($p<0.001$), no correlation was found between PD-1 expression levels and the clinical findings of the patients.

Conclusion

It is thought that comparing TIM-3 mRNA levels before and after kidney transplantation may be a useful tool in evaluating the clinical status of patients.

Keywords: Immune checkpoint regulators, PD-1, renal transplantation, TIM-3.

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Introduction

Kidney transplantation is the best choice for patients with chronic kidney failure, which affects 13.4% of people worldwide, leading to millions of deaths. However, approximately 30% of patients undergo rejection episodes during the first year after transplantation (1,2). Rejection of the kidney is caused by a complex series of events that involves both adaptive and innate immune system responses, with T cells at the center of this process (3).

CD8+ T cells contribute to tissue necrosis and tubulitis in solid organ transplantation, despite the focus of most studies being on the CD4+ T cell subgroup (4). Moreover, CD8+ T cells secrete perforin and granzymes that can cause transplant rejection (5). It was shown that in acute and chronic allograft dysfunction, the number of CD8+ T cells increase in peripheral blood (6). It can be thought that immunomodulation of the CD8+ T cell population is important in long-term allograft survival and may bring a new perspective to increasing graft survival by targeting specific metabolic processes (7).

After maturation of T cells in thymus, naive T cells start to circulate between peripheral blood and lymph nodes to encounter MHC-peptide complex, which starts an immune response cascade (8). This MHC-peptide complexes are recognized by T cell receptors (TCR) and coreceptors, CD4 and CD8, stabilizes this synapsis between TCR-MHC-peptide complex. Then costimulatory CD28 and its ligand (CD80/86) interact, and cytokines start to secrete as a result of T cell activation (9). At the end of the T cell response these effector T cells started to express negative costimulatory molecules as CTLA-4, PD-1, TIM-3, LAG-3…etc. (10). PD-1 receptor can be expressed by both CD4+ and CD8+ T cells and has two ligand called PDL-1 and PDL-2 while the primary ligand is PDL-1 (11). It was shown that in different type of T cells, PD-1/PDL-1 interaction leads to the different results (12). For example, in follicular T helper cells (Tfh) binding of PD-1 to PDL-1 causes the localization of Tfh in germinal center. Otherwise, in CD8+ T cells PD-1 shortens the interaction between CD8+T cells and target cells which leads to the saving of the target cells from CD8+ T cell attack (13).

T cell immunoglobulin and mucin domain containing protein-3 (TIM-3) is a transmembrane protein that is encoded by HAVCR2 gene located on chromosome 5 (14). The best-known ligand of TIM-3 is Galectin-9 that is a S type lectin and expressed by several cells as T cells, B cells, mast cells and nonimmune cells. TIM-3/Galectin-9 interaction plays role on negative T cell costimulatory pathway (15). Recent studies have shown that TIM-3 can modulate the alloimmune response in allograft rejection. In heart transplants, it has been stated that both PD-1 and TIM-3 expression are increased in tolerant recipients compared to people who have undergone organ rejection. The idea that these molecules are very important in transplant tolerance remains important (16).

The purpose of this study was to evaluate possible changes in PD-1 and TIM-3 expression in CD8+T cells of patients before and after kidney transplantation.

Material and Method

Patient Group

Ethical approval for this study was obtained from Health Science University Tepecik Training and Research Hospital Noninvasive Ethics Committee with the number 2022/05-31. 60 blood samples were collected from renal transplanted patients both before the operation time and between 1 and 3 months after transplantation. 30 ml heparinized blood samples were diluted by PBS (Merck, Germany) (v/v) and added carefully on 5 ml lymphocyte separating solution (Capricorn, Germany). Tubes were centrifuged at 2500 rpm for 20 minutes. Then, the lymphocyte layer was collected to a new tube and washed two times with PBS by centrifugating at 1800 and 1500 rpm for 5 min., respectively. The lymphocyte pellet was suspended in 2 ml PBS and counted on Thoma chamber and then CD8+ T cells were separated by using magnetic separation system (MACS, Miltenyi Biotech, USA).

CD8+ T Cell Separation

$1x10^7$ cells were loaded onto columns of magnetic cell separation system (MACS, Miltenyi Biotech). CD8+ T cells were separated according to the manufacturers' protocol. Purity of isolated CD8+ T cells were analyzed by flow cytometer according to the procedure explained in our preliminary study (17).

Isolation and Quantification of Total RNA and Converted CDNA

RNAs were isolated from CD8+ T cell pellets by using GeneJET RNA Purification Kit (ThermoScientific, USA) and cDNA was converted by "VitaScript™ FirstStrand cDNA Synthesis Kit" (Procomcure, Austria) according to the instruction manual.

Real Time PCR

PD-1 primers were designed according to NCBI reference sequence: NC_000002.12 and NC_000005.10 while TIM-3 and β -actin primers were

Table 1 Primer sequences

Gene	Primer Sequences	Amplicon length	References
PD-1	F: 5'-TTCCACATGAGCGTGGTCAG-3' R: 5'-CCGCAGGCTCTCTTTGATCT-3'	102 bp	NCBI, Primer blast program
TIM-3	F: 5'-CCTATCTGCCCTGCTTCTAC-3' R: 5'-CTGGTGGTAAGCATCCTTGG-3'	364 bp	(18)
β-Actin	F: 5'-CTTCCTGGGCATGGAGTCCTG-3' R: 5'-GGAGCAATGATCTTGATCTTC-3'	21 bp	(19)

designed according to the literature showed in Table 1. NCBI Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast), IDT Oligo analyzer (https://www.idtdna.com), UCSC In-Silico PCR (https://genome.ucsc.edu) programs were used to control all three primer sequences. β-actin gene was used for the internal control (NM 001101.3). Primer sequences and amplicon lengths were shown in Table 1.

Amplification was performed by PicoReal Real Time PCR System (ThermoScientific, USA) with a polymerase chain reaction (PCR) protocol: 95°C for 10 minutes, followed by 40 cycles of 95°C for 45 seconds, 58°C 45 seconds, 72°C 45 seconds and final extension 72°C 10 minutes. PCR reactions were performed in triplicate by using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark), according to the protocol of 6.25 µl SYBR Green, 0.5 µl forward and reverse primers, 2 µl cDNA and 2.75 µl pure water to a final volume of 12 µl. Relative gene expression analysis were performed according to the 2−ΔΔCT method. The results were normalized with β-actin gene expression levels. 60-95°C temperature range was used for melting curve analysis.

Statistics

IBM SPSS Statistics 21 program was used for the statistical analysis. Student t test was used to compare continuous variables, while chi-square test was used for categoric variables. Box-plots allowed to visualize gene quantifications. Spearman correlation test was used to calculate correlations. Results were expressed as mean value ± standard deviation. $p<.05$ was considered as statistically significant.

Results

Study Group

Demographic characteristics of the patients are summarized in Table 2. Six recipients were diagnosed for C4d+, CD3+ and acute tubular necrosis. Among these patients one underwent nephrectomy while the other patients were cured with intravenous immunoglobulin (IVIG) and became stable between 1 to 3 months after transplantation.

mRNA Levels of PD-1 and TIM-3 Genes

The mRNA expression levels of PD-1 and TIM-3 before and after transplantation were evaluated and calculated by using β-actin expression levels to

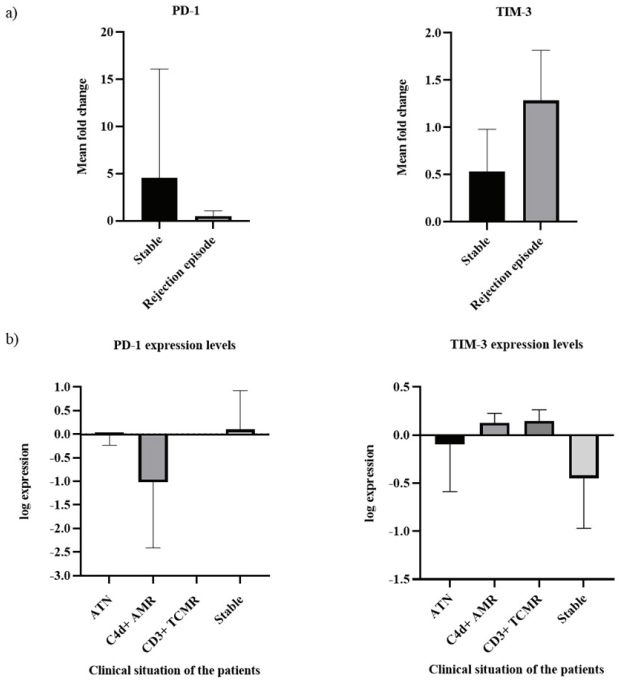

Figure 1

a) TIM-3 and PD-1 mean fold change according to the clinical status of the patients at the time of specimen collection. b) log expression values of TIM-3 and PD-1 according to the rejection types. ATN, acute tubular necrosis; AMR, antibody mediated rejection; TCMR, T cell mediated rejection.

Table 2 Patient demographics

	ATN*	C4d+	CD3+	Stable	p
No. of patients	2	2	2	24	>.05
Patients age (year, mean ± SD)	46,5±7,8	43±15,6	33±8,5	45,0±12,5	>.05
Gender (F/M)	0/2	2/0	1/1	7/17	>.05
GFR pre-Tx	6,4±0,8	6,7±1,5	13,4±10,7	8,5±3,1	>.05
GFR post-Tx**	61,5±23,3	49,2±8,8	35,5±40,3	56,1±12,7	>.05
Creatinin pre-Tx	3,5±0,6	7±2,3	4,3±2	6,9±3	>.05
Creatinin post Tx**	1 ±0,02	1,6±0,3	4,1±3,5	1,4±0,3	>.05
Cause of CKD (HT/DM/Others)	0/0/2	1/1/0	2/0/0	2/13/7	>.05
Donor (Cadaveric/Alive)	2/0	0/2	1/1	10/14	>.05
Donor age (year, mean ± SD)	34±7	47±11,3	55,5±7,7	43,8±12,2	>.05
HLA mismatches (A,B,DR,DQ; mean± SD)	1,5 ±0,5	2±1	2,5±1,5	2,4±1,4	>.05

ATN, Acute tubular necrosis; Stable, stable renal function; SD, standard deviation; F, female, M, male; GFR, glomerular filtration rate; pre-tx, before transplantation; post-tx, after transplantation; CKD, chronic kidney disease; HT, hypertension; DM, diabetes mellitus. *ATN was diagnosed at 0 time after transplantation by biopsy. **Post-tx GFR and creatinine levels were obtained 1 month after transplantation.

Table 3 Correlation between gene expression levels and demographic and clinical properties of patients

		P.gend.	P.age	D.gend.	D.age	PreTx-Cre	Post-Tx-Cre	PreTx-GFR	PostTx-GFR	HLA-MM	Rej.
PD-1	R	0,130	-0,064	-0,225	-0,072	0,026	-0,006	0,091	-0,150	0,11	-,159
	p	0,246	0,369	0,116	0,353	0,445	0,488	0,317	0,215	0,281	,400
TIM-3	R	-,404*	0,268	,393*	-0,015	-,351*	0,270	0,091	-0,145	-0,107	,558**
	p	0,014	0,076	0,016	0,469	0,029	0,075	0,316	0,222	0,288	,001

*Correlation is significant at the 0.05 level (1-tailed).
**Correlation is significant at the 0.01 level (2-tailed). P, patient; gend, gender; D, donor; Cre, creatinine; GFR, glomerular filtration rate; MM, mismatch; Rej, rejection attack

normalize the results. The pre-transplantation mRNA levels of the TIM-3 and PD-1 genes were used as control. Clearly, the mean values of PD-1 expression values were determined to be higher in stable patients. However, TIM-3 mean fold changes were evaluated higher in patients with rejection episode (Figure 1a). When we divided patients due to the rejection episode reason, PD-1 expression levels could be variable. On the other hand, the evaluation of the TIM-3 expression levels showed that patients with AMR and TCMR have increased levels of expression, while one patient had increased, and the other one had decreased levels of TIM-3 expression in patients with acute tubular necrosis (Figure 1b).

Only one stable patient had higher TIM-3 expression levels than the other stable patients. When we observed clinical status of this patient there was no certain case. Patients that have the highest PD-1 gene expression levels (9.44; 16.51; 55.33, respectively) are evaluated as stable patients. On the other hand, patients with ATN, AMR and TCMR had similar PD-1

expression levels and lower than most of the stable patients.

According to the correlation analysis, PD-1 and TIM-3 expression levels were found as non-significantly (p=0.731) negative correlated (r=-.066). Moreover, when we evaluated the correlation between patients' characteristics and PD-1, TIM-3 expression levels, rejection episode was found statistically significant positive correlation with TIM-3 expression levels. Gender of both patient and donors, and pre-tx creatinine levels were also found statistically significant positive, negative, and positive correlation, respectively (Table 3). No significant correlation was found between PD-1 expression levels and any of the clinical properties of the patients.

Discussion

Invasive methods as core needle biopsy are still the most reliable and valuable method to detect antibody and cell mediated rejection episodes after kidney transplantation (20). However, any of the invasive methods have risks of complications both for the kidney and patient (21). Studies focused on noninvasive biomarkers to predict the rejection has been developed and at the present time associations between many molecules and rejection was evaluated (22, 23). mRNA profiles of the cytokines, cytotoxic molecules, and receptor/ligands on immune cells of the renal transplant patients showed to be used for the diagnosis of the rejection (24).

TIM-3 is one of the most important immune checkpoint regulator molecules (25). Although it was first identified on the surface of helper T cells, it was determined that cytotoxic CD8+ T cells, Th17, NK cells, monocytes, dendritic cells, mast cells and microglia cells can express this molecule on their surfaces. Moreover, soluble form of TIM-3 molecule can be detected in serum and urinary samples (26). In particular, the TIM-3 molecule is involved in the termination of the Th1 immune response and induction of tolerance (27). However, expression of the TIM-3 molecule by the other immune cells leads to the idea of having different roles in different cell types, environment and cellular signaling can affect the TIM-3 mediated response (28). Renesto et al showed the TIM-3 mRNA levels of the urinary samples could be a potential biomarker to detect acute rejection (29). Otherwise, Luo et al. reached the same results in their studies in which they used peripheral blood samples (30). Shahbaz et al. compared the pre-and post- transplantation TIM-3 expression levels by using both blood and urinary samples. According to their results TIM-3 levels were found higher in patients with graft disfunction (26). Ponciano et al. compared the patients with organ rejection episode and control group, and they reported increasing level of TIM-3 (31). Renesto et al. showed higher expression levels of TIM-3 in patient with acute rejection than stable patients (29). Manfro et al. detected increased levels of TIM-3 in tissue, blood, and urinary samples in patients with acute rejection when compared to patients with graft dysfunction. They also reported the significant correlation between gene expression levels in different tissue and organs (32).

According to the previous studies, it is obvious that TIM-3 gene expression levels were detected higher in graft dysfunction and acute rejection (29). In our study we hypothesized TIM-3 expression on CD8+T cells could also be a marker for the graft failure and rejection episodes after transplantation. Therefore, in this study TIM-3 was one of our target molecules that we compared expression levels before and after kidney transplantation. According to our results we were determined that there was a significant positive correlation between graft failure and the increase in TIM-3 gene expression level of CD8+ T cells before and after transplantation.

PD-1 is expressed on NK cells, dendritic cells, both B and T lymphocytes and its ligand PDL-1 is expressed by most of the cancer cells to escape from the immune response (33). Although the role in organ transplantation and allograft rejection cannot known well, it is thought that it can be an important biomarker. Therefore, several studies were focused on the role of PD-1 in organ transplantation. Kinch et al. determined PD-1, PD-L1 and PD-L2 gene expression levels in patients who developed lymphoproliferative disorder after solid organ transplantation (kidney, heart, liver, lung), and observed positive immunostaining results for all 3 molecules in 67% (34). Bishawi et al. discovered that lack of PD-1 causes the change in peripheral lymphocyte balance and because of this allograft rejection detected in heart transplanted patients (35). In our study it was detected that PD-1 expression levels were increased in 9 patients. Two patients had the highest PD-1 levels however, these patients were stable and had good kidney functions. When the patients with rejection episode were investigated, there was no PD-1 expression change in 50% and decreased level of PD-1 in 50% of the patients. Pike et al. compared the level of PD-1 expression in CD4 and CD8 T cell populations of patients and detected increased level of PD-1 expression in patients with rejection episode (36). However, it was reported that costimulatory mediated

tolerance after lung transplantation depended on the expression of the PD-1 molecules on CD8+ T cells and if PD-1 expression is absent, the interaction of CD8 + T cells with immune cells infiltrating the graft is prolonged and causes acute rejection (37). The meaning of this finding is that acute rejection develops in cases where PD-1 expression is decreased and therefore it is parallel to our findings.

In conclusion, this study suggests that detection of TIM-3 mRNA transcripts in CD8+ T cells can be used as a potential biomarker for kidney rejection. The best way to observe patients' clinical status may be the evaluation of the TIM-3 and PD-1 expression levels after the first, third, sixth and one year of transplantation and by comparing them with the results of pre-transplantation levels can be more sensitive and reliable. Further studies can be planned to observe the expression levels of other immune checkpoint regulatory molecules that play important role in immune response.

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

Ethical Approval
Ethical approval for this study was obtained from Health Science University Tepecik Training and Research Hospital Noninvasive Ethics Committee with the number 2022/05-31. This study was conducted in line with the principles of the "Helsinki Declaration".

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all individual participants included in the study.

Availability of Data and Materials
Data sharing is not applicable.

Authors Contributions
BÇA: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

MS: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Writing-review & editing.

MP: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Writing-review & editing.

TKA: Project administration; Supervision; Writing-review & editing.

ET: Data curation; Formal analysis

MT: Data curation; Formal analysis

HİKÇ: Investigation; Methodology

İP: Project administration; Supervision

References

1. Luo Y, Shi B, Qian Y, Bai H, Chang J. Sequential Monitoring of TIM-3 Gene Expression in Peripheral Blood for Diagnostic and Prognostic Evaluation of Acute Rejection in Renal Graft Recipients. Transp proceed 2021;43(10):3669-3674.
2. Grywalska E, Smarz-Widelska I, Mertowski S, Gosik K, Mielnik M, Podgajna M et al. CTLA-4 Expression Inversely Correlates with Kidney Function and Serum Immunoglobulin Concentration in Patients with Primary Glomerulonephritides. Archivum Immunologiae et Therapiae Experimentalis 2019;67(5):335-349.
3. Ingulli E. Mechanism of Cellular Rejection in Transplantation. Ped Nephr 2010;25(1):61-74.
4. Robertson H, Wheeler J, Kirby JA, Morley AR. Renal Allograft Rejectıon-In Sıtu Demonstration of Cytotoxıc Intratubular Cells. Transplantation 1996;61(10):1546-1549.
5. Koyama I, Nadazdin O, Boskovic S, Ochiai T, Smith RN, Sykes M. Depletion of CD8 Memory T Cells for Induction of Tolerance of a Previously Transplanted Kidney Allograft. Am J of Transp 2007;7(5):1055-1061.
6. Betjes MG, Meijers RW, de Wit EA, Weimar W, Litjens NH. Terminally Differentiated CD8+ Temra Cells are Associated with the Risk for Acute Kidney Allograft Rejection. Transplantation 2012;94(1):63-69.
7. Yap M, Brouard S, Pecqueur C, Degauque N. Targeting CD8 T-Cell Metabolism in Transplantation. Front in Immunol 2015;6:547.
8. Hunter MC, Teijeira A, Halin C. T Cell Trafficking Through Lymphatic Vessels. Front in Immunol 2016;7:613.
9. Kumar BV, Connors TJ, Farber DL. Human T Cell Development, Localization, and Function Throughout Life. Immunity 2018;48(2):202-213.
10. Agarwal A, Newell KA. The Role of Positive Costimulatory Molecules in Transplantation and Tolerance. Current Opinion in Organ Transplantation 2008;13(4):366-372.
11. Bour-Jordan H, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M et al. Intrinsic and Extrinsic Control of Peripheral T-Cell Tolerance by Costimulatory Molecules of the CD28/B7 Family. Immunol Rev 2011;241(1):180-205.
12. Esensten J, Helou, YA, Chopra G, Weiss A, Bluestone JA. CD28 Costimulation: From Mechanism to Therapy. Immunity 2016;44(5):973-988.
13. Qin W, Hu L, Zhang X, Jiang S, Li J, Zhang Z et al. The Diverse Function of PD-1/PD-L Pathway Beyond Cancer. Front in Immunol 2019;10:2298.
14. Yoneda A, Jinushi M. T Cell Immunoglobulin Domain and Mucin Domain-3 as an Emerging Target for Immunotherapy in Cancer Management. ImmunoTargets and Therapy 2013;2:135.
15. Qin S, Dong B, Yi M, Chu Q, Wu K. Prognostic Values of TIM-3 Expression in Patients with Solid Tumors: A Meta-Analysis and Database Evaluation. Front in Oncol 2020;10:1288.

