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EDITORIAL

Our Dear Readers,

We are happy to publish the our journal's new issue of 2023 with valuable new articles. As known, our journal is published bimonthly and we are are so excited during all new number of journal. Every year we are trying to boost our scientific level enormously. As we have mentioned previously, we are successfully contributing to the international literature by valuable manuscripts. We are constantly working to raise our scientific bar and to increase the success of our journal by entering valuable international indexes . We would like to thank all the authors who contributed to the strengthening of our journal by sending articles from both domestic and abroad.

Sincerely Yours,

Prof. Alpaslan TANOĞLU
Editors-in-Chief

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Evaluation and epigenetic impact of B12, vitamin D, folic acid and anemia in Hashimoto's thyroiditis: a clinical and molecular docking study

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ABSTRACT

Aims: Our approach in this study is to investigate the collective effect of various parameters, including vitamin B12 (B12), vitamin D (Vit-D), folic acid, and iron deficiency, on Hashimoto's thyroiditis (HT) disease. This differs from existing literature that has examined these parameters individually.

Methods: The study evaluated age, gender, thyroid stimulating hormone (TSH), free-T4 (FT4), free-T3 (FT3), Vit-D levels, as well as autoantibodies against thyroid peroxidase (anti-TPO) and anti-tyroglobulin iron (anti-TG) levels, ferritin, B12, and folic acid parameters in a total of 30 HT patients and 37 non-HT patients. These parameters were assessed by analyzing the patients' routine blood test results using automated analysis methods.

Results: A negative correlation was found between the blood, Vit-D level and anti-TG (as the vit-D value increases, the anti-TG decreases) ($r=-0.417$; $p=0.001$; $p<0.01$). There was a statistically significant and weak correlation between blood Vit-D measurements and anti-TPO measurements ($r=-0.341$; $p=0.005$; $p<0.01$).

Conclusion: The study findings demonstrated that there was no statistically significant difference in the measurements and correlation between hemoglobin, FT3, FT4, TSH, ferritin, vitamin B12, and folic acid levels in patients with and without a diagnosis of HT ($p>0.05$). However, the study emphasized the critical role of vitamin D in the pathophysiology and treatment of HT. Furthermore, molecular docking simulations indicated that folic acid could potentially act as a potent inhibitor of human extracellular signal-regulated kinase (ERK2), which has been reported to play a key role in HT.

Keywords: Hashimoto's thyroiditis, otoimmun disease, vitamin D, anti-TPO, ferritin, vitamin B12

INTRODUCTION

Hashimoto's thyroiditis (HT) is the most common autoimmune thyroid disease. Aapproximately 20-20% of the cases are attributed to genetic predisposition, while remaining causes are linked to environmental conditions and epigenetic factors. In studies focusing on autoimmune thyroid diseases, it has been observed that HT leads to the overexpression of certain genes involved in immune function or the activation of immune cells. Furthermore, research has shown that epigenetic mechanisms, such as DNA methylation, histone modification, and miRNA, play a role in regulating specific genes, which may contribute to the autoimmune attack against thyroid tissues and leads to appearance of the disease.

The histological features of the autoimmune thyroiditis include follicular destruction, granular atrophy, and fibrosis due to lymphocytic infiltration of T cells.[1] HT is characterized by varying levels of clinical hypothyroidism and the presence of autoantibodies against thyroid peroxidase (anti-TPO) and thyroglobulin (anti-thyroglobulin). This disease is 4-10 times more common in women than men, particularly in the age range of 30-50 years.[2] The predominant antibody in autoimmune hypothyroidism, anti-TPO, is an essential biomarker, as it is present in over 90% of patients.[3]

Thyroid disorders may occurdue to deficiencies in iodine, selenium, iron, zinc, minerals and vitamins A, C, B6, B5, and D, all of which are necessary for thyroid hormone synthesis and metabolism.[4,5] Environmental factors contribute to the pathogenesis of autoimmune thyroid disease at a rate of 20-30%, while genetic factors account for 70%.[6] Polymorphisms in the vitamin D receptor

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(VDR) gene predispose individuals to conditions such as Addison's disease, type 1 diabetes, and autoimmune diseases, including Hashimoto's, under the influence of environmental conditions and epigenetic factors. Studies on cytokine genes, such as interferon-gamma (IFN-γ), interleukin-4 (IL-4), and transforming growth factor beta (TGF-β), which regulate the balance between T-helper 1 (Th1) and T-helper 2 (Th2) mechanisms, have shown a correlation with the development and severity of Hashimoto's.[7]

Although epigenetic mechanisms, such as DNA methylation, miRNA and histone modifications, have been demonstrated in some genes associated with autoimmune thyroid diseases, the precise molecular mechanisms underlying these epigenetic changes are still not fully understood.

This study aimed to evaluate the frequency of B12, vitamin D, folic acid, and iron deficiency, which play a role in the pathogenesis and epigenetic effects on HT, compared to individuals without thyroid disease.

METHODS

The study was carried out with the permission of Biruni University Clinical Researches Ethics Committee (Date: 09.04.2021, Decision No: 2021/50-40). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients who applied to the outpatient clinic between January and December 2021 and 67 patients between the ages of 21 and 72 who were diagnosed with HT were included in the study.

Patients with no known chronic disease, no history of drug use, insulin resistance and autoimmune HT were included in this study retrospectively. Anti-TPO and/or anti-TG antibody positivity was considered as the main condition in terms of autoimmune thyroiditis.

Patients who had undergone thyroidectomy, radioactive iodine (RAI) treatment, had malabsorption syndromes, gastrectomy, ileal resection, followed a vegetarian diet, were pregnancy, or had received B12, Folic acid, iron, and Vit-D supplementation in the last six months were excluded from the study.

Patients with 30 Hashimoto's and 37 non-Hashimoto's control groups were compared in terms of gender, age, TSH, FT4, FT3, Vit-D, anti-TPO, anti-TG and iron levels. Then, patients were divided into three groups according to their Vit-D levels: <10ng/mL(severe deficiency), 10-19 ng/mL (mild to moderate deficiency), and 20-50 ng/mL (optimum levels). The relationship between Vit-D and anti-TPO levels of the group was checked to understand the correlation between them. B12 values were also evaluated as 180-914 ng/L and folate deficiency found as <4.0 mcg/L between groups.[8]

G-Power Analysis

According to the literature, autoimmune Graves' disease has been reported to occur in approximately 77% of women.[9] When the expected rate for the variable was considered to be 60%, a power analysis using R (software/programming-version 3.6.2 – CRAN) determined that a minimum of 65 patients in total should be included to achieve a 90% power.

Statistical Analysis

The statistical analysis was conducted using the NCSS (Number Cruncher Statistical System) 2007 program from Kaysville, Utah, USA. Descriptive statistical methods, including mean, standard deviation, median, frequency, percentage, minimum, and maximum, were employed to evaluate the study data. The Shapiro-Wilk test and visual examinations were used to assess the normal distribution of quantitative data. The independent groups' t-test was utilized to compare two groups with normally distributed quantitative variables, while the Mann-Whitney U test was employed for non-normally distributed quantitative variables. For comparing qualitative data, the Pearson chi-square test and Fisher-Freeman-Halton exact test were utilized. Diagnostic screening tests, such as sensitivity, specificity, positive predictive value, and negative predictive value, along with receiver operating characteristic (ROC) analysis, were performed to determine the cut-off value for the parameters. Statistical significance was accepted as $p<0.05$.

- **Sensitivity:** The test can identify patients among actual patients.
- **Specificity:** The test can identify the healthy people among the real healthy people.
- **Positive predictive value (PKD):** The probability that the patient/subject has the disease/condition when restricted to those patients/subjects who test positive.
- **Negative predictive value (NKD):** The probability that the patient will not have the disease/condition when restricted to all patients/subjects who test negative.

Molecular Docking

All calculations were carried out using Schrödinger suite (Schrodinger, In. Version 2022-2, LLC). A high-resolution Human extracellular signal-regulated kinase 2 (ERK2) crystal structure (PDB ID: 5NHH, 1.94 Å) was downloaded from protein data bank and prepared. The preparation protocol includes adding missing hydrogen bonds, correction of bond orders, optimization and finally, minimization of whole

protein to avoid steric clashes. Prior to optimization step, water molecules were removed but the co-crystallized ligand (native ligand) were kept which were used as reference for binding site generation using Grid module. Folic acid and native ligand were also prepared using LigPrep module, see **Figure 1**. All possible 3D conformations, tautomers, ionization states at physiological conditions were generated. Glide XP method was used in molecular docking.