16. Wang H, Zhang X, Zheng X, Lan Z, Shi J, Jiang J et al. Prevention of Allograft Rejection in Heart Transplantation Through Concurrent Gene Silencing of TLR and Kinase Signaling Pathways. Scien Reports 2016;6(1):1-14.
17. Karahan HI, Soyöz M, Pehlivan M, Tatar E, Uslu , Gürbüz, BÇ et al. Assessment of Interleukin 2 Cytokine Expression Levels After Renal Transplantation. Transp Proceed 2019;51(4):1074-1077.
18. Wang Y, Meng J, Wang X, Liu S, Shu Q, Gao L et al. Expression of Human TIM-1 and TIM-3 on Lymphocytes from Systemic Lupus Erythematosus Patients. Scand j of Immunol 2008;67(1):63-70.
19. Han FL, Liang F, Jiang TC, Liu M. Increased Expression of CXCR5 and CXCL13 in Mice with Experimental Autoimmune Myocarditis. Eur Rev Med Pharmacol Sci 2017;21(8):1860-7.
20. Williams WW, Taheri D, Tolkoff-Rubin N, Colvin RB. Clinical Role of the Renal Transplant Biopsy. Nat Rev Neph 2012;8(2):110-121.
21. Poggio ED, McClelland RL, Blank KN, Hansen S, Bansal S, Bomback AS et al. Systematic Review and Meta-Analysis of Native Kidney Biopsy Complications. Clin J of the Am Soc of Neph 2020;15(11):1595-1602.
22. Giarraputo A, Barison I, Fedrigo M, Burrello J, Castellani C, Tona F et al. A Changing Paradigm in Heart Transplantation: An Integrative Approach for Invasive and Non-Invasive Allograft Rejection Monitoring. Biomol 2021;11(2):201.
23. Cuervo Florez M, Bruner J, Zarrinpar A. Progress and Challenges in Diagnosis and Treatment of Rejection Following Liver Transplantation. Curr Op in Organ Transp 2021;26(6):669-674.
24. Friedewald JJ, Kurian SM, Heilman RL, Whisenant TC, Poggio ED et al. Development and Clinical Validity of a Novel Blood-Based Molecular Biomarker for Subclinical Acute Rejection Following Kidney Transplant. Am J of Transp 2019;19(1):98-109.
25. Tu L, Guan R, Yang H, Zhou Y, Hong W, Ma L et al. Assessment of the Expression of the immune Checkpoint Molecules PD-1, CTLA4, TIM-3 and LAG-3 Across Different Cancers in Relation to Treatment Response, Tumor-Infiltrating Immune Cells and Survival. Int J of Canc 2020;147(2):423-439.
26. Shahbaz SK, Barabadi M, Ahmadpour P, Pourrezagholi F, Nafar M et al. Sequential Monitoring of TIM-3 mRNA Expression in Blood and Urine Samples of Renal Transplant Recipients. Transp Immunol 2019;54:9-16.
27. Das M, Zhu C, Kuchroo VK. Tim-3 and its Role in Regulating Anti-Tumor Immunity. Immunol Rev 2017;276(1):97-111.
28. Banerjee H, Nieves-Rosado H, Kulkarni A, Murter B, McGrath KV et al. Expression of Tim-3 Drives Phenotypic and Functional Changes in Treg Cells in Secondary Lymphoid Organs and the Tumor Microenvironment. Cell reports 2021;36(11):109699.
29. Renesto PG, Ponciano VC, Cenedeze MA, Camara NS, Pacheco-Silva A. High Expression of Tim-3 mRNA in Urinary Cells from Kidney Transplant Recipients with Acute Rejection. Am J of Transp 2007;7(6):1661-1665.
30. Luo Y, Shi B, Qia Y, Bai H, Chang J. Sequential Monitoring of TIM-3 Gene Expression in Peripheral Blood for Diagnostic and Prognostic Evaluation of Acute Rejection in Renal Graft Recipients. In Transp Proc 2011;43(10):3669-3674.
31. Ponciano VC, Renesto PG, Nogueira E, Rangel ÉB, Cenedeze M et al. Tim-3 Expression in Human Kidney Allografts. Transp Immunol 2007;17(3):215-222.
32. Manfro RC, Aquino-Dias EC, Joelsons G, Nogare AL, Carpio VN et al. Noninvasive Tim-3 Messenger RNA Evaluation in Renal Transplant Recipients with Graft Dysfunction. Transplantation 2008;86(12):1869-1874.
33. Varki V, Ioffe OB, Bentzen SM, Heath J, Cellini A, Feliciano J, Zandberg DP. PD-L1, B7-H3, and PD-1 Expression in Immunocompetent vs. Immunosuppressed Patients with Cutaneous Squamous Cell Carcinoma. Canc Immunol, Immunother 2018;67(5):805-814.
34. Kinch A, Sundström C, Baecklund E, Backlin C, Molin D, et al. Expression of PD-1, PD-L1, and PD-L2 in Posttransplant Lymphoproliferative Disorder After Solid Organ Transplantation. Leukemia & Lymphoma 2019;60(2):376-384.
35. Bishawi M, Bowles D, Pla MM, Oakes F, Chiang Y et al. PD-1 and PD-L1 Expression in Cardiac Transplantation. Cardiovas Pathol 2021;54:107331.
36. Pike R, Thomas N, Workman S, Ambrose L, Guzman D et al. PD1-expressing T Cell Subsets Modify the Rejection Risk in Renal Transplant Patients. Front in Immunol 2016;7:126.
37. Takahashi T, Hsiao HM, Tanaka S, Li W, Higashikubo R et al. PD-1 Expression on CD 8+ T Cells Regulates Their Differentiation within Lung Allografts and is Critical for Tolerance Induction. Am J of Transp 2018;18(1):216-225.

ORIGINAL RESEARCH

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The Impact of Salubrinal in Preventing Fetal Brain Damage in a Model of Chorioamnionitis Induced by LPS

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Abstract

Objective
Chorioamnionitis (CRY), with membrane rupture, preterm labor, prolonged labor, smoking, and bacterial or viral infection origin; is a condition that presents a risk for both maternal and neonatal sequelae. Our study aimed to investigate the effect of Salubrinal (SLB), an endoplasmic reticulum (ER) stress inhibitor, against damage to placental tissue and fetal brain in the Lipopolysaccharide (LPS) induced CRY model.

Material and Method
In this study, 24 Wistar Albino rats on the 17th gestational day; were divided into 4 groups; control, LPS (1 mg/kg intraperitoneal (ip)), LPS + SLB (1 mg/kg LPS ip and 1 mg/kg SLB ip) and SLB (1 mg/kg ip). After an experimental hysterectomy, the placenta and fetal brain tissues were taken into formaldehyde solution for histopathological analysis.

Results
According to the findings obtained; widespread congestion in the basal zone, degeneration of trophoblastic cells in the labyrinth zone, and inflammatory cell infiltrations in both basal and labyrinth zones were observed in the placental tissues of the LPS group. No pathology was detected in only the SLB group. While edema and congestion were detected in the ventricular and intermediate zones in the fetal brain tissues of the LPS group, a significant improvement was observed in these findings with SLB treatment.

Conclusion
As a result; ER stress is one of the mechanisms that play a role in placental tissue and fetal brain damage due to CRY, and SLB therapy might prevent this damage.

Keywords: Chorioamnionitis, endoplasmic reticulum stress, lipopolysaccharide, salubrinal

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Introduction

Preterm labor (PL) is defined as birth before 37 completed weeks of gestation and is the leading cause of neonatal mortality. Survivors of PL are likely to experience long-term neurological disorders (1,2).

PL usually occurs due to intrauterine infection. In pregnancies with signs of intrauterine infection, an increase in the levels of cytokines such as interleukin-1beta, interleukin-8, and tumor necrosis factor-α (TNF-α) has been observed and it has been revealed that these cytokines predispose to the formation of PL (3,4). In addition, inflammatory cytokines play a central role in the intrauterine infection process that triggers brain damage. There is a risk of white matter damage and thus, cerebral palsy in premature babies who are exposed to high concentrations of pro-inflammatory cytokines in the amniotic fluid or fetal blood (5,6). Intracerebral and systemic Lipopolysaccharide (LPS) injections induce inflammatory responses and significant white matter damage (7).

The endoplasmic reticulum (ER) is an organelle that ensures that secretory and membrane proteins fold correctly and travel to the target site after transcription (8). It acts as a quality control center for protein synthesis. When the ER cannot fulfill its function for various reasons, unfolded or misfolded proteins accumulate in the ER lumen, leading to ER stress. To cope with this stress, the ER activates intracellular signaling pathways called unfolded protein response (UPR) and tries to restore homeostasis (9).

Salubrinal (SLB) is a selective inhibitor of the de-phosphorylation of eukaryotic initiation factor 2α (eIF2α), which is critical for ER stress. Inhibition of dephosphorylation of eIF2α provides a cytoprotective effect against ER stress by reducing its workload (10,11).

This study aimed to investigate the effect of SLB, which inhibits the dephosphorylation of eIF2α in the LPS-induced chorioamnionitis model, on placental and fetal brain damage in rats.

Material and Method

Experimental Animals

The study was carried out at Süleyman Demirel University Experimental Animal Production and Experimental Research Laboratory. Ethical approval was obtained from the Animal Experiments Ethics Committee of Süleyman Demirel University with the decision of 17.10.2019/04.

Pregnant Wistar Albino female rats, 8-10 weeks old, were used in our study. The animals' environmental conditions are 12 hours of light and 12 hours of darkness, humidity (55-60%), and constant temperature (22±2 ºC) were provided in Euro type-4 cages. Standard care, nutrition, and housing conditions were provided for the experimental animals. Male and female rats were kept in the same housing environment. To detect vaginal plaque formation in rats, cotton swabs were wetted with physiological saline (SF), and a swab sample was taken from the vagina of female rats using these swabs and spread on a slide. In addition, swab samples taken to determine whether there was sperm in the vaginas of female rats were immediately diagnosed with a binocular light microscope. For the staining process, the swab samples showing sperm were kept in the open air to dry and then stained with crystal violet. 0.2 g of crystal violet was dissolved in 200 mg of distilled water for dyeing. The samples were kept in the dye solution for 1 minute and were washed with distilled water 2 times in succession for 1 minute each. After all these procedures, the samples were sealed using an aquose-mounte solution. Using the smear results, female rats were divided into 4 groups, each with 6 pregnant rats.

1- Control Group (n=6): Pregnant rats were injected with saline (0.5-1 ml volume, intraperitoneal [ip]) from their right inguinal region twice consecutively on the 17th day of the experiment.

2- LPS Group (n=6): Pregnant rats were injected with LPS (0.5-1 ml volume, 1 mg/kg, ip) in their right inguinal region on the 17th day of the experiment and then saline (0.5-1 ml volume, ip) was injected into the same region (12).

3- LPS+SLB Group (n=6): On the 17th day of the experiment, pregnant rats were given LPS (L2630, Sigma-Aldrich, MerckSa, Darmstadt, Germany) (0.5-1 ml volume, 1 mg/kg, ip) from their right inguinal region and then SLB (SML0951, Sigma-Aldrich, MerckSa, Darmstadt, Germany) (0.5-1 ml volume, 1 mg/kg, ip) were given from the same region (13).

4- SLB Group (n=6): On the 17th day of the experiment, pregnant rats were given saline (0.5-1 ml volume, 1 mg/kg, ip) from their right inguinal region and then SLB (0.5-1 ml volume, 1 mg/kg, ip) were given from the same region.

Preterm labor was performed via hysterotomy operation 6 hours after saline injection in the control group and 6 hours after LPS injection in the other

groups. Hysterotomy was performed under anesthesia by applying 10 mg/kg isoflurane to all experimental animals. Following the abdominal incision, placental tissues and fetuses were removed by clamping the artery feeding the placental tissues. Then, blood was taken from the inferior vena cava of the rats and surgical exsanguination was performed. The removed placental tissues, fetuses, and fetal brain tissues taken from fetuses were stored in formaldehyde.

Histopathological Evaluations

The collected tissues were kept in a 10% neutral formaldehyde solution for fixation for at least 48 hours. To perform the tissue tracking procedure, the tissues were washed with running water overnight after their fixation and were kept in alcohol-xylol solutions for specific periods. They were then embedded with paraffin. 5µm sections were taken from paraffin blocks with a Leica RM 2155 RT microtome (Leica Microsystem, Nussloch, Germany). After one day of drying, the slides were passed through the xylol and alcohol series. Then, tissues stained with hematoxylin-Eosin were evaluated under an imaging-assisted binocular light microscope (ECLIPSE Ni-U, Nikon, Japan), and photographs were obtained. Histopathological scoring was made according to the degree of the detected finding as (-): no finding, (+): low-level finding, (++): moderate finding, (+++): severe finding.

Results

Smear Results

To determine the animals to be included in the study, pregnancy status was checked by the presence of sperm in the vaginal canal. In crystal violet staining performed for this purpose, vaginal epithelial cells and sperm were observed in positive samples (Fig.1).

Histopathological Findings in Fetal Placenta Samples

In the histopathological evaluation of the tissues, no pathological findings were found in the fetal placenta samples of the rats in the control group. Significant histopathological changes (diffuse congestion in the basal zone, degeneration of trophoblastic cells in the labyrinth zone, and inflammatory cell infiltrates in both the basal and labyrinth zones) were observed in the fetal placenta samples of rats in the LPS group. A decrease in congestion in the basal zone and degeneration of trophoblastic cells in the labyrinth zone was detected in the fetal placenta samples of rats in the LPS+SLB group compared to those in the LPS group. In addition, inflammatory cell infiltrates in the basal and labyrinth zones seen in the LPS group had a near-normal histological appearance in the LPS+SLB group. A similar appearance was observed in the fetal placenta samples of SLB group rats as in the control group. Fetal placenta basal zone histologic images are presented in Figure 2, fetal placenta labyrinth zone histologic images are displayed in Figure 3, and the scoring table of histological evaluations is shown in Table 1.

Histopathological Findings in Fetal Brain Samples

In histopathological evaluation, the fetal brain tissues of the control group animals had a normal histological appearance. Edema and congestion were observed in the ventricular and intermediate zones in the fetal brain tissues of LPS group animals. A significant decrease in edema and congestion in the ventricular and intermediate zones was observed in the fetal brain tissues of LPS+SLB group animals compared to the LPS group. No histopathological findings were detected in the fetal brain tissues of SLB group animals, like the control group. Histological images of

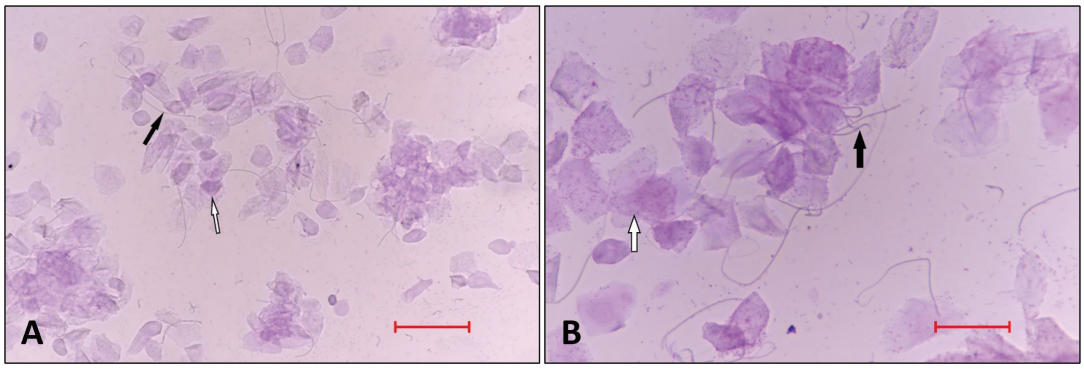

Figure 1
Positive vaginal smear.
Sperms (black arrow) and vaginal epithelial cells (white arrow).
Crystal violet, A: 20x, scale bar = 100µm, B: 40x, scale bar = 50µm.

Table 1 Histopathological evaluations of the basal zone and labyrinth zone in the placenta

Parameter/Group		Control	LPS	LPS+SLB	SLB
Basal zone findings	Congestion	-	+++	+	-
	Inflammatory cell infiltrates	-	+++	+	-
Labyrinth zone findings	Trophoblastic cell degeneration	-	+++	+	-
	Inflammatory cell infiltrates	-	++	-	-

LPS – Lipopolysaccharide; SLB – Salubrinal.

Table 2 Histopathological evaluations of the basal zone and labyrinth zone in the placenta

Parameter/Group	Control	LPS	LPS+SLB	SLB
Congestion	-	+++	+	-
Edema	-	+++	+	-

LPS – Lipopolysaccharide; SLB – Salubrinal.

fetal brains are shown in Figure 4, and the score table of histological evaluations is shown in Table 2.

Discussion

Despite the developments in treatment strategies in the last 20 years, systemic inflammation, which can develop for various reasons during pregnancy, can have a dramatic course for the mother and the fetus. Although the survival rates of these babies have increased day by day with improvements in treatment methods, severe pathological conditions, especially neurodevelopmental disorders, occur in surviving premature babies (14).

Damage mechanisms such as oxidative stress and apoptosis in systemic inflammation not only affect the vascular structures and cause damage to the endothelium, disrupting the blood supply of the tissue but also bind to receptors in the tissues through circulating cytokines and activate some intracellular pathways. With the activation of intracellular pathways, the synthesis and release of cytokines or damage markers occur. In this context, Karakuyu et al. suggested that exposure to LPS triggered cell death in lung cells by triggering ROS production (15). Bao et al. reported that after LPS administration, there was an increase in the levels of TNF-α, IL-6, and chemokine ligand 1 in the placentas of Sprague Dawley rats and that these cytokines disrupted the typical structure of the placenta and increased pathological damage (16). In our study, the labyrinth zone of the placenta facing the fetus, the basal zone facing the mother, and the brain tissue were examined separately. As a result of these histopathological examinations, degeneration of trophoblastic cells was observed in the labyrinth zone, and inflammatory cell infiltrates were observed in both the basal zone and the labyrinth zone. In the study by Yavuz et al., slight hyperemia and edema findings were obtained in the histopathological analysis of the rat cerebral cortex after 1 mg/kg intraperitoneal LPS administration. Considering the results obtained in our study, this proves the accuracy of the LPS dose and route of administration (17). Areas of congestion in the tissue should be evaluated as causing a tendency to bleed in the damaged area. The brain tissue results, which should be parallel to this placental inflammation, also support our hypothesis that inflammation first causes damage to the placenta and affects the brain tissue due to increased permeability. The fact that congestion and edema developing in the ventricular and intermediate zones are also

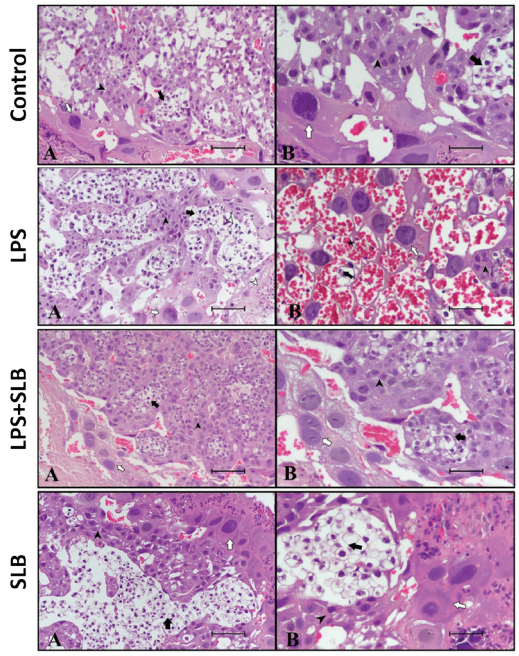

Figure 2

Fetal placenta basal zone histological images. (A) Trophoblastic giant cells (white arrow), glycogen cells (black arrow), spongiotrophoblasts (black arrowhead), inflammatory cell infiltrates (white arrowhead). H-E, 20x, scale bar; 100 µm. (B) Trophoblastic giant cells (white arrow), glycogen cells (black arrow), spongiotrophoblasts (black arrowhead), diffuse areas of congestion (black star). H-E, 40x, scale bar; 50 µm.

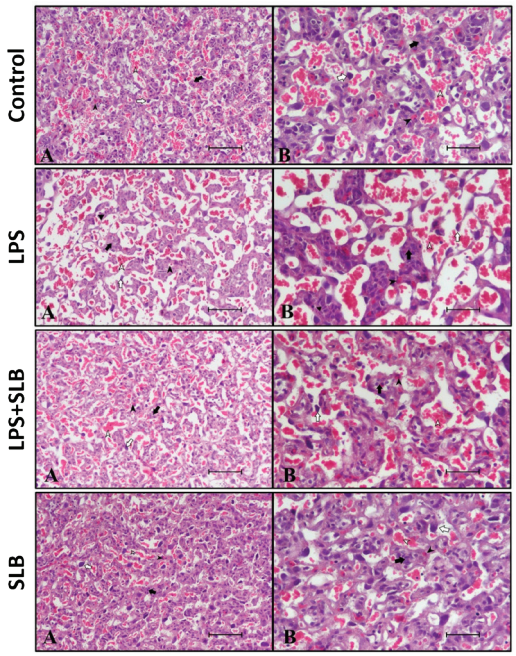

Figure 3

Fetal placenta labyrinth zone histological images. (A) Cytotrophoblasts (white arrow), syncytiotrophoblasts (black arrow), maternal vessels (white arrowhead), fetal vessels (black arrowhead), inflammatory cell infiltrates (black triangle). H-E, 20x, scale bar; 100 µm. (B) Cytotrophoblasts (white arrow), syncytiotrophoblasts (black arrow), maternal vessels (white arrowhead), fetal vessels (black arrowhead), inflammatory cell infiltrates (black triangle). H-E, 40x, scale bar; 50 µm.. LPS – Lipopolysaccharide; SLB – Salubrinal.

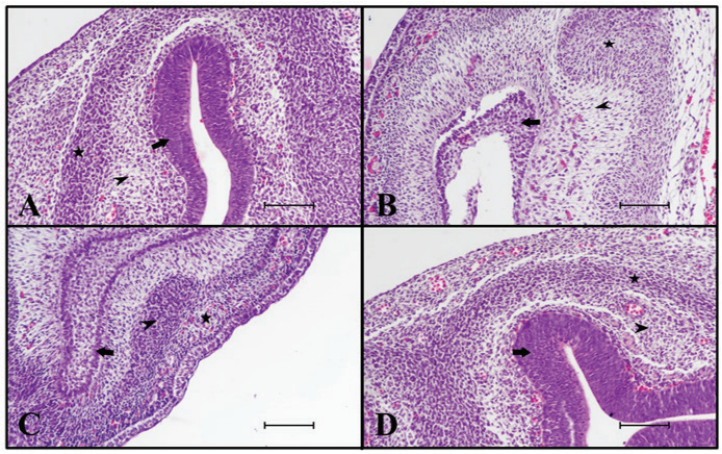

Figure 4

Histopathological images of fetal brain tissues (A) Control group; ventricular zone (arrow), intermediate zone (arrowhead), and cortical plate (star) have normal histological appearance. (B) LPS group; Edema and congestion in the ventricular zone (arrow) and intermediate zone (arrowhead), normal histological appearance in the cortical plate (asterisk). (C) LPS+SLB group; Decrease in histopathological findings in the ventricular zone (arrow), intermediate zone (arrowhead), and cortical plate (asterisk). (D) SLB group; ventricular zone (arrow), intermediate zone (arrowhead), and cortical plate (star) have a normal histological appearance. H-E, 20x, scale bar = 100 µm.

observed in the fetoplacental region is evidence that increased permeability may also damage cerebral tissue. According to the study conducted by Moradi Vastegani et al., it was found that the permeability of the blood-brain barrier (BBB) was increased in LPS-treated rats compared to the control group (18). This confirms that the increase in permeability of the BBB in the baby, through cytokines that cause systemic inflammation, plays a significant role in the progression of the damage.

Our findings concluded that SLB treatment prevented LPS-induced placental and fetal brain damage and did not show any signs of damage in the SLB alone group. SLB has an essential role in ER stress as a blocker of the dephosphorylation of eIF2α. According to the results obtained from our histopathological analysis, the regression of the damages shows that ER stress plays a role in the pathogenesis of the mentioned damage.