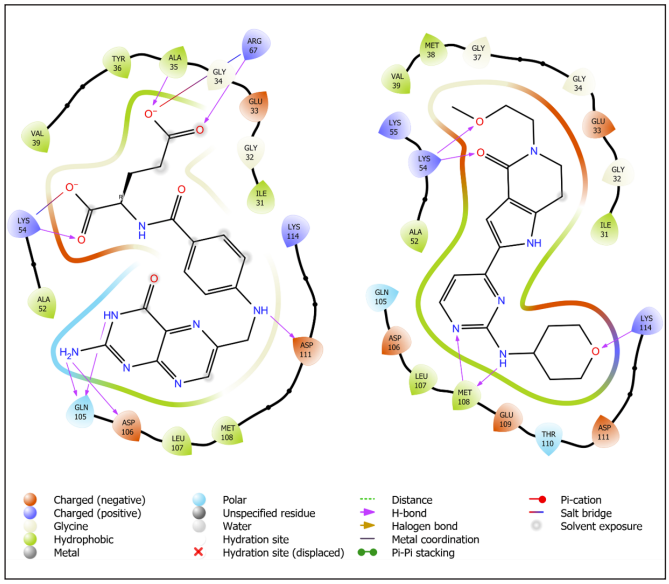

Figure 1. Ligand interaction diagram of folic acid (left) and co-crystallized ligand with ERK2.

RESULTS

The study was conducted with a total of 67 cases, 74.6% (n=50) female and 25.4% (n=17) male, who applied to the outpatient clinic between January 2021 and December 2021 (**Figure 2**).

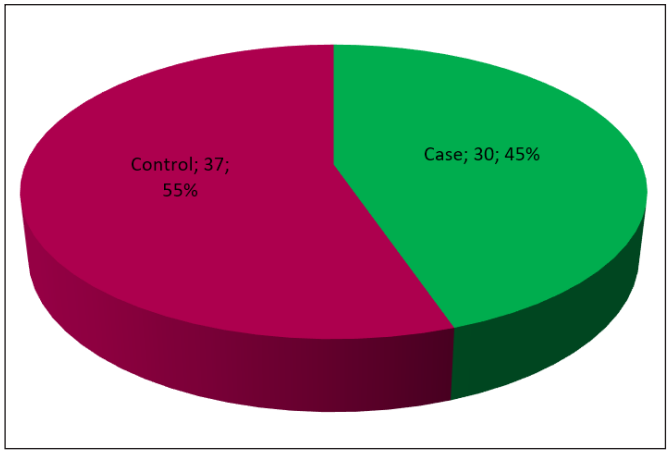

Figure 2. Distribution of groups

The ages of the cases ranged from 21 to 75, with a mean age of 43.61±13.19 (**Table 1, 2**).

Table 1. Evaluation of descriptive characteristics by groups

	Total	Grup		
		Patients (n=30)	Control (n=37)	
Sex				[a]0.140
Female	50 (74.6)	25 (83.3)	25 (67.6)	
Male	17 (25.4)	5 (16.7)	12 (32.4)	
Age				[b]0.374
Avr±Ss	43.76±13.19	45.37±13.38	42.46±13.06	
Median (Min-Max)	42 (21-75)	44.5 (28-75)	42 (21-68)	

aPearson Chi-Square Test, bStudent T Test

The ages and genders of the cases did not show a statistically significant difference according to the groups (p>0.05).

Anti-TPO measurements in the patient group were statistically significantly higher than those in the control group (p=0.001; p<0.01).

Anti-thyroglobulin measurements in the patient group were statistically significantly higher than those in the control group (p=0.001; p<0.01).

Vit-D measurements (**Figure 3**) in the patient group were statistically significantly lower than those in the control group (p=0.004; p<0.01).

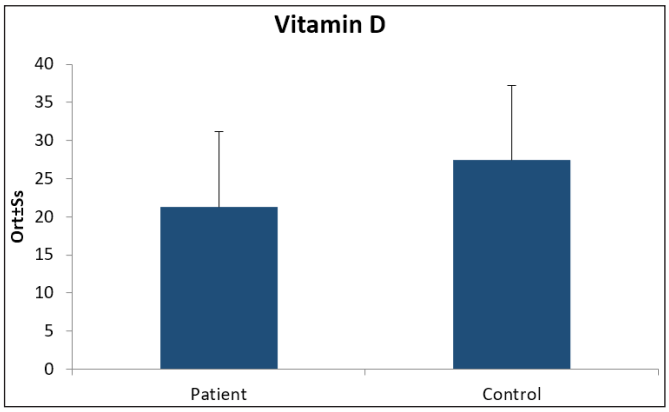

Figure 3. Distribution of vitamin-D measurement by groups

Haemoglobin, FT3, FT4, TSH, Ferritin, Vitamin B12, and Folic acid measurements of the cases did not show a statistically significant difference according to the groups (p>0.05).

For the 20.1 cut-off value of the anti-thyroglobulin level, sensitivity is 92.86%; specificity is 83.78%; positive predictive value is 81.20%, and negative predictive value is 93.30% (**Table 3**). In the obtained ROC curve (**Figure 4**), the standard error of 92.5% was determined as 3.6% for the underlying area. In addition, a statistically significant correlation was found with the 20.1 cut-off value of the anti-thyroglobulin level in predicting the disease (p=0.001; p<0.01).

Table 2. Evaluation of measurements by groups

	Total	Groups		p
		Case	Control	
Haemoglobin (gr/dl)				[b]0.155
Mean±SD	13.57±1.51	13.27±1.36	13.80±1.59	
Median (Min-Max)	13.5 (10.3-16.9)	13.2 (10.3-15.8)	13.6 (10.4-16.9)	
FT3 (pg/ml)				[c]0.434
Mean±SD	3.18±0.94	3.29±1.39	3.12±0.50	
Mean±SD	3.1 (2.1-9.5)	3 (2.1-9.5)	3.2 (2.1-4)	
FT4 (ng/dl)				[c]0.645
Median (Min-Max)	1.26±0.31	1.30±0.41	1.23±0.22	
Mean±SD	1.2 (0.9-2.9)	1.2 (0.9-2.9)	1.2 (0.9-2)	
TSH (uIU/ml)				[c]0.265
Median (Min-Max)	2.69±1.75	2.89±1.92	2.54±1.63	
Mean±SD	2.2 (0-8.1)	2.9 (0-7.6)	2.1 (0.5-8.1)	
Anti-TPO (Anti-M) (IU/ml)				[c]0.001**
Mean±SD	87.90±128.9	185.87±140.97	8.47±3.75	
Median (Min-Max)	13.5 (4.5-540)	150.5 (13.6-540)	8 (4.5-21.9)	
Anti-Thyroglobulin(IU/ml)				[c]0.001**
Mean±SD	163.69±495.24	353.26±718.12	20.23±12.30	
Median (Min-Max)	19.8 (9.5-3800)	152 (14.6-3800)	17.4 (9.5-75.3)	
Ferritin (ng/ml)				[c]0.587
Mean±SD	77.00±86.25	77.81±80.78	76.41±91.13	
Median (Min-Max)	53 (6-435)	52 (8-400)	54 (6-435)	
Vitamin B12 (pg/ml)				[b]0.919
Mean±SD	426.36±155.52	424.20±164,21	428.11±150.39	
Median (Min-Max)	409 (161-828)	388 (161-828)	448 (181-755)	
Folic Asid (ng/ml)				[b]0.666
Mean±SD	8.29±4.26	8.54±4.21	8.08±4.35	
Median (Min-Max)	7.7 (1.7-20)	8.2 (2.6-18.7)	7.7 (1.7-20)	
<4 mcg/L	12 (18.2)	5 (17.2)	7 (18.9)	
≥4 mcg/L	54 (81.8)	24 (82.8)	30 (81.1)	
Vitamin D - (25 Hydroxy) (ng/ml)				[c]0.004**
Mean±SD	24.69±10.23	21.30±9.92	27.43±9.75	
Median (Min-Max)	22 (6-62)	20.5 (6-47)	25 (14-62)	
<10 ng/mL	2 (3.0)	2 (6.7)	0 (0)	
10-19 ng/mL	19 (28.4)	12 (40.0)	7 (18.9)	
20-62 ng/mL	46 (68.7)	16 (53.3)	30 (81.1)	

aPearson Chi-Square Test, bStudent T Test, cMann Whitney U Test, dFisher Freeman Halton Test, **p<0.01, *p<0.05

Table 3. Diagnostic screening tests and ROC curve results for anti-thyroglobulin, anti-TPO, and vitamin-D

	Diagnostic Scan					ROC Curve		p
	Cut off	Sensitivite	Spesifisite	Positive predictive value	Negative predictive value	Area	95% confidence interval	
Anti-thyroglobulin	≥20.1	92.86	83.78	81.20	93.90	0.925	0.855-0.996	0.001**
Anti-TPO	≥22.1	96.67	100	100	97.40	0.997	0.989-1.000	0.001**
Vitamin-D	≤22	70.00	64.86	61.80	72.70	0.706	0.578-0,835	0.004**

**p<0.01, r: Spearman Correlation Coefficient, **p<0,01

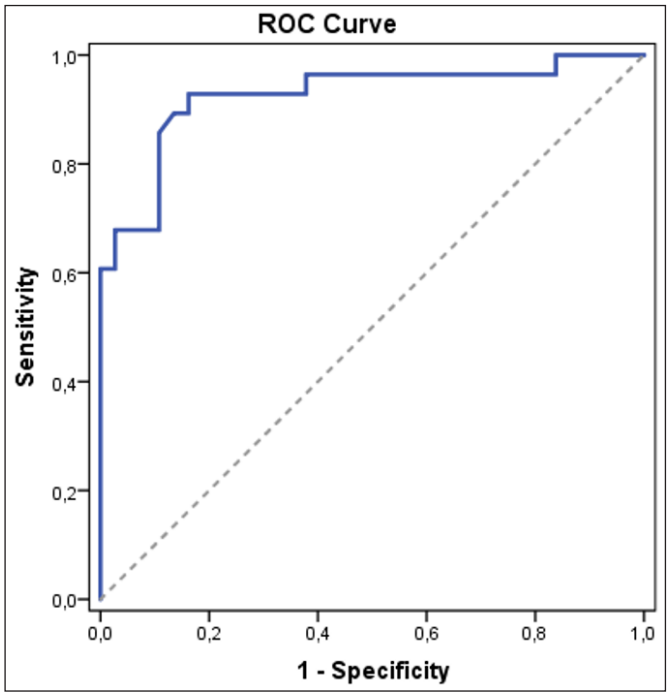

Figure 4. ROC curve for anti-thyroglobulin in predicting disease

For the 22.1 cut-off value of the anti-TPO level, sensitivity is 96.67%; specificity is 100%; positive predictive value is 100%, and negative predictive value is 97.4%. In the obtained ROC curve (Figure 5), the area under it was determined as 99.7%, with a standard error of 0.3%.

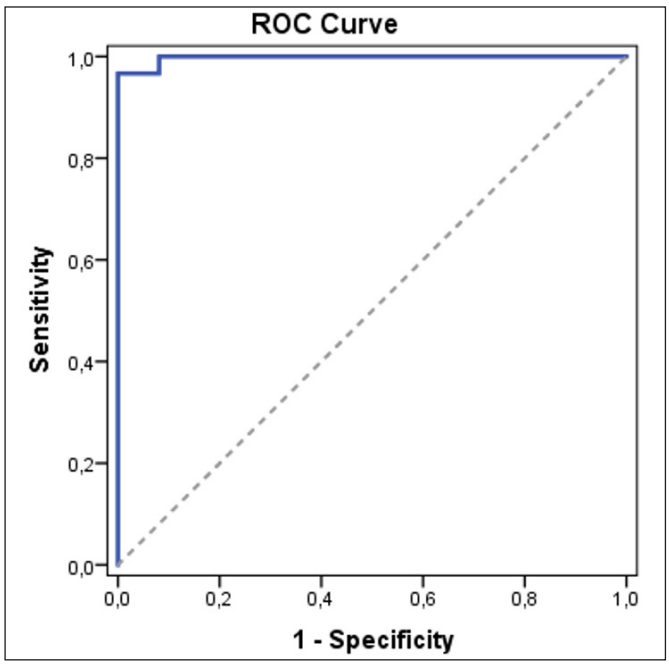

Figure 5. ROC curve for anti-TPO in predicting disease

A statistically significant correlation was found with the 22.1 cut-off value of the Anti-TPO value in predicting the disease (p=0.001; p<0.01).

For the 22 cut-off values of the Vit-D level, sensitivity is 70%; specificity 64.86%; positive predictive value is 61.80%, and negative predictive value is 72.70%. The area under the ROC curve (Figure 6) was determined as 70.6%, with a standard error of 6.5%. A statistically significant correlation was found with the 22 cut-off values of the Anti-Thyroglobulin level in predicting the disease (p=0.004; p<0.01).

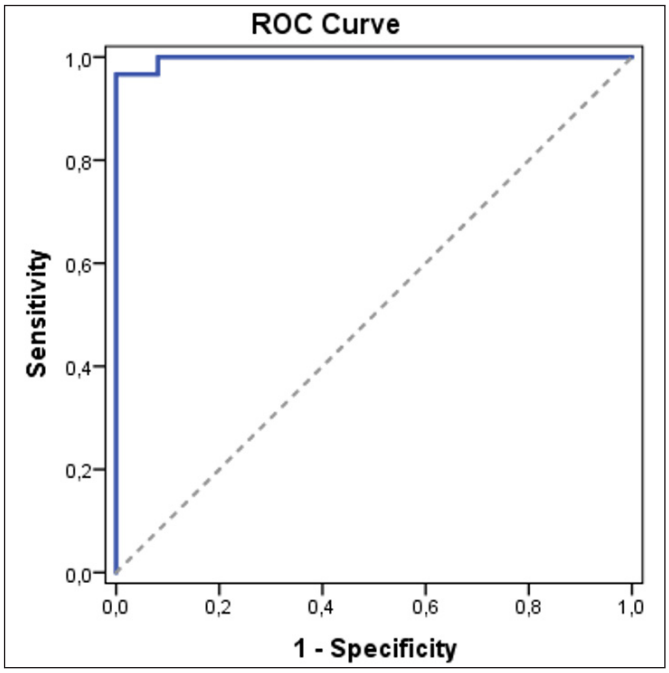

Figure 6. ROC curve for anti-TPO in predicting disease

A statistically significant weak correlation (Table 4) was found between the Vit-D measurements of the subjects and the Anti-TPO measurements (Figure 7), with a negative direction (as the Vit-D value increases, the Anti-TPO value decreases) (r=-0.341; p=0.005). ; p<0.01).

Table 4. Relationship between Vitamin D and Anti-TPO and Anti-Thyroglobulin

	Vitamin-D	
	r	P
Anti-TPO	-0.341	0.005**
Anti-thyroglobulin	-0.417	0.001**

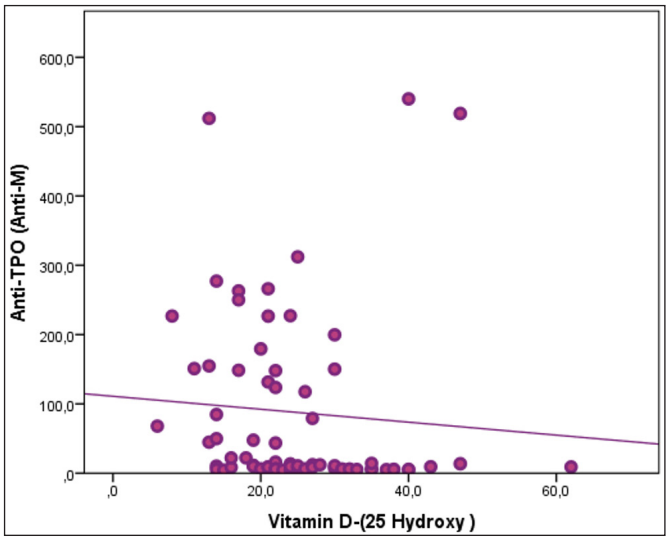

Figure 7. Relationship graph of vitamin D and anti-TPO

A statistically significant moderate correlation was found between the Vit-D measurements of the cases and the Anti-Thyroglobulin measurements (Figure 8), in the negative direction (as the Vit-D value increases, the Anti-Thyroglobulin value decreases) (r=-0.417; p=0.001; p< 0.01).

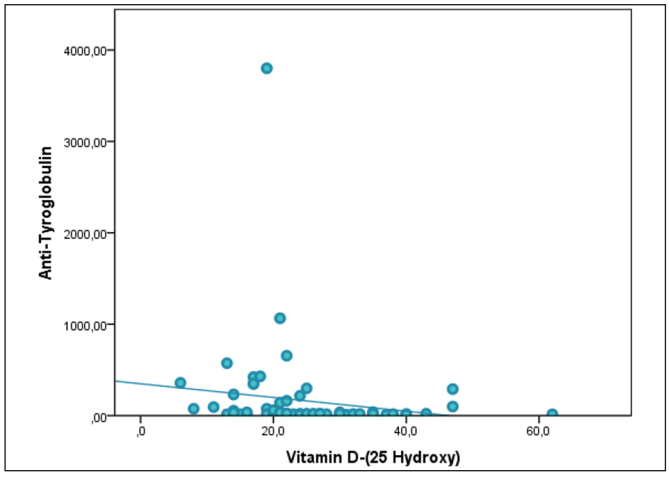

Figure 8. Relationship graph of Vitamin D and Anti-Thyroglobulin

DISCUSSION

As accepted in the literature, HT is recognized as the most common autoimmune disease worldwide. The susceptibility to HT is influenced by various factors, including genetic predispositions, environmental exposures, epigenetic factors, and immune system dysregulation.[4,5] Literature suggests that high iodine intake, iodine deficiency, selenium deficiency, as well as deficiencies in vitamin B12, iron, and vitamin D, can increase the risk of HT.[10,11] In this study, we aimed to investigate the relationship between HT severity and important factors in its pathogenesis, between vitamin D, iron, and vitamin B12.