SLB has been described as a neuroprotective agent in different nervous system pathologies, demonstrating the importance of reducing ER stress as a therapeutic target to alleviate neural damage (13). Therefore, it has been suggested that treatment with SLB provides cytoprotection related to protein kinase RNA-like endoplasmic reticulum kinase (PERK)- Eukaryotic Initiation Factor 2 alpha (eIF2α) signaling in spinal cord injury (19). It has been demonstrated that in traumatic brain injury, SLB prevents neuronal death in the cortex of mice exposed to trauma by alleviating ER stress (20).

The neuroprotective effect of SLB on ischemic injury has been reported in the middle cerebral artery occlusion and global cerebral ischemia (GCI) model. The neuroprotective effect of SLB in GCI was demonstrated as a reduction in neuron loss seven days after damage to the cornu ammonis 1 (21). This study also hypothesizes that the UPR in cornu ammonis1 cannot overcome ER stress after ischemia and that SLB contributes more to overcoming ER stress.

One of the study's limitations is that the effect of SLB in alleviating the damage to the placenta and fetal brain tissues secondary to experimental CRY was revealed only by histopathological analysis. Cellular pathways need to be shown with more studies. In addition, within the scope of our study model, we believe that a single dose of SLB after injury will not be sufficient to generalize the current results. In this context, studies evaluating the post-treatment effects of SLB for more extended treatment periods and longer follow-up periods are needed.

In line with the findings obtained in this study, we concluded that SLB alleviates LPS-induced placental and fetal brain damage. In this context, the mechanisms of placental and fetal brain damage secondary to systemic inflammation should be investigated more, ER stress-related mechanisms should be detailed, target genes should be increased, and new treatment modalities should be tried. We think the results of this study will shed light on future research on this subject.

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

Ethical Approval
The study was carried out at Süleyman Demirel University Experimental Animal Production and Experimental Research Laboratory. Ethical approval was obtained from the Animal Experiments Ethics Committee of Süleyman Demirel University with the decision of 17.10.2019/04.

The animals' environmental conditions are 12 hours of light and 12 hours of darkness, humidity (55-60%), and constant temperature (22±2 ºC) were provided in Euro type-4 cages. Standard care, nutrition, and housing conditions were provided for the experimental animals.

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Availability of Data and Materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors Contributions
PI: Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing-original draft.

SO: Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing-original draft.

MS: Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing-review & editing.

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References

1. Dicanio D, Spoto G, Alibrandi A, Minutoli R, Nicotera AG, Di Rosa G. Long-Term Predictivity of Early Neurological Assessment and Developmental Trajectories in Low-Risk Preterm Infants. Front Neurol 2022;13. doi: 10.3389/fneur.2022.958682.
2. Ophelders DRMG, Gussenhoven R, Klein L, Jellema RK, Westerlaken RJJ, Hütten MC, Vermeulen J, Wassink G, Gunn AJ, Wolfs TGAM. Preterm Brain Injury, Antenatal Triggers, and Therapeutics: Timing Is Key. Cells 2020.
3. Christiaens I, Zaragoza DB, Guilbert L, Robertson SA, Mitchell BF, Olson DM. Inflammatory Processes in Preterm and Term Parturition. J Reprod Immunol 2008.
4. Elliott CL, Loudon JAZ, Brown N, Slater DM, Bennett PR, Sullivan MHF. IL-1β and IL-8 in Human Fetal Membranes: Changes with Gestational Age, Labor, and Culture Conditions. American Journal of Reproductive Immunology 2001;46. doi: 10.1034/j.1600-0897.2001.d01-11.x.
5. Shi Z, Ma L, Luo K, Bajaj M, Chawla S, Natarajan G, Hagberg H, Tan S. Chorioamnionitis in the Development of Cerebral Palsy: A Meta-Analysis and Systematic Review. Pediatrics 2017.
6. Bo Hyun Yoon, Jong Kwan Jun, Romero R, Kyo Hoon Park, Gomez R, Choi H, Kim IO. Amniotic Fluid Inflammatory Cytokines (interleukin-6, interleukin-1β, and tumor necrosis factor-α), Neonatal Brain White Matter Lesions, and Cerebral Palsy. Am J Obstet Gynecol 1997;177. doi: 10.1016/S0002-9378(97)70432-0.
7. Wang X, Rousset CI, Hagberg H, Mallard C. Lipopolysaccharide-Induced Inflammation and Perinatal Brain Injury. Semin Fetal Neonatal Med 2006;11. doi: 10.1016/j.siny.2006.04.002.
8. Lemberg MK, Strisovsky K. Maintenance of Organellar Protein Homeostasis by ER-Associated Degradation and Related Mechanisms. Mol Cell 2021.
9. Wodrich APK, Scott AW, Shukla AK, Harris BT, Giniger E. The Unfolded Protein Responses in Health, Aging, and Neurodegeneration: Recent Advances and Future Considerations. Front Mol Neurosci 2022.
10. Lu W, Ni K, Li Z, Xiao L, Li Y, Jiang Y, Zhang J, Shi H. Salubrinal Protects Against Cisplatin-Induced Cochlear Hair Cell Endoplasmic Reticulum Stress by Regulating Eukaryotic Translation Initiation Factor 2α Signalling. Front Mol Neurosci 2022;15. doi: 10.3389/fnmol.2022.916458.
11. Matsuoka M, Komoike Y. Experimental Evidence Shows Salubrinal, an eIF2α Dephosphorylation Inhibitor, Reduces Xenotoxicant-Induced Cellular Damage. Int J Mol Sci 2015.
12. Yavuz A, Sezik M, Ozmen O, Asci H. Fingolimod Against Endotoxin-Induced Fetal Brain Injury in a Rat Model. Journal of Obstetrics and Gynaecology Research 2017;43. doi: 10.1111/jog.13444.
13. Wang Z feng, Gao C, Chen W, Gao Y, Wang H chen, Meng Y, Luo C liang, Zhang M yang, Chen G, Chen X ping, et al. Salubrinal Offers Neuroprotection Through Suppressing Endoplasmic Reticulum Stress, Autophagy, and Apoptosis in a Mouse Traumatic Brain Injury Model. Neurobiol Learn Mem 2019;161. doi: 10.1016/j.nlm.2019.03.002.
14. Song IG. Neurodevelopmental Outcomes of Preterm Infants. Clin Exp Pediatr 2023.
15. Karakuyu NF, Özmen Ö. Dekspantenol, Sıçanlarda LPS'nin Neden Olduğu Akut Akciğer Hasarında Artan VCAM-1 ve Kaspaz-3 Ekspresyonlarını Azaltarak İnflamasyonu ve Apoptozu İnhibe Eder. Kocatepe Veterinary Journal 2022; doi: 10.30607/kvj.1101065.
16. Bao J, Liu Y, Yang J, Gao Q, Shi SQ, Garfield RE, Liu H. Nicotine Inhibits LPS-Induced Cytokine Production and Leukocyte Infiltration in rat Placenta. Placenta 2016;39. doi: 10.1016/j.placenta.2016.01.015.
17. Yavuz A, Sezik M, Eris Yalcin S, Asci H, Ozmen O. Immunohistochemical Analysis of Protective Effects of Maternal Fingolimod on the Placenta and Fetal Lung and Brain in Chorioamnionitis-Induced Preterm Birth Rat Model. Immunopharmacol Immunotoxicol 2020;42. doi: 10.1080/08923973.2020.1818771.
18. Moradi Vastegani S, Hajipour S, Sarkaki A, Basir Z, Parisa Navabi S, Farbood Y, Khoshnam SE. Curcumin Mitigates Lipopolysaccharide-Induced Anxiety/Depression-Like Behaviors, Blood–Brain Barrier Dysfunction, and Brain Edema by Decreasing Cerebral Oxidative Stress in Male Rats. Neurosci Lett 2022;782. doi: 10.1016/j.neulet.2022.136697.
19. Ohri SS, Hetman M, Whittemore SR. Restoring Endoplasmic Reticulum Homeostasis Improves Functional Recovery After Spinal Cord Injury. Neurobiol Dis 2013;58. doi: 10.1016/j.nbd.2013.04.021.
20. Rubovitch V, Barak S, Rachmany L, Goldstein RB, Zilberstein Y, Pick CG. The Neuroprotective Effect of Salubrinal in a Mouse Model of Traumatic Brain Injury. Neuromolecular Med 2015;17. doi: 10.1007/s12017-015-8340-3.
21. Anuncibay-Soto B, Pérez-Rodríguez D, Santos-Galdiano M, Font E, Regueiro-Purriños M, Fernández-López A. Post-Ischemic Salubrinal Treatment Results in a Neuroprotective Role in Global Cerebral Ischemia. J Neurochem 2016; doi: 10.1111/jnc.13651.

ORIGINAL RESEARCH

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Effects of Face-to-Face Education on Reduction Noise in Hemodialysis Units: A Quasi-Experimental Study

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Abstract

Objective

This study aims to evaluate the effect of the education given to the hemodialysis personnel on noise and noise management in the hemodialysis unit on the level of noise exposure of hemodialysis patients. Another aim of the study is to determine whether hemodialysis patients are affected by noise in the hemodialysis unit.

Material and Method

This quasi-experimental study was conducted at two dialysis centers in Turkey between January and May 2022, with 101 hemodialysis patients (80%) and 50 hemodialysis unit employees (90%). Noise level measurements were made before and two weeks after the noise control education was given to the staff.

Results

In the study, before the face-to-face education, 62% of employees stated that the noisy environment sometimes distracted them, 92% had not participated in an education on noise, and 82% wanted to receive education on this subject. After the education, it was observed that the noise level decreased on all days and hours. The average of the measurements made for a total of five days after the education decreased statistically significantly compared to the pre-education period ($p<0.05$).

Conclusion

Our study showed that the noise levels in hemodialysis units are disturbing for patients, the knowledge and awareness of the personnel on the subject is low, education programs including noise prevention and reduction strategies increase the knowledge and awareness of the personnel and rapidly turn into behaviors, and education is effective in noise control.

Keywords: Face-to-face education, hemodialysis, noise, quasi-experimental study

Introduction

Environmental awareness has grown dramatically over the last two decades. Noise pollution, in addition to water and air pollution, is a significant public health issue (1,2). The effects of noise spread quickly and become more apparent as noise level increase. Noise; is a significant environmental health issue that produces a variety of illnesses based on its intensity (3,4).

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Noise-induced problems include stress response in the organism, depression, insomnia, concentration disorder, immune system weakness, anxiety, loss of appetite, hearing loss, delayed wound healing, decreased pain threshold, and deterioration in physiological parameters (3,5). While the exposure of healthy individuals to noise can cause these health problems, the exposure of patients undergoing treatment due to other health problems in hospitals is a much more important issue (6, 7). Noise in hospitals causes additional health problems for patients and negatively affects the healing process (8-11).

The standard noise intensity is 40 decibels (dB) during the day and 35 dB at night (12). Noise is defined as an unwanted and unexpected sound perceived by the ear without rhythm or harmony that causes psychosocial and physiological stress in the individual (13,14). According to the World Health Organization, the noise level in hospitals should not exceed 35 dB during the day and 30 dB at night (15). Meanwhile, the Environmental Protection Association guidelines suggest that noise levels should not exceed 45dB(A) during the day and 35dB(A) at night (16, 17). However, measurements taken in hospitals in recent years show that the noise levels are approximately 72 dB during the day and 60 dB at night (15-17).

Noise pollution in hospitals affects not only the patients but also the staff working in the hospital (18-20). Intensive care units, surgical services, and dialysis units are among the main places in hospitals where noise pollution is experienced owing to a large number of mechanical devices and the alarms they trigger (6, 21).

Noise is a significant problem for patients and staff in dialysis units (10). In these units, telephone calls, staff conversations, and; most importantly, hemodialysis machines can increase the sound intensity in these environments (10). Dialysis patients face many challenges due to chronic kidney failure. In addition, dialysis treatment for these patients is a lifelong process, often involving 4 hours a day 2-3 days a week (22). For patients who spend most of their lives in a dialysis unit, good environmental conditions also determine their health (23, 24). In addition, the number of studies that show how high the noise levels are in dialysis units and what the causes of the noise are in our country is very limited.

Therefore, it is very important to determine the level sources of noise in dialysis units and to control the noise exposure. The most effective way to change behavior in a subject is education. An important part of noise control is the education of nurses, doctors, and other staff working in the dialysis unit to deal with noise sources. Raising awareness is the first step in the fight against noise sources. Noise pollution can be combated through education, according to the literature. Based on this, this study aims to evaluate the effect of the education given to the hemodialysis personnel on noise and noise management in the hemodialysis unit on the level of noise exposure of hemodialysis patients. Another aim of the study is to determine whether hemodialysis patients are affected by noise in the hemodialysis unit.

Material and Method

Study Design and Sample

This quasi-experimental study was conducted at two dialysis centers in Turkey between January 2022 and May 2022. The inclusion criteria were individuals who have received hemodialysis treatment for at least three months, are 18 years of age and older, have a place, time, and person orientation, are literate, have no communication barriers, and agree to participate in the study. Patients with hearing problems and patients who did not volunteer to participate in the study were excluded from the study. The study was conducted with 101 (80%) hemodialysis patients and 50 (90%) hemodialysis unit employees.

Instruments

Patient Characteristics Form

This form, which was prepared by the researchers in line with the literature, includes 10 questions, including 6 questions about the sociodemographic (age, gender, marital status, etc.) characteristics of the patients and 4 questions about the disease and treatment process.

Noise Level Assessment Form

The first part of this form, which consists of two parts, there is a numerical scale (between 0-10) questioning the level of patients' discomfort with noise in the hemodialysis unit. Patients are asked to select the number that best describes the noise intensity they perceive. A level of zero indicates that the patients perceive no noise at all, while the highest number means that they perceive a very high level of noise. In the second part of this form, there are 25 statements prepared by the researchers in line with the literature, which enable the patients to evaluate their level of discomfort from various noise sources in the hemodialysis unit on a five-point Likert scale: None: 1, Somewhat: 2, Moderate: 3, High: 4, Very, High: 5. (9,10,15,18). In this form, the opinions of three expert faculty members working in nephrology in the field of internal medicine nursing and a scientific expert

nephrology nurse were taken into consideration. Using the Davis Technique, the content validity index was calculated as 0.96.

Employees Characteristics Form
This form, which was prepared by the researchers in line with the literature, consists of a total of 13 questions, including four questions about the sociodemographic (age, gender, marital status, etc.) characteristics of the employees, three questions about professional information, three questions about noise awareness in the hemodialysis unit and three questions about the level of noise disturbance.

Noise Measurement
The noise was measured using a Uni-t Ut353 Mini decibelmeter. UT350 series sound level meters can measure sound noise level and output results in dB. Depending on the application, A or C-weighted modes can be selected. These meters consume very little power and can operate continuously for up to 20 hours. They are suitable for industrial and environmental sound level measurements. At the end of each hourly measurement, the noise meters were calibrated by the researcher with the Cem Sc-05 (Sound Level Calibrator) calibration device between 94 and 114 dB as specified in the instructions, by placing the microphones at the end of the device near to the end of the calibration device, and by using two different sound intensities.

Procedure
The patient introduction form and the Noise level assessment form were applied face-to-face by the researcher to the patients who were hospitalized in the hemodialysis unit and met the research criteria. This form took approximately eight minutes to fill out. In addition, the introduction form was applied face-to-face by the researcher to the employees in the unit. It took three minutes for the employees to fill out the form. It took a total of three weeks to collect these data from patients and employees. At the end of these three weeks, the employees of the hemodialysis unit consisting of nurses, physicians, dialysis technicians, medical secretaries, and cleaning staff were divided into groups of five people so as not to disrupt the services provided in the unit and each group was given a total of 40 minutes of noise control education. The educations were conducted by the researcher in the meeting room in the hemodialysis unit and completed in two weeks.

Educational Intervention
A structured educational program was designed in the form of knowledge in noise control. This educational program has been created with a systematic approach to cover the multifaceted aspects of auditory management and aims to provide an in-depth understanding of both the theoretical principles and practical applications related to the control of environmental acoustics. It was constructed by the researchers after conducting and reviewing related literature literature (8, 9, 12, 16, 17, 19). The group teaching techniques and a PowerPoint presentation consisting of 30 slides containing visual and scientific information were used. The principal investigator (HC), performed the teaching intervention. The education lasted a total of 40 minutes in the form of discussion, question, and answer. The group education was didactic and interactive. Participants could ask questions at the time of class. An interactive portion of the teaching program was held at the end of class. In this section, personnel were encouraged to offer support to each other. The content of the education included the concept of noise, noise sources in hemodialysis units, ensuring noise awareness in hospital staff, the effects of noise on patients and hospital staff, noise reduction strategies in hemodialysis units, and evidence-based practices in reducing noise in hemodialysis units. In addition, at the end of the education, all participants were given materials in the form of written brochures containing the information explained. These brochures were also left at the hemodialysis unit. Two weeks after the education of all employees was completed, the noise level assessment form was applied to the patients for the second time.

Noise level measurements were made before and two weeks after the noise control education was given to the staff. Since the two hemodialysis centers where the study was conducted had similar physical characteristics, noise level measurements were performed in the same way. In both centers, the unit consisted of a total of 10 beds, and the nurse observation desk was located in the middle. The noise-measuring device was placed in the center of the unit. Noise sources and levels were evaluated in a 10-station hemodialysis unit over five days, covering a period of 4 times per day.

Statistical Analysis
The data obtained in the research were analyzed using SPSS (Statistical Package for Social Sciences) for the Windows 25.0 program. Descriptive statistical analyses (number, percentage, mean, standard deviation) were performed while evaluating the data. Normal distribution fit was checked with normality tests and kurtosis and skewness values. It was determined that the data met the assumption of normal distribution. In this case, the dependent sample t-test was used to

compare the average noise disturbance levels of the patients before and after the education given to the employees.

Results

The mean age of the patients who participated in the study was 57.20 ± 9.18 years and the majority (62.4%) were male. Other descriptive characteristics of the patients are given in Table 1.

The mean age of the hemodialysis unit employees who participated in the study was 32.56 ± 5.743 years, 54% were male and 44% were dialysis technicians. Other descriptive characteristics of the employees are given in Table 2.

When the noise level assessment form in which the patients who participated in the study indicated the noise sources and the degree of discomfort they were disturbed by during the hemodialysis session was evaluated, the mean score given by the patients to the noise scale between 0-10 was 7±9.23 and the level of discomfort from noise was evaluated as 5±4.18 by the patients after the education given to the employees. The answers given to the questions related to noise sources and discomfort level in the second part of the noise level assessment form are given in detail in Table 3.

When the staff participating in the study were asked about their opinions on noise in the hemodialysis unit before the education, 80% of the employees stated that the unit was noisy, 62% stated that the noisy environment sometimes distracted them, 92% stated that they had not participated in an education on noise and 82% stated that they would like to receive education on this subject (Table 4).

Noise measurement values before and after the education given to the personnel are given in Table 5. According to Table 5, it is seen that the noise level decreased after the education on all days and hours, and the noise level was the highest at noon.

The relationship between the averages of the noise measurement values before and after the education given to the personnel is given in Table 6. It is seen that the average of the measurements made for a total of five days after the education decreased statistically significantly compared to before the education ($p<0.05$).

Table 1 Descriptive characteristics of the patients (n= 101)

Characteristics		n	%
Age	57.20 ± 9.18*		
Gender	Female	38	37.6
	Male	63	62.4
Marital status	Married	84	83.2
	Single	17	16,8
Educational status	Primary	15	14.9
	Secondary	23	22.8
	High school and above	63	62.3
Income status	Income exceeds expenditure	9	8.9
	Income equal to expenditure	47	46.5
	Income less than expenditure	46	44.6
Health Insurance	Yes	92	91.1
	No	9	8.9
Presence of additional chronic disease	Yes	85	84.2
	No	16	15.8

Discussion

This study, to increase awareness and knowledge about the significance of noise in hemodialysis units, assessed the noise level on various days and hours. It revealed that patients undergoing hemodialysis were exposed to a wide range of noise sources and that staff members in the unit had little awareness of how to control noise. The results of the study indicate a significant impact of face-to-face education on the reduction of noise in hemodialysis units. However, the staff members' education on the topic decreased both the unit's noise level and the patient's level of noise disturbance. The study also contributed significant data to the literature on the topic and raised staff awareness.