Numerous studies have demonstrated the association between hypothyroidism and vitamin D deficiency in patients with HT. One study reported vitamin D deficiency in 96% of HT patients, with severe deficiency (serum levels <10 ng/mL) observed in 56% of these individuals. Furthermore, this study revealed a negative correlation between anti-thyroid peroxidase (anti-TPO) levels and vitamin D, suggesting that vitamin D deficiency may play a crucial role in autoimmune hypothyroidism.[8]

In another study investigating the effects of vitamin D supplementation in HT patients, 34 female individuals diagnosed with HT and being treated with levothyroxine for at least six months were included. These participants had normal vitamin D levels (serum 25-hydroxyvitamin D levels above 30 ng/mL). The study compared vitamin D levels after six months in two groups: one group received vitamin D supplements (n=18) and the other group did not receive vitamin D supplements (n=12). Results showed that serum vitamin D levels and titers of thyroid antibodies (anti-thyroglobulin and anti-TPO) decreased in the group that received vitamin D supplements. This indicates an inverse relationship between serum vitamin D levels and thyroid antibody titers.[10]

Although it is believed that there is an association between HT risk and vitamin D, viral infections involved in the pathogenesis of HT can lead to damage to vitamin D receptors (VDR) and reduce the utilization of vitamin D.[11] Similar findings were observed in our study. When comparing vitamin D levels between 30 patients with Hashimoto's and 37 patients without a Hashimoto's diagnosis, a significant difference in vitamin D levels was found. Our study also revealed results consistent with other studies, showing a negative correlation between vitamin D levels and anti-thyroglobulin (as vitamin D levels increase, anti-thyroglobulin levels decrease) (r= -0.417; p=0.001; p<0.01).

Furthermore, a statistically significant weak correlation was found between vitamin D and anti-thyroid peroxidase (anti-TPO) measurements (r=-0.341; p=0.005; p<0.01) (8). Considering the relationship between iron deficiency and HT, it is known that iron deficiency can decrease the levels of T3 and T4 in circulation and reduce the peripheral conversion of T4 to T3 due to the role of iron metabolism in the synthesis and metabolism of thyroid hormones.[12]

In a study examining the frequency of iron deficiency in patients with positive thyroid-specific antibodies, significant decreases were observed in hemoglobin, hematocrit, mean corpuscular volume (MCV), ferritin, iron, and transferrin saturation in patients positive for anti-TPO and anti-thyroglobulin compared to the control group. Additionally, a significant correlation was found between anti-TPO levels and serum iron, transferrin saturation, and ferritin values.[13]

However, in our study, no significant differences were found in hemogram and ferritin measurements between patients with and without a diagnosis of HT (p>0.05). Furthermore, since the HT patients in our study were under treatment and their thyroid function (FT3, FT4, and TSH measurements) was being monitored, no statistically significant differences were observed in these parameters (p>0.05).

Despite the availability of various diagnostic methods, the cytomorphological features observed in fine needle aspiration cytology (FNAC) smears remain the gold standard for HT diagnosis. HT is a common cause of hypothyroidism in women.[8]

An accurate cytological diagnosis can prevent the need for surgical intervention. However, a multidisciplinary approach that includes clinical, radiological, biochemical and cytological parameters should be used to detect subclinical hypothyroidism and guide treatment.[8,14]

The study showed that the incidence of Vit-B12 deficiency is 46% in patients with autoimmune hypothyroidism, and there is a negative correlation between Anti-TPO and Vit-B12 levels. Therefore, pernicious anaemia accompanied by autoimmune diseases or atrophic gastritis may be likely.

In a study, a relationship was found between Vitamin D deficiency, defined as 25(OH)D <10 ng/mL (~25 nmol/L), and the presence of thyroid antibodies, indicating a higher frequency of autoimmune thyroid disease, particularly HT.[16] Another study reported lower 25(OH)D levels in individuals with autoimmune thyroid disease.[17] These consistent findings once again highlight the importance of treating Vitamin D deficiency alongside HT in the management of patients.[18-20]

The significance of epigenetic mechanisms in autoimmune diseases is increasingly understood in current research. Addressing the negative impact of epigenetic changes in HT, which arises from an imbalance in immune response, necessitates restoring the balance of B12 and folic acid and achieving optimal Vitamin D levels, which play crucial roles in epigenetic mechanisms. The effect of epigenetic mechanisms and changes should not be disregarded, considering individual variations in the methylation balance of each patient. The objective of this study is to underscore the comprehensive evaluation of these vitamin and mineral deficiencies in order to reverse the adverse effects of epigenetic mechanisms and enhance the patient's clinical presentation.

In most autoimmune thyroid studies and publications, the effects of values such as Vitamin D, B12, folic acid, and anemia have been examined separately. However, in this investigation, we believe that epigenetic factors should be evaluated as a whole mechanism, and all these values should be considered together. We emphasize the importance of examining them collectively to demonstrate the comprehensive factors involved.

Molecular docking simulations can yield promising results when a high-resolution X-ray crystal structure of the target macromolecule is available.[21-23] Therefore, in the present study, molecular docking was also conducted. Previous evidence has linked HT with mitogen-activated protein kinase 1 (MAPK).[24] A recent study using network pharmacology demonstrated that MAPK is one of the key targets in treating HT.[25] This study also reported strong interactions between compounds for HT disease and MAPK1 proteins through molecular docking. Consequently, in our study, extracellular signal-regulated kinase 2 (ERK2), which belongs to the MAPK family, was selected as the primary target. To our knowledge, no literature has reported the interaction of folic acid with ERK2.

As an internal validation, the native ligand was initially docked into the binding region of ERK2. The resulting binding pose was compared with the X-ray crystal conformation, yielding a root-mean-square deviation (RMSD) value of 0.35, indicating the successful performance of the selected docking method. Docking of folic acid into the binding pocket of ERK2 produced a high score very close to that of the native ligand (-9.16 vs. -10.18 kcal/mol). As shown in **Figure 1**, folic acid interacts with ERK2 through multiple hydrogen bonds and salt bridges. LYS54 appears to be the common amino acid residue that both folic acid and the native ligand interact with. Although the bulky size of folic acid might result in steric clashes with amino acid residues, the favorable interactions in the folic acid-ERK2 complex outweigh the native ligand. These overall interactions may account for the high docking score. The observed critical interactions can provide new insights for future studies aiming to design and optimize novel therapeutic agents against HT.

While many studies have evaluated epigenetic modifications in autoimmune thyroid patients, few have investigated epigenetic modifications associated with early disease diagnosis, treatment outcomes, and the risk of recurrence during follow-up. A better understanding of these epigenetic changes can contribute to the correct diagnosis of autoimmune thyroiditis, guide appropriate treatment approaches, and yield accurate results.

Low Vitamin D levels are closely related to activated autoimmune diseases such as HT and autoimmune hepatitis. Furthermore, Vitamin D exerts an important effect on immunoregulatory cells, and deficiency is strongly associated with impaired suppressor activity of immunoregulatory cells, leading to enhanced inflammation and increased autoimmunity in autoimmune diseases.[26]

CONCLUSION

We aimed to highlight the significance of addressing vitamin D deficiency in the treatment of patients with HT and its importance in the overall management of HT.

Based on molecular docking simulations, folic acid showed strong affinity towards ERK2, which is known to play a key role in HT. This finding suggests the potential for designing novel therapeutic agents targeting ERK2 in the treatment of HT.

One limitation of our study is the relatively small number of patients, and further analysis with larger sample sizes across multiple centers is necessary to confirm the correlation and validate the docking studies. Therefore, the initial findings presented here provide a basis for conducting future clinical studies with a larger number of patients.

Furthermore, additional research in this field could contribute to a better understanding of the pathogenesis of the disease, potentially leading to the development of diagnostic and prognostic tools. Moreover, investigating the environmental and epigenetic factors involved in HT can shed light on their interplay with the disease and provide valuable insights for patient management.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Biruni University Clinical Researches Ethics Committee (Date: 09.04.2021, Decision No: 2021/50-40).

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

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The use of fetal bovine acellular dermal matrix for management of chronic wounds

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ABSTRACT

Aims: In the treatment of chronic wounds, tissue growth must be addressed and optimized. The purpose of this study is to investigate the use of the regenerative medicinal product Fetal Bovine Acellular Dermal Matrix (FBADM) in chronic wounds.

Methods: The patients were chosen sporadically and randomly based on availability to FBADM. Patients were assessed for adequate perfusion, debridement was performed, and wounds were ensured to be free of infection. FBADM was placed in the wound bed covered with a non-adherent contact layer, and a hydrogel sheet was placed to maintain adequate moisture. Offloading or compression was used as clinically indicated. Patients were then followed weekly. Digital photography was used to visually document healing progress.

Results: After 1-3 weeks wounds managed with FBADM had improved characteristics and healthy vascularized tissue that subsequently epithelialized from wound margins or grafted with split thickness skin grafts. Of the 14 wounds we achieved 69% complete healing, 24% non-complete healing after 12 weeks of FBADM application. 7% of the wounds needed skin graft surgery.

Conclusion: We found FBADM to be useful for treatment regimen of diabetic foot and leg ulcers, venous leg ulcers, surgical wounds, and wounds being prepared for skin grafting.

Keywords: Chronic wounds, extracellular matrix, tissue engineering, skin substitutes

INTRODUCTION

FBADM is a dermal repair scaffold composed of natural dermal collagen fibers. The use of FBADM has been reported to promote healing of a range of wound types. The purpose of this study is to evaluate clinical usefulness and gain experience with FBADM in chronic recalcitrant wounds. A retrospective study of thirteen complex patients with difficult to heal full-thickness 14 chronic wounds is presented. Subjects have been retrospectively investigated through their charts.

In order to understand wound healing, it is necessary to master the healing phases.[1,2] Not all wounds complete their healing phase completely or at all. Factors such as the etiology of the wound, infection status, vascular adequacy, medical or surgical intervention may play a role in this. Healing process is divided into five components of hemostasis, inflammation, proliferation, contraction and remodeling. Hemostasis begins as soon as the wound is formed, along with bleeding. Although vasoconstriction decrease the blood loss enough blood is released in the wound to stimulate Hageman factor (XII) to initiate the clotting cascade.[3] Blood fibrinogen converts to fibrin.[4] The fibrin cell forms a pathway for cell migration, primarily fibroblasts. Fibroblast is one of the major cells in the proliferative phase of wound healing.[5] Hemostasis also creates a protective layer, minimizing the risk of infection and creating the optimum environment for the subsequent healing phases. In the subsequent inflammatory phase, vasoconstriction is replaced by vasodilation. Vasodilation is the result of prostoglandin, nitric oxide and other inflammatory mediators in the environment. As plasma fluid fills the interstitial space, migration of white blood cells and diapedesis occur.[6] In the first 2 phases of wound healing, bleeding was stopped, the wound was debrided, and infection control was achieved. In wounds without a delay in healing, the proliferative phase should start after a few days. In this phase, proliferative cytokines (PDGF, interleukin1, fibroblast growth factor and chemoattractant (transforming growth factor beta) take part and prepare wound healing for the next phase by mobilizing fibroblasts and multiplying them.[7] The main

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effective cell in the contraction phase fibroblast more specifically is myofibroblast. It involves the reduction of the wound in size.[8] TGF b is very important in the natural process of the contraction phase. In remodeling phase the wound is characterized by a high level of metabolic activity. The remodeling phase is different from other phases. Hemostasis may take minutes, inflammation days, and proliferation weeks, the remodeling phase may continue for months or years. Initial mixture of Type1 and Type3 collagen density slowly changes into a wound with mainly Type1 collagen.[3]

This study aims to characterize the effectiveness of using regenerative medicinal product FBADM in chronic wounds, as well as to determine the ability of this matrix to facilitate normal tissue regeneration.

METHODS

A retrospective study of thirteen patients with recalcitrant chronic wounds treated in our advanced wound care center is presented. Subjects have been retrospectively evaluated. This study was initiated with the approval of the Clinical Researches Ethics Committee (Date:19.06.2015, North Carolina, Decision No: 00033880). All procedures were carried out in accordance with ethical rules and principles of the Declaration of Helsinki.

Patients with diabetic and venous wound etiologies were treated in our advanced wound care center. The patients were chosen sporadically and randomly based on availability to FBADM. This research is done with 13 subjects with 14 non-healing wounds those FBADM have been applied in our wound care center.

Patients were assessed for the inclusion and exclusion criteria listed below.

Inclusion criteria:

1. Be over 18 years old
2. Absence of infection according to American Society of Infectious Diseases criteria
3. Adequate vascular circulation (in the last 60 days)
   a. Ankle-brachial index between (ABI) 0.7-1.2

Exclusion criteria:

1. Be under 18 years old
2. Lack of adequate vascular circulation, ABI <0.7->1.2
3. Presence of infection according to American Society of Infectious Diseases criteria

FBADM was placed in the wound bed covered with a non-adherent contact layer, and a hydrogel sheet was placed to maintain adequate moisture. Offloading or compression was used as clinically indicated. Patients were then followed weekly. Demographics, wound etiology, wound dimensions, and associated comorbid conditions and complications were recorded. Digital photography was used to visually document healing progress.

Case 1: Post-Operative Dehiscence after Left Achilles Tendon Repair Subject

53 y\o white male with type 2 diabetes mellitus (DM) and peripheral venous insufficiency. PVI developed a wound over his left posterior Achilles after an Achilles tendon repair and heel spur resection surgery. The patient developed partial dehiscence over incision with exposed tendon. Sharp debridement was performed and post debridement measurements of wound were 5 cm×1.5 cm×1.2 cm with an area of 5.8 cm² (**Figure 1**). After one week of FBADM application the wound demonstrated healthy granulation tissue and had filled significantly. During the entire follow up time he was treated with multi layer compression wraps as well. At 8 weeks entire exposed tendon area was covered with granulation tissue (**Figure 2**). After 12 weeks the wound had totally reepithelialized (**Figure 3**).

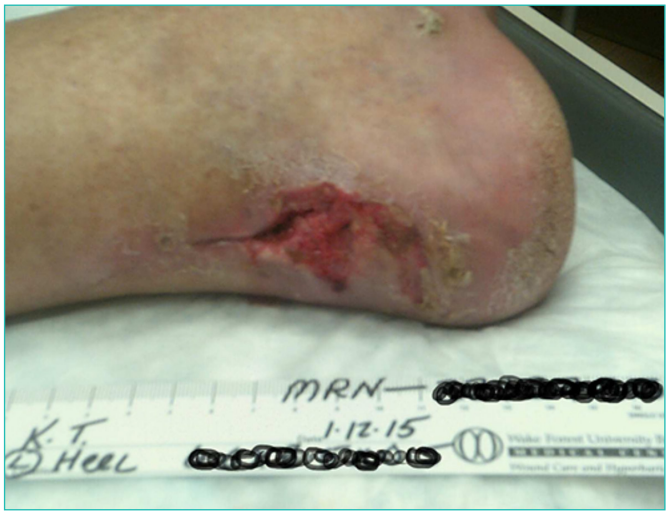


Figure 1. Wound presentation

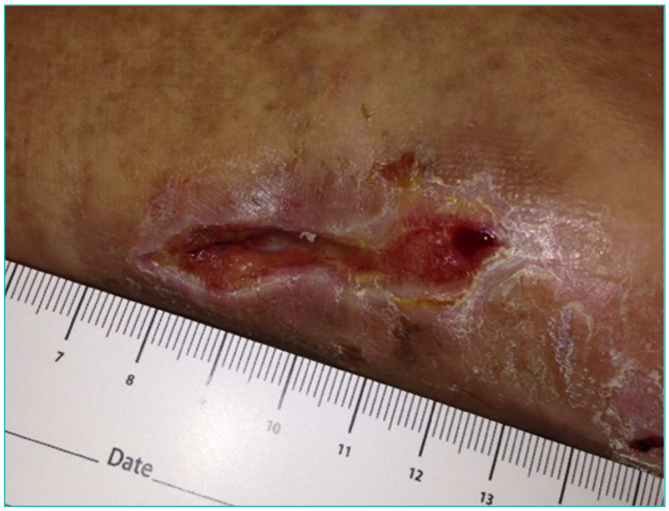


Figure 2. 8 weeks after FBADM application

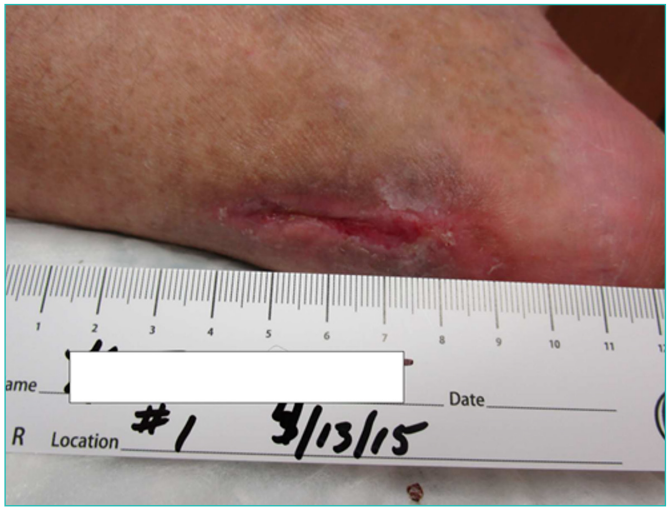


Figure 3. 12 weeks, complete re-epithelization

Case 2: Mixed Chronic Diabetic and Venous Leg Ulcer

41 y/o male with type 2 DM, morbid obesity, (Body Mass Index:38), presents with multiple wounds on both legs. Wounds have been present for the past 5 months. The patient has never received wound care but dry dressings. We decided to apply FBADM to the largest wound which located on left leg posterior which is filled with fibrotic tissue without any granulation, measured as 6.5cm x 11.5 cm × 0.4 cm with an area of 58.7 cm² (Figure 4). At one week after application (Figure 5) there was significant improvement in wound bed quality, healthy granulation, partially integrated FBADM and advancing epithelium at wound edges. Figure 6 (6 weeks) and Figure 7 (9 weeks) show rapid spontaneus epithelium advancing over wound bed from all edges. Patient had sporadic follow up due to personal issues he self reported the wound has healed approximately after 16 weeks. He applied to the wound care center after 24 weeks, the wound was totally healed with immature but intact epithelium (Figure 8). At 28 weeks wound was with mature epithelium, good quality healed skin (Figure 9).