It is seen that most of the studies on indoor noise in hospitals were conducted in intensive care units. (7, 25). There are very few studies evaluating noise in hemodialysis units (10). The fact that the mean score (7±9.23) given by the patients participating in our study to the noise scale between 0-10 is quite high shows how important the noise perceived by the patients is in hemodialysis units. In the intensive care units of hospitals, in addition to the sounds caused by medical devices and alarms used for life support, there are also sounds caused by the personnel working in this field (26, 27). Dialysis devices and alarms, which are at least as much a source of noise as hemodialysis units, are among the most important causes of noise in these units (10, 28). In addition, the sounds originating from the employees in the hemodialysis unit are also important. Many reasons such as food distribution, cleaning, nursing services, rounds, the high number of entrances and exits, and television/telephone sounds can be listed as noise sources (10, 28). When we look at the noise sources that the patients who participated in our study were disturbed by, confirm this information in the literature (Table 3). The detailed examination of noise sources and their impact on hemodialysis units provides valuable insights into the

Table 2 Descriptive characteristics of the Hemodialysis Unit employees (n= 50)

Characteristics		**n**	**%**
Age	32.56 ± 5.743*		
Gender	Female	23	46.0
	Male	27	54.0
Marital status	Married	24	48.0
	Single	26	52.0
Educational status	Secondary	3	6.0
	High school	17	34.0
	University and above	30	60.0
Occupation	Nurse	18	36.0
	Physician	6	12.0
	Dialysis Technician	22	44.0
	Medical Secretary	1	2.0
	Cleaning Staff	3	6.0
Working time	1 year and less	12	24.0
	2-5 years	28	56.0
	6-9 yıl	6	12.0
	10 years and above	4	8.0
Working Time in HD Unit	1 year and less	40	80.0
	2-5 years	8	16.0
	10 years and above	2	4.0

Table 3 The level of discomfort of the patients from the following conditions during hemodialysis (n= 101)

		Employee Pre-Education		Employee Post-Education	
		n	%	n	%
I am disturbed by noise during cleaning services	**A little**	2	2.0	22	21.8
	Moderate	27	26.7	79	78.2
	A lot	72	71.3	-	-
I am disturbed by the noise during the transportation of medical supplies	**A little**	1	1.0	30	29,7
	Moderate	30	29,7	61	60.1
	A lot	70	69.3	10	10.2
I am uncomfortable with health personnel entering and exiting the hall from outside	**A little**	1	1.0	8	7.9
	Moderate	27	26.7	67	66.3
	A lot	73	72.3	26	25.7
I am uncomfortable with patients' relatives entering and leaving the hall from outside	**A little**	2	2.0	21	20.8
	Moderate	20	19.8	62	60.6
	A lot	79	78,2	18	18.6
I am disturbed by the conversations of other patients in the room	**A little**	1	1.0	59	58.4
	Moderate	22	21.8	42	41.6
	A lot	78	77.2	-	-
I am disturbed by the conversations of nurses, physicians, and other health personnel in the hall	**A little**	-	-	5	5.0
	Moderate	25	24.8	61	60.4
	A lot	76	75,2	35	34.7
I am disturbed by the speech of the student nurses in the hall	**A little**	2	2.0	6	5.9
	Moderate	12	11.9	65	64.4
	A lot	87	86.1	30	29.7
I am disturbed by the computer, printer, and medical secretarial work in the hall	**A little**	1	1.0	9	8.9
	Moderate	19	18.8	47	46.5
	A lot	81	80.2	45	44.6
I am disturbed by the sound of the phone and phone calls at the desk	**A little**	1	1.0	5	5.0
	Moderate	14	13.9	65	64.4
	A lot	86	85.1	31	30.7
I am disturbed by the sound of cell phones and phone calls of employees or patients/ patient relatives	**A little**	1	1.0	15	14.9
	Moderate	17	16.8	53	52.5
	A lot	83	82.2	33	32.7
I am disturbed by the footsteps of the medical team and other staff in the hall	**A little**	1	1.0	58	57.4
	Moderate	16	15.8	38	37.6
	A lot	84	83.2	5	5.0
I am disturbed by the sound of hemodialysis machines and alarm sounds	**A little**	1	1.0	4	4.0
	Moderate	22	21.8	43	42.6
	A lot	78	77.2	54	53.5

Table 3 continued The level of discomfort of the patients from the following conditions during hemodialysis (n= 101)

I am disturbed by the sounds of television, music, etc.	**A little**	1	1.0	38	37.6
	Moderate	11	10.9	56	55.4
	A lot	89	88.1	7	7.0
I am disturbed by sounds coming from the patient next to me (snoring, crying, moaning, talking, etc.)	**A little**	1	1.0	14	13.9
	Moderate	16	15.8	53	52.5
	A lot	84	83.2	34	33.7
I am disturbed by the noise during care and treatment in the salon	**A little**	1	1.0	62	61.4
	Moderate	13	12.9	31	30.7
	A lot	87	86.1	8	7.9
I am disturbed by the rush of emergencies	**A little**	2	2.0	10	9.9
	Moderate	13	12.9	43	42.6
	A lot	86	85.1	48	47.5
I am disturbed by noise during breakfast, snacks, and food distribution	**A little**	1	1.0	8	7.9
	Moderate	8	7.9	52	51.5
	A lot	92	91.1	41	40.6
I am disturbed by noises coming from the corridor	**A little**	3	3.0	11	10.9
	Moderate	13	12.9	60	59.4
	A lot	85	84.1	30	29.7
I am disturbed by noises coming from the environment such as drinking tea, coffee and eating	**A little**	1	1.0	10	9.9
	Moderate	16	15.8	52	51.5
	A lot	84	83.2	39	38.6
I am disturbed by noises during the unpacking of materials and packages	**A little**	1	1.0	11	10.9
	Moderate	12	11.9	57	56.4
	A lot	88	87.1	33	32.7
I am disturbed by the noise of the air conditioners	**A little**	1	1.0	6	5.9
	Moderate	11	10.9	58	57.5
	A lot	89	88.2	37	36.6
I am disturbed by doors opening and closing	**A little**	1	1.0	6	5.9
	Moderate	10	9.9	56	55.4
	A lot	90	89.1	39	38.6
I am disturbed by the constant opening and closing of cabinets, drawers, windows, etc.	**A little**	1	1.0	5	5.0
	Moderate	9	8.9	64	63.4
	A lot	91	90.1	32	31.7
I am disturbed by ambient sounds entering the hall from the hospital surroundings (car, construction, conversations, etc.)	**A little**	1	1.0	8	8.2
	Moderate	11	10.9	11	10.7
	A lot	89	88.1	82	81.1
I am disturbed by the sound of an ambulance in the hall	**A little**	3	3.0	5	5.0
	Moderate	7	6.9	9	8.7
	A lot	91	90.1	87	86.3

Table 4 Opinions of Hemodialysis Unit employees about noise (n= 50)

		n	%
Is there a noisy working environment in your unit?	Yes	10	20.0
	No	40	80.0
Does Working in a Noisy Environment distract you?	Yes	14	28.0
	No	5	10.0
	Sometimes	31	62.0
Have you attended any education on noise and noise control?	Yes	4	8.0
	No	46	92.0
Do You Think Noise in Your Unit Affects Patients and Staff?	Yes	5	10.0
	No	7	14.0
	Sometimes	35	70.0
	I don't know	3	6.0
Do you take noise-reducing measures in your operations related to the service you provide?	Yes	20	40.0
	No	11	22.0
	Sometimes	19	38.0
Is the noise level measured in your unit?			
	Yes	10	20.0
	No	5	10.0
	I don't know	35	70.0
Would You Like to Receive Education on Noise and Noise Control?	Yes	9	18.0
	No	41	82.0

Table 5 The median of the recorded noise measurements of the pre-education and post-education

Days	**Monday**		**Tuesday**		**Wednesday**		**Thursday**		**Friday**	
Measuring Time	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
08:30	70	62	73	64	72	62	73	64	75	62
10:30	68	59	65	57	65	59	63	56	65	58
12:30	78	64	79	66	76	65	78	68	76	67
15:30	76	65	78	63	74	61	75	65	79	63

multifaceted nature of noise pollution in healthcare settings. The findings of the study underscore the significance of a comprehensive understanding of the various sources of noise and their effects on both patients and healthcare staff. The substantial mean score reported by patients on the noise scale, coupled with their discomfort levels before the educational intervention, emphasizes the pronounced impact of noise on the patient experience during hemodialysis sessions. Furthermore, the identification of specific sources of noise, such as dialysis devices, alarms, and activities of the staff, resonates with existing

Table 6 The relation between of noise measurements of the pre-education and post-education

	Min	Max	Mean	Std. Deviation	t-test	p
Pre-education	31	79	73,39	6,750	59.410*	**.000****
Post-education	26	68	58.12	4.642		

*Paired Samples t-test ** p<0.05

literature, highlighting the multitude of contributors to noise pollution within hemodialysis units (21, 26). The comprehensive exploration of noise sources and their effects, informed by patient perspectives and staff awareness, lays a strong foundation for tailored noise reduction strategies in hemodialysis units. By illuminating the diverse range of contributors to noise pollution and their implications for patient comfort and staff well-being, the study sets the stage for targeted interventions aimed at mitigating noise levels and fostering a more conducive environment for all stakeholders within the hemodialysis unit.

Noise in hemodialysis units is important not only for patients but also for the working performance and psychological well-being of all personnel working in this unit (29, 30). In addition, awareness and education of personnel on the subject are important for noise control (31). The opinions of the hemodialysis workers who participated in our study about noise in the hemodialysis unit are parallel with this information in the literature (Table 4). Furthermore, the high mean score reported by patients on the noise scale, coupled with their discomfort levels before the education, highlights the profound impact of noise on the patient experience during hemodialysis sessions. The subsequent decrease in these scores following the educational intervention not only validates the efficacy of the intervention but also emphasizes the positive influence on addressing patients' concerns and enhancing their comfort during treatment. It is noteworthy that the awareness of the personnel participating in our study on noise is low and they have not received education on the subject. This may be due to adaptation to the environment and normalization of noise. It shows that hospitals should address the issue in in-service education programs for staff. In addition to acoustic design and equipment design, another important application in the prevention and reduction of noise pollution in hospitals is staff education (20, 32, 33). While acoustic and equipment design plays a critical role in mitigating noise at its source, the heart of sustainable noise reduction lies within staff behavior and practice. Educational interventions have been identified as a cost-effective approach to noise management, fostering a culture of awareness and responsibility among healthcare workers. By increasing understanding of the impact noise has on patient outcomes and employee well-being, educational programs can catalyze the adoption of noise-reducing practices (2,6,7, 34- 36). In addition, the most cost-effective strategy mentioned in the literature is the education of healthcare workers (33, 35). The most obvious way to reduce noise is to raise staff awareness, educate them about the effects of noise, and provide them with viable alternative interventions that they can incorporate into their daily practice (31, 33). Raising staff awareness and educating healthcare workers about the effects of noise are pivotal aspects of a multifaceted approach to noise reduction in healthcare settings. By providing staff with training on the impact of noise and its consequences on patients' health and well-being, hospitals can foster a more mindful workplace. Education programs should aim to empower staff with knowledge and tools to actively reduce noise pollution (6, 21, 37).

In our study, when we look at the noise measurement values made on five days of the week and at four different times of the day, it is seen that the noise level is the highest at noon. This is an expected result since lunchtime is a busy time when the treatment of morning session patients is completed and afternoon patients are received. In addition, in our study, it is seen that the noise control education given to the staff reduced the noise measurement values made five days a week. When the relationship between the daily noise averages measured during the whole week before and after the education is examined, the statistically significant decrease in the noise level shows that the education received by the personnel on noise control has turned into behavior. The reduction in noise following the implementation of noise control education signifies a successful translation of theory into practice. By equipping staff with the knowledge and strategies to control noise, we have shown that it is possible to effect a statistically significant change in the acoustic environment of a healthcare setting.

Conclusion

Our study showed that the noise levels in hemodialysis units are disturbing for patients, the knowledge and awareness of the personnel on the subject is low, education programs including noise prevention and reduction strategies increase the knowledge and awareness of the personnel and rapidly turn into behaviors and education is effective in noise control. In line with these results, it is recommended that noise level measurements should be performed routinely by the hospital, personnel should be trained periodically with in-service education and necessary inspections should be made to ensure employee and patient safety in hemodialysis units to reduce patients' anxiety, increase their comfort and positively affect psychological and physiological parameters. In addition, conducting studies on noise and its effects on patients and employees in hemodialysis units will reveal the importance of the issue more clearly.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Ethical Approval

Ethics approval to conduct the study was obtained from Burdur Mehmet Akif Ersoy University Non-Interventional Clinical Research Ethics Committee (Decision no: 2020-3/43, Decision date: March 4, 2020). In addition, permission was received from the medical directors of the related HD center for the study. The purpose of the study was explained to each participant in a face-to-face interview by the research staff, and written consents were obtained from the patients approved to enter this study. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki.

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants included in the study.

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Availability of Data and Materials

Data are available on request due to privacy or other restrictions.

Authors Contributions

H.C: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

C.K.S: Investigation; Validation; Writing-original draft.

References

1. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and Health Impacts of Air Pollution: A Review. Frontiers in Public Health 2020;8:505570. doi:10.3389/fpubh.2020.00014.
2. Hammer MS, Swinburn TK, Neitzel RL. Environmental Noise Pollution in the United States: Developing an Effective Public Health Response. Environ Health Perspect 2014;122(2):115-9. doi: 10.1289/ehp.1307272.
3. Hahad O, Prochaska HJ, Daiber A, Thomas Münzel T. "Environmental Noise-Induced Effects on Stress Hormones, Oxidative Stress, and Vascular Dysfunction: Key Factors in the Relationship between Cerebrocardiovascular and Psychological Disorders", Oxidative Medicine and Cellular Longevity 2019;(2019):1-13. doi:10.1155/2019/4623109.
4. Lee Y, Lee S, Lee W. Occupational and Environmental Noise Exposure and Extra-Auditory Effects on Humans: A Systematic Literature Review. Geohealth 2023;7;7(6):e2023GH000805. doi: 10.1029/2023GH000805.
5. Zaman M, Muslim M, Jehangir A. Environmental Noise-Induced Cardiovascular, Metabolic and Mental Health Disorders: a Brief Review. Environ Sci Pollut Res Int 2022;29(51):76485-76500. doi: 10.1007/s11356-022-22351-y.
6. Konkani A, Oakley B. Noise in Hospital Intensive Care Units--A Critical Review of A Critical Topic. J Crit Care 2012;27(5):522.e1-9. doi: 10.1016/j.jcrc.2011.09.003.
7. de Lima Andrade E, da Cunha e Silva, DC, de Lima, EA, de Oliveira RA, Zannin PHT, Martins, ACG. Environmental Noise in Hospitals: A Systematic Review. Environmental Science and Pollution Research 2021;28:19629-19642.
8. Hsu T, Ryherd EE, Waye KP, Ackerman J. Noise Pollution in Hospitals: Impact on Patients. Journal of Clinical Outcomes Management 2012;19:301-309.
9. Xie H, Kang J, Mills GH. Clinical review: The Impact of Noise on Patients' Sleep and the Effectiveness of Noise Reduction Strategies in Intensive Care Units. Crit Care 2009;13(2):208. doi: 10.1186/cc7154.
10. Ok E, Aydin Sayilan A, Sayilan S, Sousa CN, Ozen N. Noise Levels in the Dialysis Unit and its Relationship with Sleep Quality and Anxiety in Patients Receiving HD: A pilot study. Ther Apher Dial 2022;26(2):425-433. doi: 10.1111/1744-9987.13794.
11. Gungor S, Gursel O. Effects of Noise on Vital Signs and Anxiety Levels of Patients Hospitalized in the General Surgery Intensive Care Unit. International Journal of Caring Sciences 2021;14(1);79-87.
12. World Health Organization. Environmental Noise. In: Compendium of WHO and Other UN Guidance on Health and Environment, 2022 Update. Geneva: World Health Organization; 2022 (WHO/HEP/ECH/EHD/22.01). License: CC BY-NC-SA 3.0 IGO.
13. Hsu SM, Ko WJ, Liao WC, Huang SJ, Chen RJ, Li CY, Hwang SL. Associations of Exposure to Noise with Physiological and Psychological Outcomes among Post-Cardiac Surgery Patients in ICUs. Clinics (Sao Paulo) 2010;65(10):985-9. doi: 10.1590/s1807-59322010001000011.
14. Seidman MD, Standring RT. Noise and Quality of Life. Int J Environ Res Public Health 2010;7(10):3730-8. doi: 10.3390/ijerph7103730.
15. Fortes J. "Assessing Nurses' Knowledge of Noise in the Intensive Care Unit: An Educational Intervention Project" (2021). Master's Theses, Dissertations, Graduate Research, and Major Papers Overview. 379. https://digitalcommons.ric.edu/etd/379.
16. World Health Organization. Environmental Noise Guidelines for

the European Region. (World Health Organization, 2018). Access Link: https://apps.who.int/iris/bitstream/handle/10665/279952/9789289053563-eng.pdf. Access Date: 12.02.2024
17. Environmental Protection Agency United States. EPA Guidance Documents. 2023; November; 28. Access Link: https://www.epa.gov/laws-regulations/epa-guidance-documents Access Date: 12.01.2024
18. Armbruster C, Walzer S, Witek S, Ziegler S, Farin-Glattacker E. Noise Exposure Among Staff in Intensive Care Units and the Effects of Unit-Based Noise Management: A Monocentric Prospective Longitudinal Study. BMC Nurs 2023;22:460. doi:10.1186/s12912-023-01611-3.
19. Ryherd E, Ackerman J, Zimring C, Okcu S, Waye, KP. Noise Pollution in Hospitals: Impacts on Staff. Journal of Clinical Outcomes Management 2012;19:1-10.
20. Joseph BE, Mehazabeen HUM. Noise Pollution in Hospitals- A Study of Public Perception. Noise Health 2020;22(104):28-33. doi: 10.4103/nah.NAH_13_20.
21. Andrade EL, Silva CE, de Lima EA, de Oliveira RA, Zannin PHT, Martins ACG. Environmental Noise in Hospitals: A Systematic Review. Environ Sci Pollut Res Int 2021;28(16):19629-19642. doi: 10.1007/s11356-021-13211-2.
22. Ng MSN, Chan DNS, So WKW. Health Inequity Associated with Financial Hardship Among Patients with Kidney Failure. PLoS ONE 2023;18(6):e0287510. doi: 10.1371/journal.pone.0287510.
23. Flythe JE. et al. Blood Pressure and Volume Management in Dialysis: Conclusions From a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int 2020;97:861–876.
24. Kim YJ, Choi WJ, Ham S, Kang SK, Lee W. Association Between Occupational or Environmental Noise Exposure and Renal Function Among Middle-Aged and Older Korean Adults: A Cross-Sectional Study. Sci Rep 2021;16:11(1):24127. doi: 10.1038/s41598-021-03647-4.
25. Vreman J, Lemson J, Lanting C, van der Hoeven J, van den Boogaard M. The Effectiveness of the Interventions to Reduce Sound Levels in the ICU: A Systematic Review. Critical Care Explorations 2023;5(4).
26. Kooshanfar Z, Ashrafi S, Paryad E, Salmanghasem Y, Mohammadi TK, Leili EK, Golhosseini SMJ. Sources of Noise and Their Effects on Nurses in Intensive Care Units: A Cross-Sectional Study. International Journal of Africa Nursing Sciences 2022;16: 100403.
27. Darbyshire JL, Müller-Trapet M, Cheer J, Fazi FM, Young JD. Mapping Sources of Noise in an Intensive Care Unit. Anaesthesia 2019;74(8):1018-1025.
28. Ronco C. Noise Pollution in Hemodialysis Centers. Nat Clin Pract Nephrol 2008;4(6):289–9
29. Abbasi H, Ziaei M, Salimi K, Zare S, Jokar M, Rashidi N. Noise Pollution, Annoyance, and Sensitivity; Its Impact on General Health and Aggression of Hospital Staffs. Current Psychology 2023;1-12.
30. Lo Castro F, Iarossi S, Brambilla G, Mariconte R, Diano M, Bruzzaniti V, Sttigari L, Raffaele G, Giliberti, C. Surveys on Noise in Some Hospital Wards and Self-Reported Reactions from Staff: A Case Study. Buildings 2022;12(12);2077.
31. Garside J, Stephenson J, Curtis H, Morrell M, Dearnley C, Astin F. Are Noise Reduction Interventions Effective in Adult Ward Settings? A Systematic Review and Meta-Analysis. Applied Nursing Research 2018;44:6-17.
32. Richardson A, Thompson A, Coghill E, Chambers I, Turnock C. Development and Implementation of a Noise Reduction İntervention Program: A Pre-And Post-Audit of Three Hospital Wards. Journal of Clinical Nursing 2009;18(23):3316-3324.
33. Milette I. Decreasing Noise Level in our NICU: The Impact of a Noise Awareness Educational Program. Advances in Neonatal Care 2010;10(6):343-351.
34. Taylor-Ford R, Catlin A, LaPlante M, Weinke C. Effect of a Noise Reduction Program on a Medical-Surgical Unit. Clinical Nursing Research 2008;17(2):74-88.
35. Johansson L, Knutsson S, Bergbom I, Lindahl B. Noise in the ICU Patient Room–Staff Knowledge and Clinical Improvements. Intensive and Critical Care Nursing 2016;35:1-9.
36. Peng L, Chen J, Jiang H. The İmpact of Operating Room Noise Levels on Stress and Work Efficiency of the Operating Room Team: A Protocol for Systematic Review and Meta-Analysis. Medicine (Baltimore) 2022; 21;101(3):e28572. doi: 10.1097/MD.0000000000028572.
37. Vitale E, Della Pietà C, Gualano A, et al. Background Sounds and Noises in Hospital: A Proactive Analysis of Psychological Repercussions on Patients in the “Santissima Annunziata” Hospital, Taranto, Italy. Nur Primary Care 2020;4(7):1-4.

ORIGINAL RESEARCH

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Compassion Fatigue in Pediatric Nurses and Affecting Factors

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Abstract

Objective
This study aimed to explore the factors influencing the development of compassion fatigue in pediatric nurses, focusing on socio-demographic factors such as age, type of clinic, duration of employment, and type of hospital.

Material and Method
A descriptive cross-sectional study was conducted with 108 pediatric nurses from state and university hospitals in Antalya and Burdur, Türkiye. Data were collected using an online survey that included questions on work schedule, job satisfaction, impact of patient condition on job, and work approach. Compassion fatigue was measured using the Professional Quality of Life scale. Statistical analysis was performed using SPSS version 23.0.

Results
Age, type of clinic, duration of employment, and type of hospital were significantly associated with varying levels of compassion fatigue among pediatric nurses. Nurses with 1-5 years of experience had significantly different compassion fatigue levels compared to those with 6-10 years of experience. However, no significant relationship was found between compassion fatigue scores and work shift or marital status. Nearly all nurses (99.3%) experienced low to moderate levels of compassion fatigue.

Conclusion
This study highlights the importance of considering socio-demographic factors in addressing compassion fatigue among pediatric nurses. By understanding these factors, healthcare institutions can develop targeted interventions to support nurses and improve patient care quality.

Keywords: Compassion fatigue, patient care, pediatric nursing, well-being

Introduction

Pediatric nursing is a profession characterized by intense emotional involvement, as nurses care for vulnerable and often critically ill children and their families. This emotional labor, while rewarding, can also exact a toll on nurses' well-being, leading to the phenomenon known as compassion fatigue (1). Compassion fatigue (CF) refers to the gradual decline in empathy and emotional strength that can happen

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when individuals are consistently exposed to suffering and trauma in their professional roles. (2).

Lately, recognition has been increasing recognition of prevalence, impact of CF among healthcare professionals, including pediatric nurses. Research suggests that pediatric nurses may be particularly susceptible to CF due to the inherently challenging nature of their work, which frequently involves witnessing the suffering of young patients and their families (3,4). Moreover, pediatric nurses often face unique stressors, such as caring for children with life-threatening illnesses, navigating complex family dynamics, and coping with ethical dilemmas surrounding end-of-life care (5,6).

Several factors have been identified as potential contributors to CF in pediatric nurses. High workload, long hours, and inadequate staffing levels have been consistently linked to increased levels of burnout and emotional exhaustion among healthcare professionals (5,7,8). Additionally, factors such as lack of organizational support, limited opportunities for professional development, and exposure to traumatic events can further exacerbate nurses' risk of developing CF (6,9).

Despite growing awareness of the importance of addressing CF in pediatric nursing, there remains a need for further research to better understand its prevalence, risk factors, and impact on nurses' well-being and patient care outcomes. By identifying the factors contributing to CF in pediatric nurses, healthcare organizations can implement targeted interventions to support nurses and mitigate the negative effects of CF on both individual nurses and the quality of patient care (3,10).

This study aims to subscribe to the existing literature on CF in pediatric nursing by exploring the factors that impact its development. By gaining a deeper understanding of CF and its affecting factors in the context of pediatric nursing, this research seeks to inform the development of evidence-based interventions to support the well-being of pediatric nurses and optimize patient care outcomes.