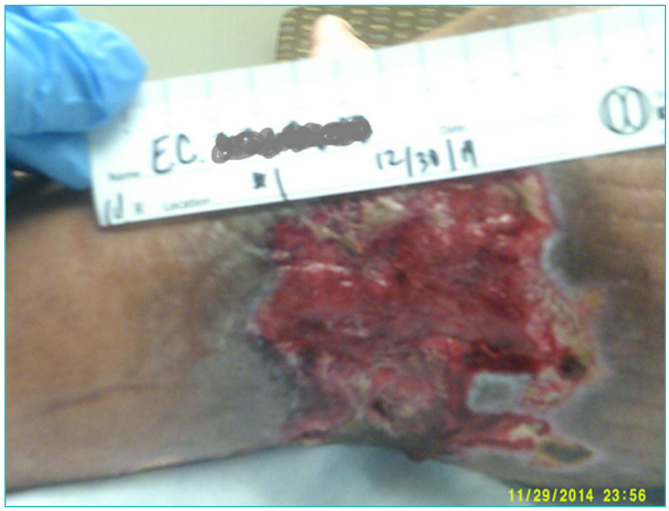


Figure 4. Wound presentation

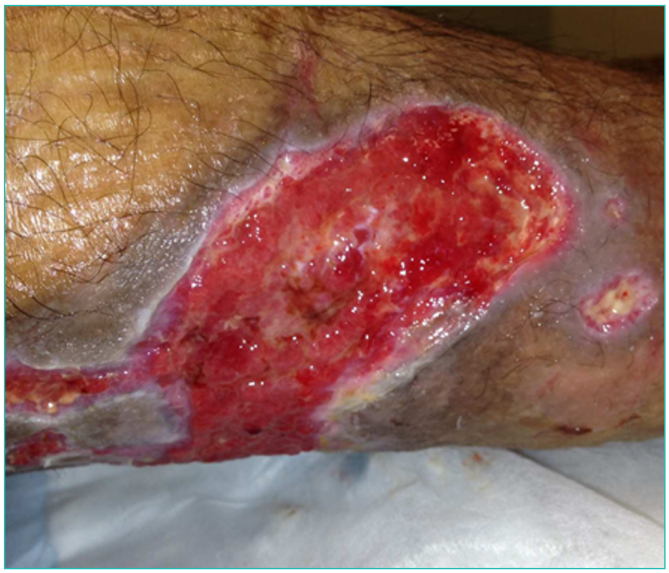


Figure 5. 1 week after FBADM application

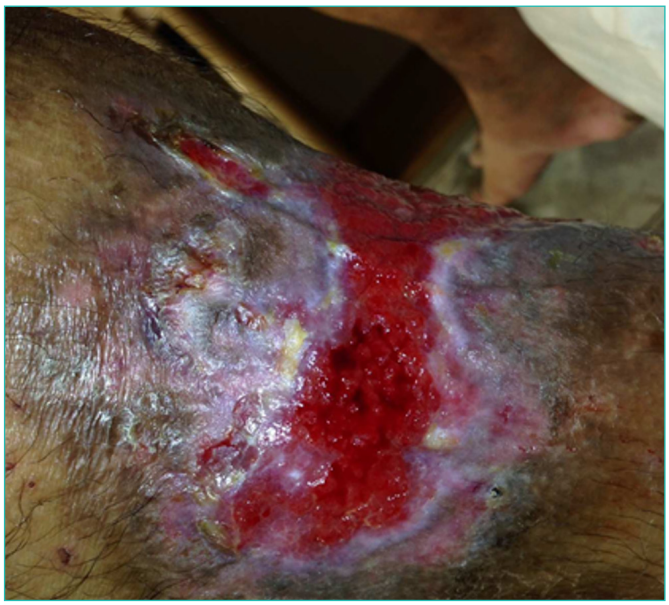


Figure 6. 6 weeks

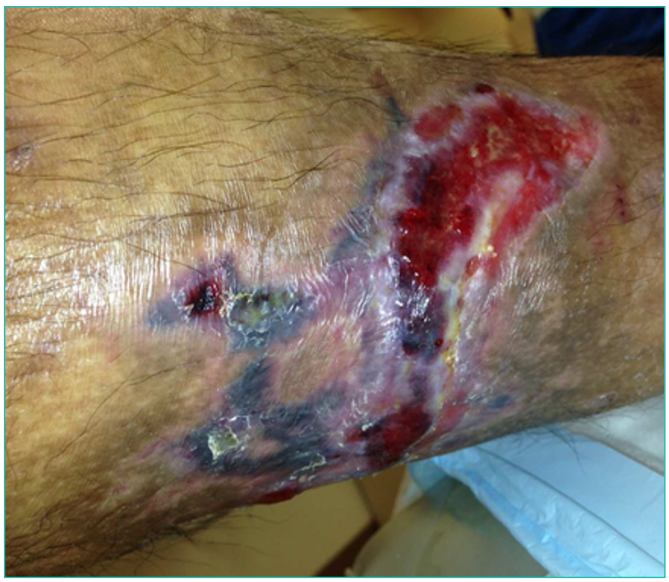


Figure 7. 9 weeks

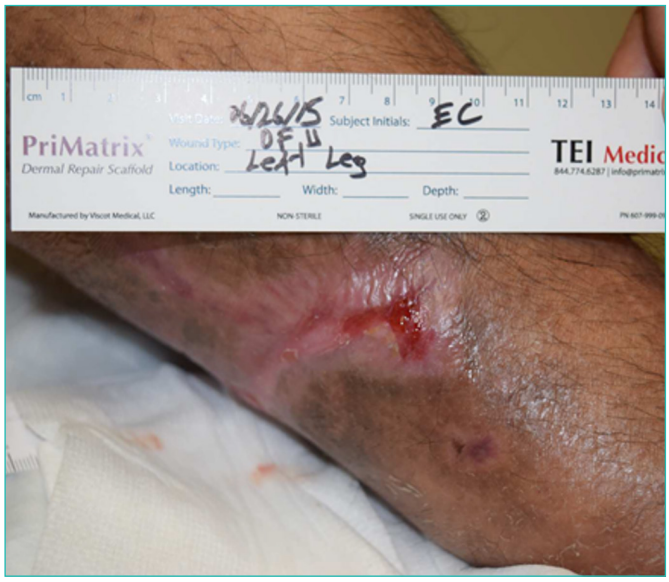


Figure 8. 24 weeks

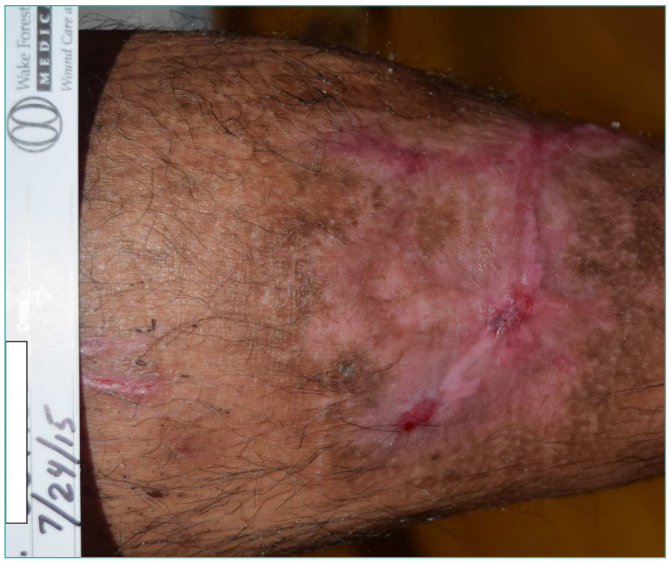


Figure 9. 28 weeks

Sharp debridement was performed and FBADM was applied along with four layer compression bandage. Wound area has reduced to 25.7 cm² and 0.2 cm² at week 4 and week 12 respectively without any complication.

RESULTS

Of the 13 patients, 69% were male, 31% female. 61% were white, 30% African-American, 9% Hispanic. Average body mass index (BMI) was 35.3, and median age was 57 (41 -77). Etiology was also varied, included diabetic foot or leg ulcers (46%), venous leg ulcers (peripheral vein insufficiency – PVI), 2 ulcers (15%) had mixed diabetic and venous insufficiency, other 2 ulcers (15%) had mixed venous and lymphedema etiology, 1 ulcer was in context of sickle cell disease and venous insufficiency and 1 ulcer was caused by scleroderma and venous insufficiency (Table 1).

Table 1. Subject demographics

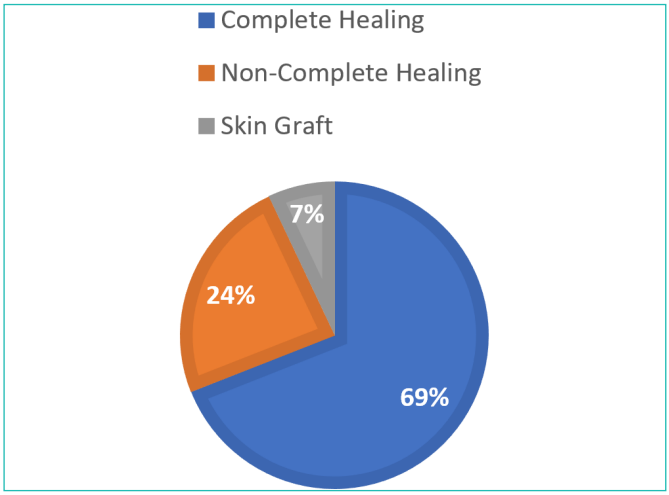
Subjects	13
Age	57 +/- 9.7 (41-77)
BMI	35.3 +/- 2.6 (21-53)
Ethnicity	
Caucasian	61%
Black	30%
Hispanic	9%
Gender	
Male	59%
Female	31%
Etiology	
DM	46%
PVI	38%
DM + PVI	15%
Lymphedema + PVI	15%
Sickle cell disease	7%
Scleroderma + PVI	7%

Wound chronicity was average 546 days. Including one of the patients had chronic ulcer of approximately 4 years (~1400 days) Excluding this patient, average wound chronicity was 73 days. Average ulcer size was 10.1 +/- 4.3 sq. cm (Table 2).

Table 2. Wound characteristics

Wound age (days)	546±159
Wound area (cm²)	10.1±5.3

After 1-3 weeks wounds managed with FBADM had improved characteristics and healthy vascularized tissue that subsequently epithelized from wound margins or grafted with split thickness skin grafts. 10 ulcers (69%) had complete healing with spontaneous epithelization in the follow up period until 12 weeks. One ulcer had improved wound bed characteristics becoming candidate for split thickness skin grafting at 4 weeks, and although the skin graft did not have 100% take after surgery, the ulcer had healed in the following 6 weeks with wound care. This patient had a previously completely failed attempt at split thickness skin grafting. One patient (7%) had some improvement, but non-complete healing. One patient (7%) had no change and one (7%) had some increase in wound size (worsening) during the 12 weeks follow up period (Graph 1).



Graph 1. Treatment results

DISCUSSION

Wound occurs as a result of deterioration of skin and soft tissue integrity by many mechanisms. Among the causes of chronic wound are factors such as insufficient angiogenesis, insufficient cell migration and insufficient innervation that interrupt the physiological healing process.[9,10]

There is always a risk of recurrence in healed chronic wounds, especially diabetic foot ulcers.[11] Extracellular matrix (ECM) is very important in wound healing. It provides structural support and is the largest dermal layer.[12] In chronic wounds, the ECM is often insufficient. Application of ADM in chronic wounds can be an alternative to ECM.[13,14] Since there is no cellular component in ADM, it does not produce an immunological response.[15] ADM creates a favorable environment for cellular proliferation and vascularization.[16] As a result of the contact of the ADM with the surrounding tissue, epithelialization and the formation of healthy granulation tissue can be triggered.[17] It is predicted that extracellular matrix equivalents such as ADM are effective in comorbid patients with complex non-healing wounds,[18,19] and will shorten the recovery time and increase the rate of recovery, and decrease the percentage of amputation, especially in patients with chronic neuropathic ulcers, compared to standard treatment.[20]

Tissue engineering aim is to produce the product that will either increase the existing organ function or replace it by passing the materials through mechanical and chemical processes. Wound healing process, is a dynamic process in which the communication and relationships of intercellular, ECM and growth factors are effective after the disruption of tissue integrity.[21] ECM consists of three-dimensional space with structural proteins, laminins, proteoglycans, hyaluronic acid, collagens, fibronectins and elastins. In addition to being a structural support for cells, ECM binds to growth factors and is a reservoir for active molecules that are effective in cell proliferation and migration after loss of tissue integrity.[22] In many chronic wounds, the increased number of inflammatory cells causes an increase in protease levels and breaks down and reduces ECM components, such as growth factors and proteins, which are essential in the healing process.[23]

During ADM construction, all living cells are removed. These matrices or scaffolds provide a collagen structure for tissue remodeling. The purpose of removing viable cells is to prevent an inflammatory or immunological response.[24] We can define the function of acellular matrices as a biological modulator that affects the biological process of wound healing.

ADM prepares a bed for cell growth and granulation tissue formation, contains receptors for fibroblasts to attach to tissue support, and stimulates angiogenesis. It contains and protects growth factors.[25] When implanted, the ADM must be fully inserted into the wound. It is thought that ADMs provide normal wound healing by forming a biological cover.[26,27] FBADM is rich in collagen type 3. This type of collagen has important role in proliferative stage of wound healing and in stimulating tissue regeneration processes. It is not denatured or cross-linked during manufacturing process.[28]

In recent years, a wide range of skin substitutes has been developed. These products have been largely used as a reconstructive option for skin loss and defects in chronic wounds. Although an optimal skin substitute is not yet available, these products address the various challenges of wound healing.[29]

FBADM remains morphologically identical to natural tissue structure, which provides a preferred environment for cellular migration and proliferation. The mechanical property of this matrix is similar to the adjacent environment.[30] The main advantage of FBADM is represented by the lack of morbidity of the donor areas, less surgical time related to the procedure, and faster rehabilitation. These innovative biomaterials provide easier and less stressful possibilities for non healing wounds.[31] This ECM derived from decellularized fetal bovine dermis intended for the treatment of non healing ulcers, second-degree burns, and surgical wounds, rich in type III collagen, which is the first type of collagen synthesized during both embryonic development and wound healing.[32,33] In addition to providing elasticity to the ECM, type III collagen has been shown to promote migration of fibroblasts,[34] and to be an essential regulator of ECM deposition and organization,[35] FBADM becomes incorporated into the wound and rapidly degrades and has shown success for the treatment of acute full-thickness wounds.[36] FBADM has several advantages relating to its source tissue and manufacturing process, which may have contributed to the limited inflammatory response.[37]

Despite all the FBADM benefits, complications such as hematoma, seroma, necrosis, and infection must be considered. Also, FBADM is an expensive product and this can make it unsuitable for many patients. More work should be done to achieve cheaper FBADM to make it a cost-effective choice. Another potential disadvantage of FBADM is its limited size options. The studies on FBADM is inadequate, and more research on the results of FBADM usage in chronic wounds is needed.

This study, included recalcitrant ulcers of various etiologies with overall satisfactory results. According to the findings FBADM can stimulate tissue regeneration and reset wound healing to allow progression through stage beyond the inflammatory phase where most of the wounds become chronic.

CONCLUSION

We found FBADM to be useful for treatment regimen of diabetic foot and leg ulcers, venous leg ulcers, surgical wounds, and wounds being prepared for skin grafting. Future comparative and prospective evaluations are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Wake Forest University Faculty of Medicine Clinical Researches Ethics Committee (Date: 19.06.2015, Decision No: 00033880)

Informed Consent: All patients signed and free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The interaction of dexamethasone with sugammadex and rocuronium during general anesthesia in rhinoplasty surgeries

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ABSTRACT

Aims: Sugammadex is a cyclodextrin specifically designed to reverse the action of rocuronium through encapsulation. Theoretically, it is possible that sugammadex can encapsulate cortisone. There have been conflicting results regarding clinical dexamethasone-sugammadex interactions in patients under general anesthesia. The primary outcome of the present study is to investigate any possible alteration in the efficacy of sugammadex as a reversal of rocuronium due to dexamethasone injection in rhinoplasty surgery. The secondary outcome is evaluation of clinical observation sugammadex in these groups of patients.

Methods: Adult patients with the American Society of Anesthesiology (ASA) II risk class undergoing rhinoplasty were included. All patients received standard general anesthesia with neuromuscular blockade using rocuronium. Patients were allocated to either the dexamethasone group or control group. The anesthesiologist measured the time interval between sugammadex injection and the recording of the 90% train of four. Additionally, the duration required for extubation after sugammadex injection was recorded. Finally, the signs of residual respiratory insufficiency and muscle weakness were checked in the post-anesthesia care unit until the 2nd-hour post-surgery.

Results: Sixty-one patients were enrolled in the study. The dexamethasone group included 30, and the control group included 31 patients. The comparison of demographic and surgical characteristics of these two groups showed no statistical difference. The duration required for extubation was higher in dexmethasone group compared to control group (p=0.001). The total rocuronium administration dose was higher in dexmethasone group (p=0.01). The time required for the recovery of the head, upper, and lower extremity lifting was longer in the dexamethasone group (p=0.001, 0.003, and 0.047, respectively).

Conclusion: The present study demonstrated an interaction between sugammadex and dexamethasone, which affected the reversal of neuromuscular blockade during rhinoplasty surgeries.

Keywords: Dexamethasone, reversal time, rocuronium, sugammadex

INTRODUCTION

It is important to terminate the effects of neuromuscular blocker (NMB) drugs that provide muscle relaxation, which is one of the components of general anesthesia. Sugammadex is a cyclodextrin in a circular structure consisting of eight glucose molecules and gammacyclodextrin designed to encapsulate rocuronium for terminating the effects of non-depolarizing NMB drugs, especially those with steroid structure.[1] It antagonizes the effect of rocuronium, which is a nondepolarizing blocker, in a very short time, regardless of duration and dose, and completely eliminates undesirable results such as prolonged effect or residual block.[2-5]

Dexamethasone shares the same steroidal ring as rocuronium, leading to a possible encapsulation from sugammadex. If dexamethasone is encapsulated by sugammadex, less sugammadex will be available to reverse the rocuronium, resulting in a longer turnaround time. Dexamethasone is one of the most widely used corticosteroids for the treatment of many clinical conditions such as laryngeal, cerebral and surgical edema, as well as in combination with analgesics for multimodal analgesia and for the prevention of postoperative nausea and vomiting (PONV).[6-9]

Rhinoplasty is one of the most common cosmetic surgical procedures performed under general anesthesia.[10] Edema and ecchymosis may develop after rhinoplasty surgery. The edema and ecchymosis around the eyes and nose lead to anxiety for the patients and physicians. In addition, the presence of ecchymosis may prolong the duration of social isolation and the absence of work after surgery.