Material and Method

Study Design

This descriptive cross-sectional study was carried out within state and university hospitals located in the Antalya and Burdur provinces, involving pediatric nursing staff.

Participant and Sample Size

A known population sampling approach was employed, encompassing four state hospitals and one university hospital in Turkey. The study population consisted of 198 nurses employed in pediatric services across state and university hospitals located in Antalya and Burdur. The patient-to-nurse ratios were 1:10 in state hospitals and 1:7 in the university hospital. The sample size calculation was recalculated with a 95% confidence interval, resulting in a sample size of 131 (calculated using Open Epi program version 3). Out of the calculated sample size, 108 nurses voluntarily participated in the research, representing an 82.4% response rate, which can be considered sufficient to represent the population. The sample selection process was not based on convenience sampling; rather, it followed a known population sampling approach to ensure a representative sample of nurses from the target population.

Data Collection Procedures and Tools

In the wake of providing a clear explanation of the research's objectives and methodologies, data were gathered from consenting nurses via an online survey. On average, each participant spent approximately 15 minutes completing the survey.

The Participant Information Inventory

This study utilized a survey comprising 17 questions across various categories, including work schedule, job satisfaction, impact of patient condition on job, and work approach. While creating these questions, a study in the literature was taken as a basis (11).

Compassion Fatigue

The Professional Quality of Life was used to measure compassion fatigue. This scale is a 30-item self-report tool consisting of three distinct subscales. Participants rate the frequency of experiencing each item over the past 30 days on a 5-point Likert scale (ranging from 1 = never to 5 = very often). Subscale scores are obtained by summing the responses for each 10-item subscale. Stamm (12) categorized CF scores as low (22 points and below), moderate (23–41), or high (42 points and above). Scores range from 0 to 50, with higher scores indicating greater levels of Burnout and CF (12). Employees with elevated scores are advised to seek support (13). Cronbach's alphas for the subscales are declared as 0.81 for CF (12). The Turkey version also demonstrated good internal consistency ($\alpha = 0.80$). In this study, Cronbach's alpha for CF was 0.81.

Statistical Analysis

The data underwent analysis using the SPSS version 23.0 software. Descriptive statistics, encompassing

numbers and percentages, were employed to provide an overview of the data. For a more nuanced examination, non-parametric tests were utilized. Analyzed using the Mann–Whitney U-test, Kruskal–Wallis. These statistical approaches are well-suited for analyzing data that may not meet the assumptions of parametric tests. Due to the violation of the assumption of homogeneity, the Games-Howell post-hoc analysis was employed as a corrective measure, rather than using the Bonferroni correction. A significance level of $p<0.05$ was employed to ascertain statistical significance, ensuring a rigorous scrutiny of the results.

Results

The research analyzed data from 108 participants. Of the participants, 75.9% were female, 57.4% were Bachelor's degree, 36.1% were single, 55.1% of the married participants did not have children, 48.1% were employed at university hospitals, and 16.7% were employed at private hospitals. 35.2% of the participants had 1-5 years of work experience, and 13.0% consisted of those who only worked day shifts. 60.2% of the participants reported considering leaving their jobs. 42.6% of the participants worked in general pediatric clinics. The comparison of the mean scores of CF of the participating nurses with their socio-demographic characteristics is shown in Table 1.

When examining the CF levels concerning participants' socio-demographic variables, considerable differentiations emerged in CF concerning participants' age, type of clinic they worked in, duration of employment, condition, and place they worked (Table 2). Regarding nurses' levels of CF by age, advanced analysis following the categorization revealed significant differences between the three age groups ($p < 0.001$). There were statistically significant differences

Table 1 Comparison of compassion fatigue score averages according to participant variables (n = 108)

Variables	n	%	X ± SD	Median (min-max)	Test Value	p	Post hoc test*
Gender							
Male	26	24.1	22.44 ± 6.38	26(11-44)	2241.5	0.014	
Female	82	75.9	26.51 ± 8.25	28 (10-43)			
Age (years)							
20-24[a]	41	38.0	18.06 ± 7.62	21(15-38)	31.659	<0.001	c>a,b*
25-30[b]	38	35.2	23.51 ± 7.44	24(10-45)			
31 or older[c]	29	26.8	27.40 ± 6.58	27(9-41)			
Education							
High school[a]	31	28.7	25.43 ± 8.64	23(15-48)	5.165	0.032	a>b>c*
Bachelor's degree[b]	62	57.4	23.09 ± 8.14	20(10-39)			
Postgraduate degree[c]	15	13.9	20.08 ± 6.27	21(12-42)			
Marital status							
Married	69	63.9	23.66 ± 8.10	22(10-43)	6215.3	0.094	
Single	39	36.1	24.72 ± 8.21	23(14-36)			
Having children							
Yes	31	44.9	25.20 ± 6.74	28(8-45)	7258.1	<0.001	
No	38	55.1	20.14 ± 7.68	21(11-44)			
Total							
	108	100.0	26.38 ± 8.91	27(8-48)			

X= Mean, SD: Standart Deviation, * Games-Howell Post Hoc Correction

among other age groups (p < 0.05). Advanced analyses were conducted to identify the specific years that contributed to differences in the age-based assessment of nurses. The Games-Howell correction was applied due to the data's non-normal distribution. To adjust for multiple comparisons, a new significance level was computed by dividing the original level by 3, reflecting the three comparisons in the advanced analysis (0.05/3 = 0.017). The results indicated significant differences between individuals aged 20–24 years and those aged 25–30 years (p < 0.001), as well as between individuals aged 20–24 years and those aged 31 years or older (p < 0.001). No statistically significant differences were found among the aged 25–23 years and those aged 31 years or older (p > 0.024).

It was found that the duration of employment significantly influenced the level of CF among nurses. Advanced analysis was performed to find out which one this statistical difference occurred. The Games-Howell correction was applied due to the data's non-normal distribution. To adjust for multiple comparisons, a new significance level was computed by dividing the original level by 6, reflecting the three comparisons in the advanced analysis (0.05/6 = 0.008). The results showed that individuals with less than one year of working experience had statistically significantly lower compassion fatigue (p<0.001). It was determined that nurses with 6-10 years of working experience had a statistically significantly higher level of compassion fatigue than other groups (p<0.001). It was found that there was no difference in terms of compassion

Table 2 Comparison of participant variables and compassion fatigue scores

Variables	n	%	X ± SD	Median (min-max)	Test Value	p	Post hoc test*
Hospital Type							
Public Hospital[a]	38	35.2	25.41 ± 7.15	26 (11-48)	6879.2	0.001	a>b,c*
University Hospital[b]	52	48.1	21.18 ± 8.34	23 (14-40)			
Private Hospital[c]	18	16.7	19.42 ± 7.69	21 (9-45)			
Experience years							
<1 year[a]	12	11.1	18.60 ± 7.02	19 (8-38)	17.256	0.001	c>a>b,d*
1–5 years[b]	38	35.2	20.25 ± 7.38	22 (11-48)			
6–10 years[c]	41	38.0	27.34 ± 7.77	25 (17-41)			
>11 years[d]	17	15.7	21.16 ± 8.54	21 (10-39)			
Work Shift							
Day	14	13.0	24.14 ± 6.58	25 (9-34)	7168.0	0.569	
Day and night	94	87.0	25.31 ± 7.33	27 (10-33)			
Tend to quit nursing							
Yes	65	60.2	29.15 ± 7.07	27 (15-48)	5479.50	<0.001	
No	43	39.8	21.65 ± 8.18	23 (11-37)			
Type of Clinic							
General Pediatric Clinic[a]	46	42.6	27.48 ± 8.11	27 (12-40)	28.124	0.014	a>b,c,d*
Intensive Care Unite[b]	28	25.9	25.25 ± 7.68	23 (10-35)			
Pediatric Emergency Department[c]	21	19.4	21.04 ± 8.45	22 (11-38)			
Pediatric Surgery Clinic[d]	13	12.1	22.35 ± 8.64	22 (13-34)			
Total							
	108	100.0	26.38 ± 8.91	27 (8-48)			

X= Mean, SD: Standart Deviation, * Games-Howell Post Hoc Correction

Table 3 Distribution of Compassion Fatigue Levels

Compassion Fatigue Level	n	%
Low	40	37.0
Moderate	66	61.1
High	2	1.9

fatigue between nurses with 1-5 years of experience and nurses with more than 11 years of experience (p=0.028).

The mean score for compassion fatigue among pediatric nurses in this study was 23.89 ± 6.98. These results indicate that the majority of pediatric nurses in this study reported low to moderate levels of compassion fatigue. Further details on the distribution of compassion fatigue levels according to the cut-off points are provided in Table 3.

Discussion

The findings of this study shed light on the significant impact of socio-demographic factors on CF among nurses, highlighting several key points that resonate with existing literature. The results indicate that age, type of clinic, duration of employment, and type of hospital influence nurses' CF levels.

Age emerged as a crucial factor, aligning with previous studies that found a significant relationship between age and CF (14,15). Specifically, our study revealed that younger nurses, particularly those aged 20-25, experience higher levels of CF compared to their older counterparts. This finding is consistent with the notion that younger nurses may be less experienced in coping with the emotional demands of patient care, leading to increased susceptibility to CF (15).

The type of clinic and duration of employment also emerged as significant factors in CF levels. Nurses working in general pediatric clinics and those with shorter or longer durations of employment (1-5 years and 21 years and above) reported higher levels of compassion fatigue. This finding is in line with previous research suggesting that the nature of the clinical setting and the length of exposure to stressors can contribute to CF (16, 17).

The hospital category was also discovered to correlate with levels of CF. Nurses working in university hospitals reported higher levels of compassion fatigue. These findings echo previous studies that have identified workplace environments as important factors in CF (18, 19).

It is noteworthy that while several socio-demographic factors were found to be associated with compassion fatigue, no significant relationship was found between work shifts and marital status. This finding is consistent with some previous studies (20, 21) but contrasts with others (20, 22), suggesting that the relationship between these factors and CF may vary across different populations and contexts.

The mean score for compassion fatigue among pediatric nurses in this study was 23.89 ± 6.98. These results indicate that the majority of pediatric nurses in this study reported low to moderate levels of compassion fatigue. Comparing this finding with existing literature, our results are consistent with previous studies that have also reported predominantly low to moderate levels of compassion fatigue among nurses (1,11). However, it is important to note that the level of compassion fatigue can vary depending on the population studied, the measurement tools used, and the healthcare setting. It is also worth noting that while the majority of nurses in our study reported low to moderate levels of compassion fatigue, even low levels of compassion fatigue can have a significant impact on nurses' well-being and patient care outcomes. Therefore, it is crucial for healthcare organizations to implement strategies to support nurses and mitigate the negative effects of compassion fatigue.

In general, the outcomes of this research add to our comprehension of the intricate relationship between sociodemographic variables and CF among nursing professionals. By identifying specific factors that influence compassion fatigue, healthcare organizations can develop targeted interventions to support nurses in managing this challenging aspect of their work.

Despite the valuable insights gained from this study, several limitations should be considered.

The cross-sectional design of this study limits the ability to establish causal relationships between socio-demographic factors and compassion fatigue. Longitudinal studies would offer a more comprehensive understanding of how these factors evolve over time. Future research could incorporate objective measures of CF to validate the findings. Moreover, since the study sample comprised only nurses in a particular setting, the findings may not be broadly applicable to other populations or healthcare settings.

Limitations

Despite the valuable insights gained from this study, several limitations should be considered. Firstly, the cross-sectional design limits the ability to establish causal relationships between socio-demographic factors and compassion fatigue. Longitudinal studies would provide a more comprehensive understanding of how these factors evolve over time. Secondly, the study's sample was limited to pediatric nurses in specific hospitals in Antalya and Burdur, Turkey. This may limit the generalizability of the findings to other populations or healthcare settings. Future research should aim to include a more diverse sample to enhance the generalizability of the results. Additionally, the use of self-reported measures, such as the Professional Quality of Life scale, introduces the possibility of response bias. Participants may have provided socially desirable responses, leading to an overestimation or underestimation of compassion fatigue levels.

Conclusion

In conclusion, this study highlights the significant impact of socio-demographic factors on CF among nurses. Age, type of clinic, duration of employment, and type of hospital were found to be associated with varying levels of CF. These results underscore the significance of considering socio-demographic agents in the development of targeted interventions to support nurses in managing CF. Through attending to these elements, healthcare institutions can bolster the welfare of their nursing workforce and improve the caliber of patient care.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Ethical Approval

Ethical considerations were carefully addressed throughout the study process. Approval was secured from Ethics committee of Mehmet Akif Ersoy University (Date: 07.02.2024; Decision no: GO/2024-105) prior to commencing the research. Permission was obtained from the author to use the scale. Furthermore, permissions were obtained from entire participants, affirming their voluntary involvement and ensuring ethical research practice. Written permission was obtained from the institution where the data of the study was collected. The study was conducted in line with the principles of the Helsinki Declaration.

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants or legal guardians included in the study.

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Availability of Data and Materials

Data available on request from the authors.

Authors Contributions

H.İ.T: Idea/Concept; Design; Supervision/Consulting.; Analysis and/or Interpretation; Literature Search; Writing the Article; Critical Review.

References

1. Berger J, Polivka B, Smoot EA, Owens H. Compassion Fatigue in Pediatric Nurses. J Pediatr Nurs 2015;30(6):e11-e17.
2. Taşdemir Hİ, Aydın R, Dursun Ergezen F, Taşdemir D, Ergezen Y. Unveiling the Burden of Compassion Fatigue in Nurses. Nurs Ethics 2023;09697330231200571.
3. Robins P, Meltzer L, Zelikovsky N. The Experience of Secondary Traumatic Stress Upon Care Providers Working Within a Children's Hospital. J Pediatr Nurs 2009;24:270–279.
4. Sacco TL, Copel LC. Compassion Satisfaction: A Concept Analysis in Nursing. Nurs Forum 2018;53(1):76-83.
5. Sekol MA, Kim SC. Job Satisfaction, Burnout, and Stress Among Pediatric Nurses in Various Specialty Units at an Acute Care Hospital. J Nurs Educ Pract 2014;4:115–124.
6. Zander M, Hutton A, King L. Coping and Resilience Factors in Pediatric Oncology Nurses. J Pediatr Oncol Nurs 2010;27:94–108. http://dx.doi.org/10.1177/1043454209350154.
7. Sinclair S, Raffin-Bouchal S, Venturato L, Mijovic-Kondejewski J, Smith-MacDonald L. Compassion Fatigue: A Meta-Narrative Review of the Healthcare Literature. Int J Nurs Stud 2016;57:20-31.
8. Embriaco N, Papazian L, Kentish-Barnes N, Pochard F, Azoulay E. Burnout Syndrome Among Critical Care Healthcare Workers. Curr Opin Crit Care 2007;13(5):482-488.
9. Ondrejková N, Halamová J. Qualitative Analysis of Compassion Fatigue and Coping Strategies Among Nurses. Int J Nurs Sci 2022;9(4):467-480.
10. Xie W, Chen L, Feng F, Okoli CT, Tang P, Zeng L, Wang J. The Prevalence of Compassion Satisfaction and Compassion Fatigue Among Nurses: A Systematic Review and Meta-Analysis. Int J Nurs Stud 2021;120:103973.

11. Oktay D, Ozturk C. Compassion Fatigue in Nurses and Influencing Factors. Perspect Psychiatr Care 2022;58(4).
12. Stamm BH. The Concise ProQOL Manual. 2nd ed. Pocatello, ID: ProQOL.org; 2010. Available from: http://proqol.org/uploads/ProQOL_concise_2ndEd_12-2010.pdf.
13. Yesil A, Ergun U, Amasyali C, Er F, Olgun NN, Aker AT. Validity and Reliability of the Turkish Version of the Professional Quality of life Scale. Arch Neuropsychiatry 2010;47(2):111-118.
14. Alharbi J, Jackson D, Usher K. Personal Characteristics, Coping Strategies, and Resilience Impact on Compassion Fatigue in Critical Care Nurses: A Cross-Sectional Study. Nurs Health Sci 2020;22(1):20-27.
15. Cocker F, Joss N. Compassion Fatigue Among Healthcare, Emergency and Community Service Workers: A Systematic Review. Int J Environ Res Public Health 2016;13(6):618.
16. opeland D, Henry M. The Relationship Between Workplace Violence, Perceptions of Safety, and Professional Quality of Life Among Emergency Department Staff Members in a Level 1 Trauma Centre. Int Emerg Nurs 2018;39:26-32.
17. Sinclair S, Raffin-Bouchal S, Venturato L, Mijovic-Kondejewski J, Smith-MacDonald L. Compassion Fatigue: A Meta-Narrative Review of the Healthcare Literature. Int J Nurs Stud 2017;69:9-24.
18. Maillet S, Read E. Work Environment Characteristics and Emotional Intelligence as Correlates of Nurses' Compassion Satisfaction and Compassion Fatigue: A Cross-Sectional Survey Study. Nurs Rep 2021;11(4):847-858.
19. Zhan Y, Liu Y, Chen Y, Liu H, Zhang W, Yan R, Li S. The Prevalence and Influencing Factors for Compassion Fatigue Among Nurses in Fangcang Shelter Hospitals: A Cross-Sectional Study. Int J Nurs Pract 2022;28(5):e13054.
20. Cavanagh N, Cockett G, Heinrich C, Doig L, Fiest K, Guichon JR, Doig CJ. Compassion Fatigue in Healthcare Providers: A Systematic Review and Meta-Analysis. Nurs Ethics 2020;27(3):639-665.
21. Sorenson C, Bolick B, Wright K, Hamilton R. Understanding Compassion Fatigue in Healthcare Providers: A Review of Current Literature. J Nurs Scholarsh 2016;48(5):456-465.
22. Salmond E, Salmond S, Ames M, Kamienski M, Holly C. Experiences of Compassion Fatigue in Direct Care Nurses: A Qualitative Systematic Review. JBI Evidence Synthesis 2019;17(5):682-753.

CASE REPORT

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Isolated Hypoglossus Nerve Injury After Septoplasty

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Abstract

Cranial nerve injuries due to intubation are rare. While isolated nerve injuries may develop, injuries may occur in which more than one cranial nerve is affected. The most important step in these nerve injuries is to determine which cranial nerve or nerves are injured and then determine the treatment method. The current case presents isolated hypoglossal nerve injury after surgery.

Keywords: Anesthesia, cranial nerves, hypoglossal nerve, neck ınjuries

Introduction

Isolated cranial nerve injury is a very rare complication of general anesthesia. Specific hypoglossal nerve palsy affects the mobility of the tongue and basic swallowing and speech functions. Hypoglossal nerve injury is most often associated with the placement and/or positioning of the endotracheal tube (1). Early diagnosis of these complications is important due to possible differential diagnoses such as ischemic stroke, intracranial hematoma, carotid artery dissection, airway obstruction, or airway trauma.

In the present case, isolated hypoglossal nerve injury noticed in the postoperative period after septoplasty is presented in light of the current literature.

Case Report

A 28-year-old male patient applied to our clinic with a complaint of nasal congestion. Physical examination of the patient observed that the septum was deviated to the left side and both inferior turbinates were hypertrophied. Otherwise, otorhinolaryngological examination was normal. The patient was recommended surgery for septoplasty and submucosal resection of both inferior turbinates. The patient had no history other than smoking for 10 pack years, and no abnormalities were found in examinations performed for general anesthesia preparation. The patient was discharged on the 1st day. Three days after the operation, the patient who attended our clinic to remove the nasal packages was noticed to have evident speech impairment. On physical examination, there was slight atrophy in the left half of the tongue and a shift to the left when the tongue was protruding (Figure 1). The patient, whose cranial nerve examinations and cranial contrast-enhanced diffusion magnetic resonance imaging were normal, was diagnosed with isolated left hypoglossus nerve injury. The reason for this was thought to be intubation trauma or tube compression of the left

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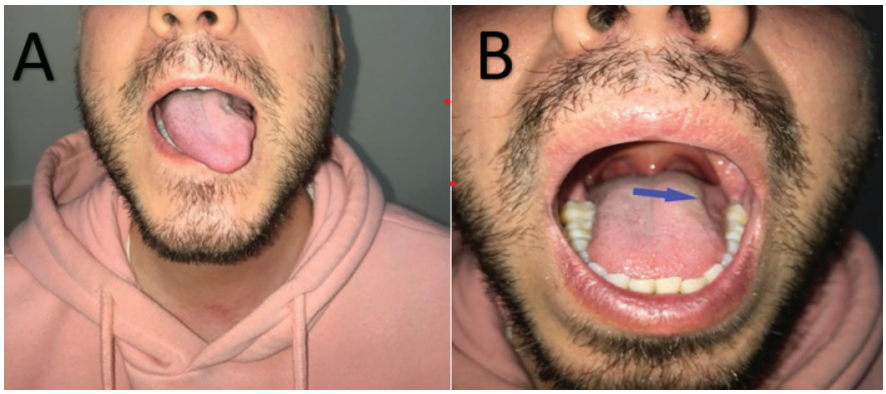

Figure 1
When the patient protrudes his tongue, the tongue deviated towards the damaged side (A) and dark-blue arrow indicates the atrophic area posterolateral to the tongue (B).

hypoglossal nerve. The patient started on 1 mg/kg methylprednisolone. The treatment was tapered and terminated within 2 weeks. The patient was called for regular weekly otorhinolaryngological examination. The patient's tongue movements started were mild at the end of the 1st month and completely returned to normal at the end of the 3rd month. Informed consent was obtained from the patient.

Discussion

The hypoglossal nerve is the 12th cranial nerve originating from the medulla oblongata in the brainstem. It innervates all external and internal muscles of the tongue, except the palatoglossus, which is innervated by the vagus nerve. It is a nerve that only has motor functions. The hypoglossal nerve descends along the internal carotid artery, passes medially over the external carotid artery (2 to 5 cm above the carotid bifurcation), and courses medial to the posterior belly of the digastric muscle to stimulate the tongue muscles (2). In hypoglossal nerve injury, one side of the tongue is usually affected, and when the patient sticks out their tongue, the tongue deviates towards the damaged side. While tumors are in first place (about half) in the etiology of unilateral hypoglossal nerve palsy, other causes include idiopathic, trauma, stroke, hysteria, surgery, multiple sclerosis, infection and Guillain-Barre neuropathy (3).

Hypoglossal nerve injury is a rare perioperative complication that has physical, social, and psychological impacts on affected patients. The hypoglossal nerve can be injured alone or together with the lingual nerve or recurrent laryngeal nerve (Tapia syndrome) (4). There was no physical examination finding of lingual or vagal nerve damage in our patient. Additionally, hypoglossal nerve damage may occur as a symptom of another intracranial event. Therefore, early recognition is important. Our case was evaluated with contrast-enhanced cranial magnetic resonance imaging in the postoperative period to exclude intracranial events included in the differential diagnosis. Since no abnormality was observed in the magnetic resonance images and no additional pathology was observed in the physical examination of the patient, this situation was evaluated as nerve neuropraxia due to nerve compression during intubation or intubation trauma caused by the laryngoscope. In order to prevent possible psychological effects on the patient, he was given information about similar patients in the medical literature.

Isolated hypoglossal nerve injury associated with tracheal intubation via laryngoscopy is usually unilateral and is considered a rare postoperative complication with multifactorial causes. Other causes include use of LMA, cricoid pressure, and direct compression of the hypoglossal nerve below the angle of the mandible during mask ventilation (5). It is noteworthy that in most case reports, unilateral hypoglossal nerve injury develops on the left side (5). In our case, there was left-sided paralysis. In otorhinolaryngological operating rooms, in most cases the mechanical ventilator is located on the patient's left side because the otolaryngologist is located on the right side of the patient. The fact that the anesthesiologist keeps the intubation tube safe by positioning it on the opposite side to the surgeon (left side) may explain the frequency of left-sided hypoglossal paralysis in otorhinolaryngology cases. Another reason associated with mechanic ventilator may be that since the mechanical ventilator is on the left, unwanted manipulations made by the surgeon in the head and neck region during the intraoperative

period may cause the intubation tube to be displaced to the left.As a result, manipulations by the anesthetist and/or surgeon may cause the tube to be placed on the left side.

Complete recovery of hypoglossal nerve function is usually achieved within the first six months. This progressive recovery of function is suggestive of neuropraxic nerve damage, which is typical of compression injury (6). Although there are no clearly proven treatment options for nerve injuries in these cases in the literature, the combination of steroids and vitamin complexes generally accelerates nerve healing(4). Due to the side effects of steroids, only vitamin B complex use is mentioned in the literature (7). We treated our case with steroid therapy in accordance with the literatüre (4,8).

Conclusion

Unilateral hypoglossal nerve palsy is a rare complication after surgery. As in the case we presented, paralysis is reversible since it is mostly at the level of neuropraxia. Steroid treatment is thought to be effective in restoring nerve function.

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

Ethical Approval
Since the current article is a case-report ethics committee approval was not received. However informed consent form was obtained from the patient.

Consent to Participate and Publish
There are no human subjects in our study.

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Authors Contributions
FY: Conceptualization; Data curation; Formal analysis; Methodology; Validation; Visualization; Writing-original draft

EO: Conceptualization; Formal analysis; Methodology; Validation; Resources; Supervision; Writing-review & editing.

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References

1. Leuzinger K, Misra L. Unilateral Hypoglossal Nerve Palsy in a Patient with a Difficult Airway Requiring Prolonged Intubation. Case Rep Anesthesiol 2021;2021:8842503. Published 2021 Feb 18. doi:10.1155/2021/8842503
2. Kojima A, Saga I, Ishikawa M. Intraoperative Hypoglossal Nerve Mapping During Carotid Endarterectomy: Technical Note. World Neurosurg 2018;113:249-253. doi: 10.1016/j.wneu.2018.02.123. Epub 2018 Mar 1. PMID: 29501517
3. Keane JR. Twelfth-nerve palsy. Analysis of 100 Cases. Arch Neurol 1996;53(6):561-6. doi: 10.1001/archneur.1996.00550060105023. PMID: 8660159.
4. Yavuzer, R., Başterzi, Y., Æzköse, Z. et al. Tapia's Syndrome Following Septorhinoplasty. Aesth. Plast. Surg 2004;)(28):208–211 https://doi.org/10.1007/s00266-003-3037-7
5. Venkatesh B, Walker D. Hypoglossal Neuropraxia Following Endotracheal Intubation. Anaesth Intensive Care 1997 Dec;25(6):699-700. doi: 10.1177/0310057X9702500635. PMID: 9452858.
6. Tesei F, Poveda LM, Strali W, Tosi L, Magnani G, Farneti G. Unilateral Laryngeal and Hypoglossal Paralysis (Tapia's Syndrome) Following Rhinoplasty in General Anaesthesia: Case Report and Review of the Literature. Acta Otorhinolaryngol Ital 2006;26(4):219-21. PMID: 18236639; PMCID: PMC2640002.
7. Alpay HC, Kaygusuz İ, Karlidağ T, Kaplama ME. Septorinoplasti Sonrası Tek Taraflı İzole Hipoglossal Sinir Paralizisi: İki Olgu Sunumu. KBB-Forum 2009;8(4).
8. Lagalla G, Logullo F, Di Bella P, Provinciali L, Ceravolo MG. Influence of Early High-Dose Steroid Treatment on Bell's Palsy Evolution. Neurol Sci 2002;23(3):107-12. doi: 10.1007/s100720200035. PMID: 12391494.

CASE REPORT OLGU SUNUMU

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Valproik Asit Kullanımında Nadir Bir Yan Etki: Ödem

A Rare Side Effect of Valproic Acid Use: Edema

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Öz

Valproik asit; epilepsi, bipolar bozukluk ve migren gibi hastalıkların tedavisinde kullanılır. Valproik asitin en sık görülen yan etkileri saç dökülmesi, gastrointestinal sistem ilişkili semptomlar ve kilo alımıdır. Akut karaciğer yetmezliği, pankreatit, kanama diyatezi, ensefalopati ciddi yan etkileridir. Valproat kullanan hastalarda periferik ödem gelişmesi nadir görülen bir yan etkidir. Çoğunlukla uzun dönem yan etkisi olarak görülmekle beraber, kısa dönem kullanımlarda da bildirilen vakalar mevcuttur. Akut başlayan periferik ödem, geniş ayrıcı tanı spektrumuyla kapsamlı araştırmayı gerektirir. Olgumuzda 16 yaşındaki erkek hastada akut alt ekstremite ödemi, valproik asit kullanımına bağlı nadir görülen ilaç yan etkisi olarak karşımıza çıkmıştır.

Anahtar Kelimeler: Valproik asit, Ödem.

Abstract

Valproic acid is used in the treatment of diseases such as epilepsy, bipolar disorder and migraine. The most common side effects of valproic acid are hair loss, gastrointestinal system-related symptoms and weight gain. Acute liver failure, pancreatitis, bleeding diathesis, and encephalopathy are serious side effects. Peripheral edema is a rare side effect in patients using valproate. Although it is mostly seen as a long-term side effect, there are also cases reported in short-term use. Acute onset peripheral edema requires comprehensive investigation with a wide spectrum of differential diagnosis. In our case, acute lower extremity edema in a 16-year-old male patient occurred as a rare drug side effect due to valproic acid use.

Keywords: Valproic acid, Edema.

Giriş

Bilateral alt ekstremitelerde ve genital bölgede akut gelişen ödem varlığında birçok farklı sistemin patolojilerini düşünüp, ayrıcı tanı yapmak gerekir. Bu tabloya sahip hastalar, nefrotik sendrom, hipoalbuminemi, nefritler, konjestif kalp yetmezliği, karaciğer patolojileri, lenfatik veya venöz obstrüksiyon gibi altta yatan durumlar açısından değerlendirilmelidir. Biz bu vaka sunumunda uzun dönem valproik asit kullanımında nadir görülen bir ilaç yan etkisi olan, ani başlayan bilateral alt ekstremite ödemi ile karşımıza çıkan bir hastayı sunmak istedik.

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Olgu Sunumu

16 yaşında erkek hastaya, iki yaşındayken epilepsi tanısıyla çocuk nöroloji uzmanı tarafından valproik asit tedavisi başlanmış. İzlemde nöbet tekrarı olmamasına rağmen yapılan elektroensefalografik değerlendirmede epileptik deşarjların devam etmesi nedeniyle valproik asit tedavisine devam edilmiştir. Son 5 yıldır 10 mg/kg/gün dozunda olacak şekilde, son iki yıldır 500 mg/ gün sodyum valproat kullanan hasta tarafımıza bir hafta önce testislerde aniden başlayan ve sonrasında günler içerisinde her iki bacağa yayılan şişlik ile başvurdu. Şişliğe eşlik eden ağrı, kızarıklık, ısı artışı yoktu. Ateş ve üriner sistem yakınmaları bulunmuyordu. Geçirilmiş enfeksiyon öyküsü yoktu. Yürüme zorluğu, çabuk yorulma, bacaklarda soğukluk, solukluk tariflemiyordu. Daha önce benzer şikâyeti hiç olmamıştı. Ailede bilinen böbrek veya karaciğer hastalığı yoktu.

Fizik muayenesinde genel durumu iyi, bilinç açık, oryante, koopere idi. Kalp tepe atımı 80 /dk; tansiyon arteriyel sağ kolda 110/72 mmHg, sol kolda 115/76 mmHg idi. Testisler bilateral ödemli idi. Hassasiyet, kızarıklık, ısı artışı yoktu. Bacaklarda pretibial bölgede +2 derecede gode bırakan ödem mevcuttu. Homans bulgusu negatifti. Posterior tibial ve dorsalis pedis başta olmak üzere tüm bilateral alt ekstremite nabızları eşit ve +2 grade palpabl idi. Göz çevresinde ödemi yoktu. Solunum sesleri eşit ve doğaldı. Kardiyovasküler sistem muayenesinde patoloji yoktu. Nörolojik muayenede özellik yoktu.

Nefrotik ve nefritik sendromları ayırt etmek için yapılan idrar tetkiklerinde dansite 1015, spot idrar protein negatif, spot idrarda protein/kreatinin oranı: 0,05; mikroskobik bakıda özellik yoktu. Serum kreatitin: 0,5 mg/dl; albümin: 4,2 g/dl; AST: 20 U/L; ALT: 18 U/L; trombosit sayısı: 171.000 /µl; TSH:0,9 µIU/ml; sT4:0,8 ng/dl; total kolesterol: 120 mg/dl; trigliserit: 140 mg/dl; HDL: 48 mg/dl; LDL: 90 mg/dl; serum valproik asit düzeyi: 35 µg/ml idi. Elektrokardiyografide normal sinüs ritmi izlendi. Kalp yetmezliği, efüzyon taraması açısından çocuk kardiyoloji tarafından yapılan ekokardiyografik değerlendirmede patoloji saptanmadı. Çekilen akciğer grafisinde özellik yoktu. Vasküler obstrüksiyon veya yetmezlik açısından yapılan alt ekstremite venöz ve arteriyel Doppler ultrasonografide derin ven trombozu veya venöz yetmezliğe rastlanmadı. Lenfatik drenajda patoloji saptanmadı. Takibinde çocuk nöroloji tarafından valproat kesildi. Ödemler 1 ay içinde belirgin şekilde geriledi, normale döndü.

Tartışma ve Sonuç

Valproat ve onun valproik asit, sodyum valproat ve valproat semisodyum formları, öncelikle epilepsi ve bipolar bozukluğu tedavi etmek ve migren tipi baş ağrılarını önlemek için kullanılan ilaçlardır. Valproik asitin en sık görülen yan etkileri saç dökülmesi, gastrointestinal sistem ilişkili semptomlar ve kilo alımıdır. Akut karaciğer yetmezliği, pankreatit, kanama diyatezi, ensefalopati ciddi yan etkileridir (1,2). Nadir görülen yan etkiler arasında periferik ödem de yer almaktadır (2-6). Akut başlayan periferik ödem, geniş ayrıcı tanı spektrumuyla kapsamlı araştırmayı gerektirir. Olgumuz, periferik ödemin valproatın nadir fakat klinik olarak önemli bir advers ilaç reaksiyonu olduğunu ileri süren, kısıtlı sayıda literatüre katkıda bulunmaktadır. İlk çalışmalar, valproatın neden olduğu karaciğer hasarının sonucu olarak valproata bağlı ödemi bildirmiştir (7). Ancak daha yeni vaka sunumlarında hepatotoksisite olmayan veya kan ilaç düzeyi terapotik dozun altındaki hastalarda valproata bağlı ödem saptanmıştır (1,2). Valproatın periferik ödem yapma mekanizması tam olarak bilinmemektedir. Benzodiazepin grubu gibi γ-aminobütirik asit (GABA) sistemini etkileyen ilaçların bu nadir yan etkiye neden olabileceği bildirilmiştir (8). Benzodiazepinlerden farklı olarak valproat, GABA'nın kullanılabilirliğini artırır (6,9). GABA reseptörlerinin periferik dokularda bulunması, GABAerjik ilaçların bölgesel vasküler direnç üzerinde de etkili olabileceğini düşündürmektedir(10). Bu mekanizma daha önceki vaka raporlarında öne sürülmüştür ancak henüz çalışmalarla kanıtlanmamıştır (2).

Valproat kullanan hastalarda periferik ödem gelişmesi durumunda klinisyenlerin bu nadir ilaç yan etkisinden haberdar olması gerektiği düşüncesindeyiz. Mekanizması bilinmemekle birlikte literatürde çoğunlukla uzun süreli valproik asit kullanımında alt ekstremitede ve testislerde ödem gelişen vakalar bildirilmekle beraber (1,3,4,6); kısa süreli kullanımda da gelişen az sayıda vaka mevcuttur (2,6). İlaç kesilince ödemlerin geriledi̇ği gözlenmiştir (1,2,4). Bizim vakamızda da ilaç kesiminden sonra 1 ay içinde bacaklarda ve skrotumdaki ödem tamamen geriledi̇ği gözlemlenmiştir.

Sonuç olarak bu yan etkinin saptandığı hastalarda ilacın değiştirilmesi veya kesilmesi düşünülmelidir. Tedavi alan hastalar ödem konusunda bilgilendirilmeli ve takiplerinde ödem açısından fizik muayene yapılmalıdır.

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Veriler, gizlilik veya diğer kısıtlamalar nedeniyle yalnızca yazarlardan talep edilebilir.

Yazar Katkıları
MB: Çalışmanın planlanması; Araştırma; Görselleştirme; Makalenin Yazımı.

İCM: Görselleştirme; Makalenin düzenlenmesi.

Kaynaklar

1. Prajapati H, Kansal D, Negi R. Magnesium Valproate-Induced Pedal Edema on Chronic Therapy: A rare Adverse Drug Reaction. Indian Journal of Pharmacology 2017;49(5):399.
2. Lin S-T, Chen C-S, Yen C-F, Tsei J-H, Wang S-Y. Valproate-related peripheral oedema: A Manageable But Probably Neglected Condition. International Journal of Neuropsychopharmacology 2009;12(7):991-3.
3. Chuang K, Patel S. Valproate-Induced Lower Extremity Swelling. Federal Practitioner 2020;37(8):388.
4. Maneerot T, Jenraumjit R, Kijsawatpaiboon S, Reungyos J. Low-Dose Valproate-Related Peripheral Edema in Patients With Bipolar Disorder: Two Case Reports. Journal of Clinical Psychopharmacology 2019;39(4):412-3.
5. Ettinger A, Moshe S, Shinnar S. Edema Associated with long-Term Valproate Therapy. Epilepsia 1990;31(2):211-3.
6. Haviv Y, Kuper A. Severe Peripheral Oedema Associated with Valproic Acid. Clinical Drug Investigation 2000;19:385-7.
7. Zimmerman HJ, Ishak KG. Valproate-Induced Hepatic Injury: Analyses of 23 Fatal Cases. Hepatology 1982;2(5):591S-7S.
8. Mathew T, D'Souza D, Nadimpally US, Nadig R. Clobazam-Induced Pedal Edema:"An Unrecognized Side Effect of a Common Antiepileptic Drug". Epilepsia 2016;57(3):524-5.
9. Bourin M, Chenu F, Hascoet M. The Role of Sodium Channels in the Mechanism of Action of Antidepressants and Mood Stabilizers. Current Drug Targets 2009;10(11):1052-60.
10. Takemoto Y. Effects of Gamma-Aminobutyric Acid on Regional Vascular Resistances of Conscious Spontaneously Hypertensive Rats. Clinical and Experimental Pharmacology and Physiology 1995;22:S102-Sl04.

REVIEW

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The Efficacy of Some Herbal Therapies Preferred by Turkish MS Patients

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Abstract

Multiple sclerosis (MS), that is the most common non-traumatic cause of disability among young adults, is a chronic, progressive, inflammatory and neurodegenerative disease of the central nervous system. Despite recent remarkable advances in treatment, there is no cure for MS. Many unmet needs of persons with MS encourages them to use complementary and alternative medicines, especially herbal medicines, as a promising therapeutic option. Here, potential benefits and mechanisms of action of some herbal medicines preferred by Turkish MS patients are reviewed.

Keywords: Herbal therapy, medicinal plants, multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic, progressive, inflammatory and neurodegenerative disease of the brain and spinal cord characterized by myelin destruction and axonal loss. MS has a complex immunopathogenesis. It is presumed that, in MS, breakdown of peripheral tolerance mechanisms - through the defective/decreased T-regulatory (Treg) and B-regulatory lymphocytes, and less anti-inflammatory myeloid cells - changes the immune milieu towards a pro-inflammatory one which causes activation of previously dormant naive T cells, myeloid cells and B-cells via molecular mimicry, novel autoantigen presentation, CNS-sequestered antigens that leak to deep cervical lymph nodes or bystander activation (1,2). Naive T-cells differentiate into either pro-inflammatory effector T cell subsets including T helper (Th) 1 and Th 17 or anti-inflammatory Th 2 phenotype; depending on the cytokine environment which is modified by local antigen presenting cells and other immune cells.3,4 Th1 and Th17 are key players in MS immunopathogenesis. Th1 cells (driven by interferon-γ (IFNγ), interleukin (IL) 12, IL18) secretes IL2, tumor necrosis factor- α (TNFα) and IFNγ, and activates macrophages, natural killer cells, B cells and cytotoxic CD8+ Tcells. Th17 cells, differentiation induced by tissue growth factor-β (TGFβ), IL1, and IL6; secretes IL17 A-F, IL21, and IL22 and promotes the recruitment of other immune cells in inflammatory region (3,4). Activated T cells and inflammatory myeloid cells pass the blood-brain barrier (BBB), and infiltrates brain and spinal cord. Once in central nervous system, infiltrating myeloid cells release various pro-inflammatory cytokines, TNFα, IL1, IL6, and IL8, macrophage inflammatory protein 2, CXC-chemokines, which amplifies Th17 responses cause activation of resident microglia, effector T cell subsets' reactivation

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by myelin antigen, clonal expansion of T-effector cells and release of more inflammatory mediators which ultimately initiates an inflammatory cascade ending up with myelin, axon and oligodendrocyte destruction (1-4). The underlying chronic inflammation affects neurons and axons through a series of events such as oxidative stress, mitochondrial energy failure, ionic dyshomeostasis, and compromised protective and regenerative mechanisms eventually leading to neurodegeneration (1,2). This ongoing chronic inflammation and neurodegeneration increases the burden of disease over time and expose patients to a variety of neurological symptoms that are reflected in many aspects of their lives as increased disability. Relentless progressive course of the disease urge sufferers to use many complementary and alternative methods to relieve or control the symptoms due to MS.

Complementary and alternative medicine (CAM) use has gaining an increasing interest all over the world with a prevalence ranging from 9.8% to 76% (5). Studies have shown that individuals' preference for using CAM is affected by different factors. The most common causes are benefit expectation, dissatisfaction with conventional therapies, safety of CAM, easy access, affordability, internal health locus of control, and tradition (5). The methods and approaches the CAM umbrella covers are variable, including herbal therapy among many. Herbal therapy includes a range of pharmacologically active compounds and is employed for the treatment of a various diseases including MS. Research shows that more than one third of MS patients use at least one CAM at least once to improve the health and manage symptoms of MS; being more prevalent among women, those who have higher education and report worse health status (6,7). Homeopathy and diets are reported as the most common, and a perceived benefit reported by more than half (7). Though herbal therapy is practiced traditionally in Türkiye, its specific use by MS patients are reported in a few studies. In those, the ratio of herbal medicine use was between 10-49 %, and patients' knowledge about CAM was scarce in some (8,13). No relation has been reported between CAM use and socio-demographic features such as age, gender, education level, and disease related features such as disease duration, disability level and quality of life measures (8-10). Most commonly reported agents were capers, ginger, garlic, cumin seeds, primrose, flaxseed, St. John's wort, ginseng and turmeric (10-13).

The mechanisms of action of herbal therapy mainly depends on the bioactivities of the secondary metabolites. These often interact with major targets in cells, including enzymes, receptors, ion channels and pumps, or cytoskeletal elements (mostly tubulin or microtubules) and modulate these targets via alkaloids, phenolic compounds (e.g. flavonoids), terpenoids (e.g. saponins), polysaccharides or other secondary metabolites (14). Quite a lot of these plant-derived metabolites, either in original forms or synthetic derivatives, are used in medicine with established practices (14). Regarding MS, clinical and experimental studies have documented the beneficial effects of some herbal regimens on BBB permeability, inflammatory cell infiltration of the CNS, and T-cell polarization involved in MS pathogenesis; and some have been shown to have antiinflammatory and neuroprotective effects. Besides, many is used to relieve MS related symptoms such as fatigue, depression, cognitive impairment, spasticity, neuropathic pain, and urinary tract complications. In this review, different herbal medicines preferred by Turkish MS patients are reviewed in terms of their possible positive pharmacological effects.

Capers: Caper, a perennial shrub from the family of Capparaceae L, is typical of the Mediterranean flora known for its edible, brine flower buds and fruits and also for its therapeutic properties in various human ailments. The preferences for use in folk medicine are listed as antibacterial, antifungal, hepatoprotective, anthelmintic, antidiabetic, anti-inflammatory, anti-oxidant, anti-cancer, and antihyperlipidemic effects which have been ascribed to the phenolic acids, phytosterols, flavonoids, alkaloids, natural sugars, vitamins, and organic acids in content (15,16). The most widely studied *Capparis* species for their therapeutic and nutritional properties are Capparis ovata, *Capparis spinosa*, and *Capparis decidua*. Among these, *Capparis ovata* is the one whose impact was specifically evaluated in the experimental allergic encephalomyelitis models of multiple sclerosis. Caper is one of the most commonly used herb among MS patients. Though there is no human trial evaluating its efficacy on MS disease progress, in several experimental studies *Capparis ovata* has been found to inhibit and/or ameliorate experimental allergic encephalomyelitis (EAE) effectively in murine MS model and has been suggested as a new immune-regulatory and anti-inflammatory agent (17,18). Different studies report the effectiveness of Capparis ovata to inhibit the expression of pro-inflammatory and inflammatory genes, which are essential key players in MS pathophysiology, at a significant level including TNFα, IL6, IL17, glial fibrillary acidic protein, NFκβ (nuclear factor kappa-light-chain-enhancer of activated B cells), CC-chemokine ligand 5, CXC-chemokine 9,10, and tyrosine-protein phosphatase

nonreceptor type 11 and hypoxia-inducible factor-1 and also to enhance the up-regulation of the myelin basic protein and myelin-associated glycoprotein expression (16-19).

Ginger: Ginger (*Zingiber officinale* Roscoe) is a perennial herbaceous plant from the Zingiberaceae family, cultivated in sub-tropical regions and often ingested as a spice or dietary supplement and in folk medicine (20-22). It contains more than 400 different compounds and rich from terpenes, phenolic compounds, polysaccharides, lipids, organic acids, and raw fibers (20-22). Ginger is often considered a panacea due to its numerous effects including anti-pyretic, anti-inflammatory, antihyperglycaemic, antiapoptotic, anti-tumour, antioxidant, anti-diabetic, anti-clotting and analgesic properties. Health benefits are ascribed to its active phytoconstituents such as gingerols, shogaols, zingerone, paradols beside other phenolic compounds and flavonoids. In particular, gingerol has been reported to have anti-oxidative, anti-inflammatory, immune-modulatory and neuroprotective effects (20-22). The anti-inflammatory effect of ginger, either in the form of pure active ingredient or as an extract, has been studied in experimental MS models and have been found to effectively ameliorate the clinical and pathological disease severity (23,24). It has been shown to exhibit its effects chiefly via Th1, Th17, and B cell response down-regulation, chemokines and chemokine receptors expression down-regulation, and Treg response up-regulation (22-24). Besides, it modulates Th2-, Th9-, Th22 cell-related responses along with modulation of the pro-inflammatory and anti-inflammatory cytokines production, toll like receptor-related signaling and adhesion molecules expression (22,25). The immunomodulatory properties along with anti-oxidative features poses the ginger to be considered to have a therapeutic potential for the management of MS.

Garlic: Garlic (*Allium sativum L*) belongs to the lily family, and is recognized for anti-microbial, anti-neoplastic, anti-diabetes, anti-atherosclerosis, hepatoprotective and anti-inflammatory effects which are attributed to its organosulfur compounds. Among these are 'allicin, alliin, diallyl sulfide, diallyl di- and trisulfide, E/Z-ajoene, and S-allyl-cysteine (SAC)' (26). SAC has been shown to ameliorate clinical signs and severity of the EAE along with attenuation of inflammatory cell infiltration, axonal demyelination, and axonal loss in lumbar spinal cord (27). The anti-inflammatory effects of garlic is suggested to occur by reduced activation of microglia and astrocytes, decreased expression of IL1β, NFκB, Toll-like receptor-4, nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 in microglial cells and increased number of Treg cells (28,29). In addition, it has also been found to be a potent reactive-oxygen scavenger with its enhancing and regulatory effects on the antioxidant enzymes (28).

Black Cumin: *Nigella sativa* Linn is an annual indigenous herbaceous plant from the Ranunculaceae family and known as black seed or black cumin (30). Seed and oil of N. sativa have a long history of folkloric usage and regarded as a valuable traditional remedy to treat all ailments except to prevent from death (31). Black cumin seeds are rich from various chemical components including fixed and essential oil, alkaloids, carvacrol, proteins, saponin, terpenoids, quinones (such as thymoquinone, nigellone, and thymohydroquinone), minerals and vitamins (30). Beneficial effects attributed to N. sativa are mainly due to quinone ingredients of which thymoquinone is the major and most abundant bioactive component (31,32). Among many, some of the reported biological properties of N. sativa are antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, cardio-protective and neuroprotective properties (31,32). In experimental studies N. sativa oil and extracts has been postulated to modulate cellular and humoral immune responses (32). The mechanisms underlying the immunomodulatory effects of thymoquinone results from its inhibitory effect on NFκβ, mitogen-activated protein kinase (MAPK) and janus kinase (JAK)/signal transduction and activator of transcription (STAT) signaling pathways (33). By this way, thymoquinone inhibits NFκβ-mediated neuroinflammation and production of inflammatory mediators (NO, PGE2, TNF-a, and IL1b) (33-35). It also attenuates neuroinflammation by decreasing a set of cytokines including IL1, IL6, IL12, monocyte chemoattractant protein 1 and 5, granulocyte colony-stimulating factor, and CXCL10/IFN-γ' induced protein 10 in microglia cells in rats (36). In EAE, protective treatment with N. sativa has been shown to reduce days of relapses significantly, ameliorate the clinical manifestations and decrease the severity of the disease (37-39).

Evening primrose: Evening primrose (*Oenothera biennis L*) is a wild medicinal plant from the Onagraceae family. *Oenothera biennis L* is the most commonly and best-studied species in the Oenothera L family which originated from Central America and now naturalized worldwide (40-41). Evening primrose have been preferred for treating a wide variety of ailments including premenstrual syndrome, asthma, eczema, inflammation, arthritis, metabolic disorders, headaches and eruptions of the skin (42). Evening primrose oil, extracted from its seeds, is rich from

omega-6 essential fatty acids (FA); 70-74% of its components are linoleic acid and about 8-10% γ-linolenic acid along with other fatty acids (palmitic, oleic, & stearic acids) and campesterol and β-sitosterol steroids (41). Linoleic acid and γ-linolenic acid are precursors of anti-inflammatory eicosanoids, major components of myelin and the neuronal cell membrane and contributes to the fluidity and flexibility of cell membranes. Evening primrose oil supplementation increases the plasma levels of γ-linolenic acid and its metabolite dihomo-γ-linolenic acid which lead to production of anti-inflammatory eicosanoids (41,43). Besides, 15-hydroxyeicosatrienoic acid, metabolized from dihomo-γ-linolenic acid inhibits the conversion of arachidonic acid to leukotriene A4, hence prevents the pro-inflammatory action of leukotrienes (41,43). Moreover, γ-linolenic acid has been reported to suppress inflammation mediators such as IL1, IL6, TNFα by inhibiting NFκβ activation (44).

In hemp seed oil/evening primrose oil treated C57BL/6 mice with EAE, Rezapour-Firouzi et al have shown a significant increase in the expression of IL10 and mTOR complex 2 (RICTOR) genes along with a remarkably reduced cell infiltration and promoted remyelination (45). Moreover, evening primrose/hemp seed oil administration prevented the development of EAE and the attenuated the severity of the disease in EAE induced models (45). Evening primrose/hemp seed oil treatment significantly inhibited the expression of the regulatory-associated protein of mammalian target of rapamycin (RAPTOR), interferon-gamma, IL17, signal transducer and activator of transcription factors (STAT3) genes and promoted the expression of regulatory-associated companion of mammalian target of rapamycin (RICTOR), forkhead box P3 (FoxP3) and IL10 genes (46).

In a clinical study on 100 relapsing-remitting MS patients, the effects of evening primrose/hemp seed oil and olive oil were evaluated. At the end of the study, patients on the evening primrose/hemp seed oil group has been reported to show significantly better disability scores and reduced relapse rate compared to olive oil group (47). In another study on 52 MS patients, evening primrose oil consumption for 3 months along with their immunomodulator drugs for their disease provided better outcomes involving cognitive function, pain, fatigue, and overall life satisfaction compared to placebo (48).

Flaxseed: Flaxseed, *Linum usitatissimum*, whose Latin name means very useful, is one of the oldest crops grown widely all over the world (49). It has been reported to have potential health imparting benefits at reducing the risk of some disease including cardiovascular diseases, cancer, arthritis, menopausal symptoms and osteoporosis with its antitumoral, antioxidant, and anti-inflammatory effects (49-51). Flaxseed is the richest plant source of polyunsaturated fatty acids (PUFA); linoleic acid (omega-6) and α-linolenic acid (omega-3,) with a ratio of approximately 0.3:1, which are dietary essential fatty acids that cannot be synthesized by humans (49,52). It also contains monounsaturated FAs and at a much lesser amount saturated FAs. Omega-3 PUFAs (docosahexaenoic acid, eicosapentaenoic acid and alpha-linolenic acid) exhibits anti-inflammatory properties. Besides, it is also a good reservoir of lignans, phytoestrogens, phenolic compounds, dietary and protein fiber, minerals, and vitamins (A, C, and E) (49). Flaxseed shows direct antiatherogenic, anti-inflammatory and immunomodulatory effects through leukocyte adhesion inhibition, the proinflammatory eicosanoids production decrease, and cellular migration and proliferation inhibition with abundant omega-3 it contains (53). Although results of two recent meta-analysis are controversial, flaxseed supplementation has been reported to modulate the levels of some circulating inflammatory biomarkers including C-reactive protein, IL6, TNFα, NFκβ, macrophage marker mac-3, vascular cell adhesion protein 1, E-selectin, and intercellular adhesion molecule 1(53-55).

Data from a large international MS cohort suggest a significant association for omega 3 supplementation with flaxseed, showing reduced relapse rate and reduced likelihood of worsening relapse rate (56). However, a recent Cochrane analysis, evaluating the efficacy of PUFAs and monounsaturated fatty acids, states the uncertainty of evidence in terms of disability worsening, relapse rate, or overall health status in persons with MS (57).

St. John's wort: St. John's wort (*Hypericum perforatum L.*) is a perennial herb from the family Hypericaceae (58). Traditionally, it has been used orally and topically for the treatment of several human ailments, including burns, wounds, hemorroids, diarrhea, and ulcers and depression (58). St. John's wort contains naphthodianthrones (hypericin, pseudohypericin, protohypericin, protopseudohypericin, and cyclopseudohypericin), flavonoids (rutin, hyperoside, quercetin, quercitrin and luteolin), acylphloroglucinols (hyperforin and adhyperforin), proanthocyanidins, procyanidines, several amino acids, and tannins (59). St. John's wort preparations are widely investigated for their antidepressant activity but also for their antioxidant, anxiolytic, anticancer, and anti-inflammatory activities (58,59). Hyperforin

Table 1 Summary of he effects of herbal medicines used in multiple sclerosis and EAE models

Plant		Compound	Study group	Effect	Reference
Caper	*Capparis ovata* *Capparis spinosa*	β-sitosterol	EAE Human PBMC	↓ TNFα, IL-6, NF-κB, CCL5, CXCL9, CXCL10, HIF1A gene expression ↓ IL-17 & ↑ IL-4 gene expression	17-19 16
Ginger	*Zingiber officinale*	Gingerol	EAE	↓ Clinical disease score, ↓ inflammatory cell infiltration in CNS ↓ Th1, Th17 & B cell-related response ↑Treg cell response, TGF-β expression ↓ IL-17, IL-27, IL-33 TNF-α, CCL20, CCL22, CCR6, CCR4 expression	20-25
Garlic	*Allium sativum L*	S-allyl-cysteine	EAE	↓ Clinical disease score, ↓ inflammatory cell infiltration in CNS ↓ IL1β, NFκB, TLR-4, Nrf2 & heme oxygenase 1 in microglial cells ↑ Treg cell frequency	27 -29
Black Cumin	*Nigella sativa*	Thymoquinone	EAE	↓ Clinical disease score, ↓ inflammatory cell infiltration in CNS ↓ Reactive astrocytes number, ↑ remyelination ↓ NF-κB, MAPK, JAK/STAT signaling pathway ↓NO, PGE2, TNFα, IL1, IL6, IL12	32-39
Evening primrose	*Oenothera biennis L*	Linoleic acid, γ-linolenic acid	EAE Human	↑ IL10, RICTOR, FoxP3 gene expression ↓ IL1, IL6, IL17,TNFα IF-γ, NFκβ, RAPTOR, STAT3 expression ↓ Cell infiltration, ↑ remyelination ↓ Relapse rate, disability Better quality of life	44-46 47, 48
Flaxseed	*Linum usitatissimum*	Linoleic acid, α-linolenic acid	Acute inflammation Human	↓ NFκβ, reactive oxygen species ↓ cRP, IL6, VCAM-1, no effect on TNFα, E-selectin, ICAM-1 ↓ hs-CRP & TNFα; no effect on IL6 & cRP ↓ Relapse rate & disability No evidence of certain effects on disability & relapse rate	51 54 55 56 57
St. John's wort	*Hypericum perforatum L.*	Hyperforin	EAE	↓ Clinical disease score, ↓ inflammatory cell infitration in CNS ↓ Th1 & Th17 cells differentiation ↑ Th2 and Treg cell differentiation	60,61
Ginseng	*Panax ginseng C.A. Meyer*	Ginsenosides	EAE Human	↓ BBB permeability ↓ IL1, IL6, NFκβ; ↑ IGF-1, TGFβ, and VEGF-1 ↓ Th1 & Th17 cells; ↑ Treg cells Effect on fatigue: controversial results	64-67 68,69
Turmeric	*Curcuma longa*	Curcumin	EAE Human	↓ Clinical severity of EAE ↓ Astrocytes proliferation, ↑ myelinogenesis, oligodendrocyte activity ↓ IL1, IL6, IL17, IL21, IL23, NF-kB, TNF-α , IFN-γ, STAT3, RORγt ↑ IL4, IL10, FOXP3 & TGF-β ↑ Treg cells ↑ FoxP3, TGF-β, IL-10 expression	70-75 76-78

is the major lipophilic constituent responsible for the anti-depressant and anti-inflammatory activities of *H. perforatum* (58,59).

In EAE models St. John's wort extract and hyperforin has been shown to reduce the incidence and severity of the disease, through decreasing the inflammatory cell infiltration (60,61). Furthermore, it inhibits Th1 and Th17 cells differentiation and promotes Th2 and Treg cell differentiation through regulation of their transcription factors Foxp3, T-bet, ROR-γt and GATA3 (60).

Ginseng: Ginseng, called the king of all herbs, is a deciduous perennial plant from the Panax genus and

belongs to the Araliaceae family (62,63). The plant is indigenous to the East Asia and consists of about 20 species or variants among which '*Panax ginseng C.A*. Meyer (Asian or Korean ginseng), *Panax quinquefolius L* (American ginseng) and *Panax notoginseng*' (Sanchi ginseng) are the most widely used ones as traditional remedy for thousands of years (62,63). The major bioactive compounds of the genus Panax are ginsenosides or ginseng saponins and gintonins (63). Ginsenosides have been reported to show their effect by interaction with membrane ion channels, cell membranes, and extra-/intracellular receptors, thus promoting alterations at the transcriptional level with resultant immune-regulatory, anti-oxidant, anti-cancer and anti-inflammatory, anti-nociceptive, anti-apoptotic and neuroprotective effects (62-64). Neuroprotective effects are proposed to be via cholinergic recovery, regulation of brain-derived neurotrophic factor, and activation of the Akt/mTOR signal pathway in addition to the main mechanisms described above (64).

In EAE models ginseng has been shown to ameliorate the clinical severity of disease through BBB permeability, negative regulation of pro-inflammatory cytokine expressions (TNFα, IL1β, and IL6), Th1 and Th17 cells suppression, Treg cells upregulation, the p38 MAPK/ NFκβ signaling pathway downregulation, and growth factors (IGF-1, TGFβ, and VEGF-1) expression enhancement (65,66). Besides, ginseng and its non-saponin fraction has also been reported to enhance innate immunity and attenuate cytokine production via Toll-like receptor (TLR)4/myeloid differentiation primary response 88 (MyD88)/nuclear factor (NF)-κB signaling pathway inhibition. All these studies suggest that P. ginseng and related materials (extract, fraction, components, etc.) might have a potential and be a good candidate for multi-targeted approaches in the treatment of MS (67). However, in two double-blind, placebo-controlled clinical trials comparing the effect of ginseng on fatigue in multiple sclerosis patients controversial results has been reported though both state its use as safe (68,69).

Turmeric: Recognized as 'spice of life' or 'golden spice' turmeric (*Curcuma longa* Linn) contains curcumin as the main active component which can easily pass through all cell membranes and BBB due to its lipophilic properties (70). Curcumin has a plethora of broad therapeutic potential with anti-oxidant and anti-inflammatory and neuroprotective properties and have been assessed in many neurodegenerative diseases (70,71). Curcumin's anti-inflammatory activity is due to the modulation of inflammatory responses through down-regulation of cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) activities, inhibition of NFκβ pathway, inhibition of TNFα, IL1, IL2, IL6, IL8, and IL12, MCP, and migration inhibitory protein production; activation of adenosine monophosphate (AMP)-activated protein kinase/silent mating type information regulation 2 homolog 1 (AMPK/SIRT1) axis,and down-regulation of the Janus kinase 2 /signal transducers and activators of transcription 3 (JAK2/STAT3) signaling pathway (70-73). Treatment with curcumin has been shown to reduce the clinical severity of EAE significantly and also ameliorate neuroinflammation by down-regulating Th17 production, inhibiting Th1 differentiation and increasing Th2 polarization (70-76). Curcumin has been shown to reduce the inflammatory cells infiltration and demyelination (70-72,74). Besides, reduced astrocytes proliferation, improved myelogenesis due to enhanced repair mechanisms, and activity and differentiation of oligodendrocytes have also been reported with curcumin treatment (71,72). These ameliorative effects of curcumin is mainly via changed cytokines expression profile in favor of anti-inflammatory mediators. The decrease of the pro-inflammatory gene expression of NFκβ, IL1, IL17, IFNγ, TNFα, MCP-1 RAR-related orphan receptor gamma (RORγt), and STAT3, and a robust increase of the anti-inflammatory gene expression IL4, IL10, FoxP3 and TGFβ have been convincingly reported (70-72,75).

In two placebocontrolled studies MS patients who were on interferonβ-1a treatment were supplemented with curcumin for 6 months. It has been shown that curcumin treatment resulted in significant decrease in the proportion of Th17 cells and expression level of RORγt and IL-17; and also an increased expression of FoxP3, TGF-β, and IL-10 as well as restoration of Treg cells frequency and function (76,77). In another study the effect of curcumin as an add-on therapy on new/ enlarging T2 lesions in relapsing remitting MS patients under treatment with subcutaneous interferon-β failed to reveal any beneficial effect in comparison to placebo group. However, the authors also note that the study drop-out rate was higher than expected and therefore no definite conclusion can be drown (78).

In these clinical and in-vitro and in-vivo trials curcumin's outstanding anti-inflammatory and neuroprotective effects has been shown convincingly and has been suggested as a promising nutraceutical agent with a therapeutic potential in the management of MS, however in demand of more clinical trials (74).

Aloysia citrodora (Lemon verbena), *Andrographis paniculata* (creat or green chiretta), *Boswellia papyrifera* (Sudanese frankincense), *Camellia*

sinensis (Green Tea), *Ginkgo biloba*, *Ruta graveolens* (common rue or herb-of-grace), and *Vaccinium spp*. (Cranberry) and Cannabis sativa are other herbal plants tried to relieve symptoms of MS (42). Among these, only extracts of Cannabis sativa L has been developed by the pharmaceutical industry and was approved as a legal therapeutic option in MS-related spasticity, neuropathic pain, and urinary symptoms in some countries. In numerous studies cannabinoids, bioactive constituent of cannabis-cannabidiol and tetrahydrocannabinol, have been shown to reduce cytokine production, induce apoptosis, inhibit cell proliferation and enhance T regulatory cell function (78-80). In a recent meta-analysis covering 3161 MS patients, results favored cannabinoid use for spasticity, pain and bladder dysfunction but with limited efficacy (81). In Türkiye cannabis use is illegal and under strict legal regulations.

Conclusion

In this article, the efficacy and mechanism of action of some herbal therapies, mainly preferred by Turkish MS patients are reviewed. A growing body of information has been gathered about the beneficial effects of herbal therapy to alleviate or suppress inflammation, oxidative stress and hence promote neuroprotection from some experimental and clinical studies. Also herbal therapy is reported to be natural, safe and reliable for the treatment of many medical conditions (42). However, the evidence is scarce to recommend their use, except a few (6). Given the complex nature of the disease and the polypharmacy the patients are on, concurrent use of herbs with MS drugs may be risky and may cause severe side effects. In conclusion, MS patients should be informed about herbal remedies and should be advised to be cautious.

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References

1. Dendrou CA, Fugger L, Friese MA. Immunopathology of Multiple Sclerosis. Nat Rev Immunol 2015 Sep 15;15(9):545-58. doi: 10.1038/nri3871.
2. Haase S, Linker RA. Inflammation in Multiple Sclerosis. Ther Adv Neurol Disord 2021;14:17562864211007687. doi: 10.1177/17562864211007687.
3. Kunkl M, Frascolla S, Amormino C, Volpe E, Tuosto L. T Helper Cells: the Modulators of Inflammation in Multiple Cclerosis Cells 2020;9(2):482. doi: 10.3390/cells9020482.
4. Ivanova EA, Orekhov AN. T Helper Lymphocyte Subsets and Plasticity in Autoimmunity and Cancer: An Overview. Biomed Res Int 2015;2015:327470. doi: 10.1155/2015/327470.
5. Tangkiatkumjai M, Boardman H, Walker DM. Potential Factors that Influence Usage of Complementary and Alternative Medicine Worldwide: A Systematic Review. BMC Complement Med Ther 2020;20(1):363. doi: 10.1186/s12906-020-03157-2.
6. Yadav V, Bever C Jr, Bowen J, Bowling A, Weinstock-Guttman B, Cameron M, Bourdette D, Gronseth GS, Narayanaswami P. Summary of Evidence-Based Guideline: Complementary and Alternative Medicine in Multiple Sclerosis: Report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 2014;82(12):1083-92. doi: 10.1212/WNL.0000000000000250.
7. Pucci E, Cartechini E, Taus C, Giuliani G. Why Physicians Need to Look More Closely at the use of Complementary and Alternative Medicine by Multiple Sclerosis Patients. Eur J Neurol 2004;11(4):263-7. doi: 10.1046/j.1468-1331.2003.00758.x.
8. Gedizlioğlu M, Yumurtaş S, Trakyalı AU, Yıldırım F, Ortan P, Köşkderelioğlu A. Complementary and Alternative Therapy Use in Multiple Sclerosis: A Cross-Sectional Survey. Turk J Neurol 2015; 21(1): 13-15. Doi:10.4274/tnd.46354
9. Duru Aşiret G, Düğer Ü, Kapucu S, Tuncer Kurne A, Karabudak R. Multiple Skleroz Hastalarının Tamamlayıcı ve Alternatif Tedavi Kullanım Durumlarının Belirlenmesi. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi 2017;6:1-6.
10. Çelik RGG, Öztürk M, Altın S, Köseoğlu M, Sariahmetoğlu H, Tütüncü M, et al. The Prevalence of Alternative-Complementary Therapies in Patients with Multiple Sclerosis. Turk J Neurol 2019; 25(3): 153-58. doi: 10.4274/tnd.2019.04207
11. Dağlı S, Ünlü MD, Demirci S. Complementary and alternative medicine use in Multiple Sclerosis. 2nd International Turkic World Congress of Multiple Sclerosis 2020 Abstract Book, pp: 31.
12. Aksoy S, Şengül Y, Karaman HIÖ. Multipl Skleroz Hastalarına Tamamlayıcı ve Alternatif Tıp Yöntemlerine Başvurunun Demografik ve Klinik Korelasyonlarının Değerlendirilmesi. 2nd International Turkic World Congress of Multiple Sclerosis 2020 Abstract Book, pp: 16-17.
13. Dayapoğlu N, Tan M. Use of Complementary and Alternative Medicine Among People with Multiple Sclerosis in Eastern Turkey. Neurology Asia 2016; 21(1): 63-71.
14. Wink M. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. Medicines (Basel) 2015;2(3):251-86. doi: 10.3390/medicines2030251.
15. Gazioglu I, Semen S, Acar OO, Kolak U, Sen A, Topcu G. Triterpenoids and Steroids Isolated from Anatolian Capparis Ovata and Their Activity on the Expression of Inflammatory Cytokines. Pharm Biol 2020;58(1):925-31. doi: 10.1080/13880209.2020.1814356.
16. Moutia M, El Azhary K, Elouaddari A, Al Jahid A, Jamal Eddine J, Seghrouchni F, et al. Capparis Spinosa L. Promotes Anti-Inflammatory Response In Vitro Through the Control of Cytoki-

ne Gene Expression in Human Peripheral Blood Mononuclear Cells. BMC Immunol 2016;17(1):26. doi: 10.1186/s12865-016-0164-x.
17. Ozgun-Acar O, Celik-Turgut G, Gazioglu I, Kolak U, Ozbal S, Ergur BU, et al. Capparis Ovata Treatment Suppresses Inflammatory Cytokine Expression and Ameliorates Experimental Allergic Encephalomyelitis Model of Multiple Sclerosis in C57BL/6 Mice. J Neuroimmunol 2016;298:106-16. doi: 10.1016/j.jneuroim.2016.07.010.
18. Ozgun-Acar O, Gazioglu I, Kolak U, Sen A, Topcu G. A Potential Therapeutic Role in Multiple Sclerosis for Stigmast-5,22-Dien-3b-ol Myristate Isolated from Capparis Ovata. Eurobiotech J 2017;1(3):241-46.
19. Sen A, Topcu G, Ozgun O, Kolak U, Hacibekiroglu I, Celik G, et al. Anti-Neuroinflammatory Effect of Butanolic Fraction of Capparis Ovata Water Extract Used as an Alternative and Complementary Treatment for Multiple Sclerosis. J Neuroimmunol 2014;275(1-2):172-73.
20. Choi JG, Kim SY, Jeong M, Oh MS. Pharmacotherapeutic Potential of Ginger and its Compounds in Age-Related Neurological Disorders. Pharmacol Ther 2018;182:56-69. doi: 10.1016/j.pharmthera.2017.08.010.
21. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, et al. Bioactive Compounds and Bioactivities of Ginger (Zingiber Officinale Roscoe). Foods 2019;8(6):185. doi: 10.3390/foods8060185.
22. Jafarzadeh A, Nemati M. Therapeutic Potentials of Ginger for Treatment of Multiple Sclerosis: A Review with Emphasis on its Immunomodulatory, Anti-Inflammatory and Anti-Oxidative Properties. J Neuroimmunol 2018;324:54-75. doi: 10.1016/ j.jneuroim.2018.09.003.
23. Han JJ, Li X, Ye ZQ, Lu XY, Yang T, Tian J, et al. Treatment with 6-Gingerol Regulates Dendritic Cell Activity and Ameliorates the Severity of Experimental Autoimmune Encephalomyelitis. Mol Nutr Food Res 2019;63(18):e1801356. doi: 10.1002/mnfr.201801356
24. Jafarzadeh A, Mohammadi-Kordkhayli M, Ahangar-Parvin R, Azizi V, Khoramdel-Azad H, Shamsizadeh A, et al. Ginger Extracts Influence the Expression of IL27 and IL-33 in the Central Nervous System in Experimental Autoimmune Encephalomyelitis and Ameliorates the Clinical Symptoms of Disease. J Neuroimmunol 2014;276(1-2):80-8. doi: 10.1016/ j.jneuroim.2014.08.614.
25. Ho SC, Chang KS, Lin CC. Anti-Neuroinflammatory Capacity of Fresh Ginger is Attributed Mainly to 10-Gingerol. Food Chem 2013;141(3):3183-91. doi: 10.1016/j.foodchem.2013.06.010.
26. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, et al. Bioactive Compounds and Biological Functions of Garlic (Allium sativum L.). Foods 2019;8(7):246. doi: 10.3390/foods8070246.
27. Zeinali H, Baluchnejadmojarad T, Fallah S, Sedighi M, Moradi N, Roghani M. S-allyl Cysteine Improves Clinical and Neuropathological Features of Experimental Autoimmune Encephalomyelitis in C57BL/6 Mice. Biomed Pharmacother 2018;97:557-63. doi: 10.1016/j.biopha.2017.10.155.
28. Song H, Cui J, Mossine VV, Greenlief CM, Fritsche K, Sun GY, et al. Bioactive Components from Garlic on Brain Resiliency Against Neuroinflammation and Neurodegeneration. Exp Ther Med 2020;19(2):1554-59. doi: 10.3892/etm.2019.8389.
29. Abdullah A, Maged M, Hairul-Islam M I, Osama I A, Maha H, Manal A, et al. Activation of Aryl Hydrocarbon Receptor Signaling by a Novel Agonist Ameliorates Autoimmune Encephalomyelitis. PLoS One 2019;14(4):e0215981. doi: 10.1371/journal.pone.0215981.
30. Sıcak Y, Erdoğan Eliuz EA. Chemical Content and Biological Activity Spectrum of Nigella Sativa Seed Oil. KSU J Agric Nat 2019;22(5):928-34. doi: 10.18016/ksutarimdoga.vi.537674.
31. Dalli M, Bekkouch O, Azizi SE, Azghar A, Gseyra N, Kim B. Nigella sativa L. Phytochemistry and Pharmacological Activities: A Review (2019-2021). Biomolecules 2021;12(1):20. doi: 10.3390/biom12010020.
32. Majdalawieh AF, Fayyad MW. Immunomodulatory and Anti-Inflammatory Action of Nigella Sativa and Thymoquinone: A Comprehensive Review. Int Immunopharmacol 2015;28(1):295-304. doi: 10.1016/j.intimp.2015.06.023
33. Ali MY, Akter Z, Mei Z, Zheng M, Tania M, Khan MA. Thymoquinone in Autoimmune Diseases: Therapeutic Potential and Molecular Mechanisms. Biomed Pharmacother 2021;134:111157. doi: 10.1016/j.biopha.2020.111157
34. Wang Y, Gao H, Zhang W, Zhang W, Fang L. Thymoquinone Inhibits Lipopolysaccharide-Induced Inflammatory Mediators in BV2 Microglial Cells. Int Immunopharmacol 2015;26(1):169-173. doi: 10.1016/j.intimp.2015.03.013
35. Velagapudi R, Kumar A, Bhatia HS, El-Bakoush A, Lepiarz I, Fiebich BL, et al. Inhibition of Neuroinflammation by Thymoquinone Requires Activation of Nrf2/ARE Signalling. Int Immunopharmacol 2017;48:17-29. doi: 10.1016/j.intimp.2017.04.018
36. Taka E, Mazzio EA, Goodman CB, Redmon N, Flores-Rozas H, Reams R, et al. Anti-Inflammatory Effects of Thymoquinone in Activated BV-2 Microglial Cells. J Neuroimmunol 2015;286:5-12. doi: 10.1016/j.jneuroim.2015.06.011
37. Fahmy HM, Noor NA, Mohammed FF, Elsayed AA, Radwan NM. Nigella Sativa as an Anti-Inflammatory and Promising Remyelinating Agent in the Cortex and Hippocampus of Experimental Autoimmune Encephalomyelitis-Induced Rats. J Basic Appl Zool 2014;67(5):182-95. doi:10.1016/j.jobaz.2014.08.005.
38. Mohamed A, Afridi DM, Garani O, Tucci M. Thymoquinone Inhibits the Activation of NF-KappaB in the Brain and Spinal Cord of Experimental Autoimmune Encephalomyelitis. Biomed Sci Instrum 2005;41:388-93.
39. Noor NA, Fahmy HM, Mohammed FF, Elsayed AA, Radwan NM. Nigella Sativa Amliorates Inflammation and Demyelination in the Experimental Autoimmune Encephalomyelitis-Induced Wistar Rats. Int J Clin Exp Pathol 2015;8(6):6269-86.
40. Yüzbaşıoğlu İS. Oenothera Parodiana (Onagraceae): A New Alien Species Record for the Flora of Turkey. Biological Diversity and Conservation 2014;7(2):122-126.
41. Timoszuk M, Bielawska K, Skrzydlewska E. Evening Primrose (Oenothera Biennis) Biological Activity Dependent on Chemical Composition. Antioxidants (Basel) 2018;7(8):108. doi: 10.3390/antiox7080108.
42. Farzaei MH, Shahpiri Z, Bahramsoltani R, Nia MM, Najafi F, Rahimi R. Efficacy and Tolerability of Phytomedicines in Multiple Sclerosis Patients: A Review. CNS Drugs 2017;31(10):867-89. doi: 10.1007/s40263-017-0466-4.
43. Kapoor R, Huang YS. Gamma Linolenic Acid: An Antiinflammatory Omega-6 Fatty Acid. Curr Pharm Biotechnol 2006;7(6):531-4. doi: 10.2174/138920106779116874.
44. Cao D, Luo J, Zang W, Chen D, Xu H, Shi H, Jing X. Gamma-Linolenic Acid Suppresses NFκβ Signaling Via CD36 in the Lipopolysaccharide-Induced Inflammatory Response in Primary Goat Mammary Gland Epithelial Cells. Inflammation 2016;39(3):1225-37. doi: 10.1007/s10753-016-0358-7.
45. Rezapour-Firouzi S, Kheradmand F, Shahabi S, Tehrani AA, Mazloomi E, Mohammadzadeh A. Regulatory Effects of Hemp Seed/Evening Primrose Oil Supplement in Comparison With Rapamycin on the Expression of the Mammalian Target of Rapamycin-Complex 2 and Interleukin-10 Genes in Experimental Autoimmune Encephalomyelitis. Res Pharm Sci 2019;14(1):36-45. doi: 10.4103/1735-5362.251851.
46. Rezapour-Firouzi S, Mohammadian M, Sadeghzadeh M, Mehranfar S, Mazloomi E. The Effects of Evening Primrose/Hemp Seed Oil Compared to Rapamycin on the Gene Expression of Immunological Parameters in Experimental Autoimmune Encephalomyelitis Splenocytes. Iran J Allergy Asthma Immunol 2020;19(2):183-92. doi: 10.18502/ijaai.v19i2.2771.
47. Rezapour-Firouzi S, Arefhosseini SR, Mehdi F, Mehrangiz EM, Baradaran B, Sadeghihokmabad E, et al. Immunomodulatory and Therapeutic Effects of Hot-Nature Diet and Co-Supplemented Hemp Seed, Evening Primrose Oils Intervention in Multiple

Sclerosis Patients. Complement Ther Med 2013;21(5):473-80. doi: 10.1016/j.ctim.2013.06.006.
48. Majdinasab N, Namjoyan F, Taghizadeh M, Saki H. The Effect of Evening Primrose Oil on Fatigue and Quality of Life in Patients with Multiple Sclerosis. Neuropsychiatr Dis Treat 2018;14:1505-12. doi: 10.2147/NDT.S149403.
49. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and Flaxseed Oil: An Ancient Medicine & Modern Functional Food. J Food Sci Technol 2014;51(9):1633-53. doi: 10.1007/s13197-013-1247-9.
50. Rafieian-Kopaei M, Shakiba A, Sedighi M, Bahmani M. The Analgesic and Anti-Inflammatory Activity of Linum usitatissimum in Balb/c Mice. J Evid Based Complementary Altern Med 2017;22(4):892-96. doi: 10.1177/2156587217717416.
51. Chera EI, Pop RM, Pârvu M, Soriţău O, Uifălean A, Cătoi FA, et al. Flaxseed Ethanol Extracts' Antitumor, Antioxidant, and Anti-Inflammatory Potential. Antioxidants (Basel) 2022;11(5):892. doi: 10.3390/antiox11050892.
52. Benatti P, Peluso G, Nicolai R, Calvani M. Polyunsaturated Fatty Acids: Biochemical, Nutritional and Epigenetic Properties. J Am Coll Nutr 2004;23(4):281-302. doi: 10.1080/07315724.2004.10719371.
53. Dupasquier CM, Dibrov E, Kneesh AL, Cheung PK, Lee KG, Alexander HK, et al. Dietary Flaxseed Inhibits Atherosclerosis in the LDL Receptor-Deficient Mouse in Part Through Antiproliferative and Anti-Inflammatory Actions. Am J Physiol Heart Circ Physiol 2007;293(4):H2394-402. doi: 10.1152/ajpheart.01104.2006.
54. Askarpour M, Karimi M, Hadi A, Ghaedi E, Symonds ME, Miraghajani M, et al. Effect of Flaxseed Supplementation on Markers of Inflammation and Endothelial Function: A Systematic Review and Meta-Analysis. Cytokine 2020;126:154922. doi: 10.1016/j.cyto.2019.154922.
55. Rahimlou M, Jahromi NB, Hasanyani N, Ahmadi AR. Effects of Flaxseed Interventions on Circulating Inflammatory Biomarkers: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr 2019;10(6):1108-119. doi: 10.1093/advances/nmz048.
56. Jelinek GA, Hadgkiss EJ, Weiland TJ, Pereira NG, Marck CH, van der Meer DM. Association of Fish Consumption and Ω 3 Supplementation with Quality of life, Disability and Disease Activity in an International Cohort of People with Multiple Sclerosis. Int J Neurosci 2013;123(11):792-800. doi: 10.3109/00207454.2013.803104.
57. Parks NE, Jackson-Tarlton CS, Vacchi L, Merdad R, Johnston BC. Dietary Interventions for Multiple Sclerosis-Related Outcomes. Cochrane Database Syst Rev 2020;5(5):CD004192. doi: 10.1002/14651858.CD004192.pub4.
58. Kuo CH, Chou YC, Liao KC, Shieh CJ, Deng TS. Optimization of Light Intensity, Temperature, and Nutrients to Enhance the Bioactive Content of Hyperforin and Rutin in St. John's Wort. Molecules 2020;25(18):4256. doi: 10.3390/molecules25184256.
59. Murch SJ, Saxena PK. St. John's wort (Hypericum perforatum L.): Challenges and Strategies for Production of Chemically-Consistent Plants. Can J Plant Sci 2006;86(3): 765-71. Doi:10.4141/P05-179
60. Nosratabadi R, Rastin M, Sankian M, Haghmorad D, Mahmoudi M. Hyperforin-Loaded Gold Nanoparticle Alleviates Experimental Autoimmune Encephalomyelitis by suppressing Th1 and Th17 Cells and Upregulating Regulatory T Cells. Nanomedicine 2016;12(7):1961-71. doi: 10.1016/j.nano.2016.04.001.
61. Selek S, Esrefoglu M, Meral I, Bulut H, Caglar HG, Sonuc G, et al. Effects of Oenothera Biennis L. and Hypericum Perforatum L. Extracts on Some Central Nervous System Myelin Proteins, Brain Histopathology and Oxidative Stress in Mice with Experimental Autoimmune Encephalomyelitis. Biotech Histochem 2019;94(2):75-83. doi: 10.1080/10520295.2018.1482001.
62. Hou M, Wang R, Zhao S, Wang Z. Ginsenosides in Panax Genus and Their Biosynthesis. Acta Pharm Sin B 2021;11(7):1813-34. doi: 10.1016/j.apsb.2020.12.017
63. Ratan ZA, Haidere MF, Hong YH, Park SH, Lee JO, Lee J, et al. Pharmacological Potential of Ginseng and its Major Component Ginsenosides. J Ginseng Res 2021;45(2):199-210. doi: 10.1016/j.jgr.2020.02.004.
64. Lu J, Wang X, Wu A, Cao Y, Dai X, Liang Y, Li X. Ginsenosides in Central Nervous System Diseases: Pharmacological Actions, Mechanisms, and Therapeutics. Phytother Res 2022;36(4):1523-1544. doi: 10.1002/ptr.7395.
65. Lee MJ, Chang BJ, Oh S, Nah SY, Cho IH. Korean Red Ginseng Mitigates Spinal Demyelination in a Model of Acute Multiple Sclerosis by Downregulating p38 Mitogen-Activated Protein Kinase and Nuclear Factor-κB Signaling Pathways. J Ginseng Res 2018;42(4):436-46. doi: 10.1016/j.jgr.2017.04.013.
66. Lee MJ, Choi JH, Kwon TW, Jo HS, Ha Y, Nah SY, Cho IH. Korean Red Ginseng Extract Ameliorates Demyelination by Inhibiting Infiltration and Activation of Immune Cells in Cuprizone-Administrated Mice. J Ginseng Res 2023;47(5):672-680. doi: 10.1016/j.jgr.2023.05.001.
67. Oh J, Kwon TW, Choi JH, Kim Y, Moon SK, Nah SY, Cho IH. Ginsenoside-Re Inhibits Experimental Autoimmune Encephalomyelitis as a Mouse Model of Multiple Sclerosis by Downregulating TLR4/MyD88/NF-κB Signaling Pathways. Phytomedicine 2024;122:155065. doi: 10.1016/j.phymed.2023.155065.
68. Kim E, Cameron M, Lovera J, Schaben L, Bourdette D, Whitham R. American Ginseng does not Improve Fatigue in Multiple Sclerosis: A Single Center Randomized Double-Blind Placebo-Controlled Crossover Pilot Study. Mult Scler 2011;17(12):1523-6. doi: 10.1177/1352458511412062
69. Etemadifar M, Sayahi F, Abtahi SH, Shemshaki H, Dorooshi GA, Goodarzi M, et al. Ginseng in the Treatment of Fatigue in Multiple Sclerosis: A Randomized, Placebo-Controlled, Double-Blind Pilot Study. Int J Neurosci 2013;123(7):480-6. doi: 10.3109/00207454.2013.764499.
70. Benameur T, Giacomucci G, Panaro MA, Ruggiero M, Trotta T, Monda V, et al. New Promising Therapeutic Avenues of Curcumin in Brain Diseases. Molecules 2021;27(1):236. doi: 10.3390/molecules27010236.
71. Mohajeri M, Sadeghizadeh M, Najafi F, Javan M. Polymerized Nano-Curcumin Attenuates Neurological Symptoms in EAE Model of Multiple Sclerosis Through Down Regulation of Inflammatory and Oxidative Processes and Enhancing Neuroprotection and Myelin Repair. Neuropharmacology 2015;99:156-67. doi:10.1016/j.neuropharm.2015.07.013
72. Xie L, Li XK, Funeshima-Fuji N, Kimura H, Matsumoto Y, Isaka Y, et al. Amelioration of Experimental Autoimmune Encephalomyelitis by Curcumin Treatment Through Inhibition of IL17 Production. Int Immunopharmacol 2009;9(5):575-81. doi: 10.1016/j.intimp.2009.01.025.
73. Sadek MA, Rabie MA, El Sayed NS, Sayed HM, Kandil EA. Neuroprotective Effect of Curcumin Against Experimental Autoimmune Encephalomyelitis-Induced Cognitive and Physical Impairments in Mice: An Insight into the Role of the AMPK/SIRT1 Pathway. Inflammopharmacology 2023. doi: 10.1007/s10787-023-01399-3.
74. Mavaddatiyan L, Khezri S, Abtahi Froushani SM. Molecular Effects of Curcumin on the Experimental Autoimmune Encephalomyelitis. Vet Res Forum 2021 Winter;12(1):47-52. doi: 10.30466/vrf.2019.98789.2356.
75. Khosropour S, Shahvarooghi E, Rezaeizadeh H, Esmaeelzadeh M. Curcumin and Its Semisynthetic Derivative F-Curcumin Ameliorate the Expression of Cytokines in Autoimmune Encephalomyelitis Mouse Models of Multiple Sclerosis. Iran J Allergy Asthma Immunol 2023;22(6):575-587. doi: 10.18502/ijaai.v22i6.14646.
76. Dolati S, Ahmadi M, Rikhtegar R, Babaloo Z, Ayromlou H, Aghebati-Maleki L, et al. Changes in Th17 Cells Function After Nanocurcumin Use to Treat Multiple Sclerosis. Int Immunop-

harmacol 2018;61:74-81. doi: 10.1016/j.intimp.2018.05.018.
77. Dolati S, Babaloo Z, Ayromlou H, Ahmadi M, Rikhtegar R, Rostamzadeh D, et al. Nanocurcumin Improves Regulatory T-Cell Frequency and Function in Patients with Multiple Sclerosis. J Neuroimmunol 2019;327:15-21. doi: 10.1016/j.jneuroim.2019.01.007.
78. Petracca M, Quarantelli M, Moccia M, Vacca G, Satelliti B, D'Ambrosio G, Carotenuto A, Ragucci M, Assogna F, Capacchione A, Lanzillo R, Morra VB. Prospective Study to Evaluate Efficacy, Safety and Tolerability of Dietary Supplement of Curcumin (BCM95) in Subjects with Active Relapsing Multiple Sclerosis Treated with Subcutaneous Interferon Beta 1a 44 mcg tiw (CONTAIN): A Randomized, Controlled Trial. Mult Scler Relat Disord 2021;56:103274. doi: 10.1016/j.msard.2021.103274.
79. Jones E, Vlachou S. A Critical Review of the Role of the Cannabinoid Compounds Δ9-Tetrahydrocannabinol (Δ9-THC) and Cannabidiol (CBD) and Their Combination in Multiple Sclerosis Treatment. Molecules 2020;25:4930. doi:10.3390/molecules25214930.
80. Slaven M, Levine O. Cannabinoids for Symptoms of Multiple Sclerosis: Benefits to Patients Still Unclear. JAMA Netw Open 2018;1(6):e183484. doi: 10.1001/jamanetworkopen.2018.3484.
81. Oláh A, Szekanecz Z, Bíró T. Targeting Cannabinoid Signaling in the Immune System: “High”-ly Exciting Questions, Possibilities, and Challenges. Front Immunol 2017;8:1487. Doi:10.3389/fimmu.2017.01487.
82. Torres-Moreno MC, Papaseit E, Torrens M, Farré M. Assessment of Efficacy and Tolerability of Medicinal Cannabinoids in Patients with Multiple Sclerosis: A Systematic Review and Meta-Analysis. JAMA Netw Open 2018;1(6):e183485. doi: 10.1001/jamanetworkopen.2018.3485.