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It was reported that dexamethasone administration in rhinoplasty significantly decreased eyelid edema and ecchymosis.[11]

Additionally, as part of the multi-modal analgesia, dexamethasone is combined with non-steroidal anti-inflammatory agents to prevent PONV and laryngeal edema.

The relationship between dexamethasone and sugammadex has been investigated in vitro,[12] and in vivo,[13,14] but conflicting results have emerged. In vitro, dexamethasone dose-dependent inhibition of the reversal process of rocuronium by sugammadex was observed in functionally innervated human muscle cells,[12] whereas some studies showed no effect in real clinical conditions.[13-15]

The aim of this study was to investigate whether dexamethasone caused any changes in the efficacy of sugammadex as a reversal of rocuronium in patients undergoing rhinoplasty. The secondary outcome is to evaluate the results of clinical observations and to contribute to the literature.

METHODS

The research protocol was approved by İstanbul Yeni Yüzyıl University Clinical Researches Ethics Committee (Date: 23.11.2016, Decision No:003). All study participants received detailed information about the study, and they all consented to enrollment in this study. All aspects of the study were conducted according to the Declaration of Helsinki.

The patients aged between 18 and 65 who were undergoing rhinoplasty at İstanbul Yeni Yüzyıl University Hospital Ear-Nose-Throat Surgery Clinic constituted the target population of this study. All patients had normal hematological and cardiopulmonary screening test results and gave written consent to participate in the study. Subsequently, we selected those with the American Society of Anesthesiology (ASA) scores I and II among these patients. Patients who went through a combined surgery, those with systemic arterial hypertension, anti-coagulative medication use, diabetes mellitus, neuromuscular disease, and a history of peptic ulcer treatment during the last five years were excluded. Additionally, patients who have been receiving corticosteroid treatment for other indications were excluded.

Standart anesthesia management was performed in all patients. However it was at the discretion of the anesthesiologist whether to use dexamethasone intraoperatively and this decision was not influenced or changed by the investigators. Thus the patients were allocated to either the dexamethasone group or control group per assigned anesthesiologist's decision.

Anesthesia Induction and Maintenance

All procedures were performed under general anesthesia. Standard anesthesia monitoring was established for every patient with non-invasive arterial blood pressure, electrocardiogram, pulse oximeter, and end-tidal capnography. In addition, a train-of-four (TOF)-Watch SX device (Organon Teknika BV, Boxtel, Netherlands) was used for neuromuscular conduction monitoring. The skin electrodes were placed over the ulnar nerve at the wrist of the arm.

Anesthesia was induced with 2 μg/kg fentanyl and 2mg/kg propofol. Rocuronium IV 0.6 mg/kg was administered, and the TOF value was allowed to reach 0 (TOF 0). After TOF 0, the patients were intubated. Subsequently, the peripheral nerve stimulator was switched to TOF mode (0.2 ms duration, 2Hz frequency) every 15 seconds. The number of thumb twitches recorded indicated the level of neuromuscular block. Patients were covered, and care was taken to stabilize the skin temperature of the thenar region at 32-34°C.

Anesthesia was maintained with 50% oxygen, 2% sevoflurane, and 0.25 μg/kg/min remifentanil IV infusion. During the surgery, when the TOF value was 25% (TOF25), one-fourth of the induction dose of rocuronium was repeated. At the end of the procedure, patients were left until TOF25 was reached. When TOF25 was reached, sugammadex [IV; 2 mg/kg (current body weight)] was administered. When the TOF values of all of the patients were 90% (TOF90), they were extubated. At the end of the surgery, all patients were administered 1 mg/kg tramadol IV combined with paracetamol 10mg/kg for postoperative pain management. Patients were transferred to the post-anesthesia care unit (PACU) and stayed there until a modified Aldrete score (MAS) of higher than 9 was reached.

Rhinoplasty Procedure

In all patients, a closed approach was used with an interseptocolumellar incision and resection of the depressor muscle of the nasal septum. A transcartilaginous bilateral incision of the alars 6 mm from the caudal margin of the lateral middle crura was performed. The cartilage was exposed for resection by dissection of the vestibular skin off the cartilage. Dieresis of the lower lateral cartilage with extension to the nasal dorsum was performed to expose the nasal structures. The perichondrium was opened by a single incision in the midline of the upper lateral cartilage, with the detachment of the perichondrium in continuity with the periosteum of the nasal bone. On the subperichondrial and subperiosteal plane under direct

sight, the osteocartilaginous hump was assessed. Bone resection was performed with a down-biting diamond rasp, and septal cartilage was resected with a scalpel blade. Resection of the caudal portion of the septum, a lateral osteotomy with low to low fracture, the closure of the mucosa with simple stitches of polyglactin 5.0, and application of a dressing with microporous tape were the final steps of the procedure.

Study Variables

The time between TOF25 and TOF90 was recorded in seconds (TOF25-90). Additionally, the time from the cessation of anesthetic gases until extubation, the time between the beginning of the surgical incision and the last skin suture (i.e. surgical duration) was also recorded. Also, upper and lower extremity and head lift times were included in the analysis.

Statistical Analysis

We considered a 15% increase in the time from sugammadex administration to TOF 90 to be clinically relevant. A power calculation with a power of 0.80 at a=0.05 indicated a minimum 26 patients for each group. Because a dropout rate of%15-20 was expected a total of 62 patients with 31 patients in each group were included in the study.

Descriptive statistics of the categorical variables were given as numbers or percentages; continuous variables were provided as means±standard deviation (SD) or as medians (minimum-maximum). The Chi-square test was used to evaluate categorical variables. In addition, the paired t-test or Wilcoxon signed-rank test was used to compare the means/medians of variables as appropriate.

RESULTS

A total of 62 patients were enrolled in the study. One patient in dexamethasone group was excluded from the analyses due to surgical complication. Finally, data from the remaining 61 patients (30 patients in dexamethasone group and 31 patients in control group) were analyses for the study.

The mean age of the patients in the dexamethasone group was 31±10.07 and in the control group 30.77±6.63 years. There was no significant difference among the groups regarding patient age (p=0.510). The mean body mass index of the dexamethasone group was 23.48±2.55. and 22.93 ±2.74 in the control group (p=0.425). The ASA score comparison between the two groups revealed no significant difference. The duration of the surgical procedure was 156.77±23.72 minutes in the dexamethasone group and 156.67±30.23 minutes in the control group (p=0.988) (Table 1).

Table 1. Demographic data and duration of surgeries in the study groups (Values are presented as mean±SD or number with percentage)

	Dexamethasone group (n=31)	Control group (n=30)	p value
Age (years)	31.55±10.07	30.77±5.63	0.510
Height (cm)	171.94±6.55	172.53±7.27	0.737
Weight (kg)	69.68±10.37	68.47±10.35	0.650
BMI (kg/m²)	23.48±2.55	22.93±2.74	0.425
ASA I/II	26 (83.9%) / 5 (16.1%)	30 ((100%) / 0 (0%)	
Duration of surgery (min)	156.77±23.72	156.67±30.32	0.988

Intraoperative additional rocuronium requirement was 25.64±12.09 mg in the dexamethasone group and 14.19±12.46 mg in the control group. The required dose for rocuronium was significantly higher in the dexamethasone group compared to the control group (p=0.001). Time to TOF 90 after sugammadex administration was 111.27±33.02 seconds in the dexamethasone group and 87.90±14.01 seconds in the control group. The difference was statistically significant between the groups (p=0.001). Additionally, the extubation time was 152.57±57.48 seconds in the dexamethasone group and 102.58±30.22 seconds in the control group (p=0.001) (Table 2).

Table 2. Intraoperative measurements of additional rocuronium doses, times of TOF 0.9 and extubation. (Values are presented as mean±SD)

	Dexamethasone group (n=31)	Control group (n=30)	p value
Additional rocuronium (mg)	25.64±12.09	14.19±12.46	0.001
Time to TOF 0.9 (sec)	111.27±33.02	87.90±14.01	0.001
Time to extubation (sec)	152.57±57.48	102.58±30.22	0.001

The duration of the head-lifting, upper-extremity, and lower-extremity lifting were measured. The time required for head lifting was 168.33±63.77 in the dexamethasone group and 114.68±41.71 minutes in the control group (p=0.001). Similarly, the time required for the upper and lower extremity lifting was longer in the dexamethasone group (p=0.003 and p=0.047, respectively) (Table 3).

Table 3. Timing of clinical entities (head, upper and lower extremity lifting) (Values are presented as mean±SD)

	Dexamethasone group	Control group	p value
Head-lift (min)	168.33±63.77	114.68±41.71	0.001
Upper extremity -lift (min)	99.68±27.41	128.83±42.76	0.003
Lower extremity -Lift (min)	151.67±55.39	128.65±27.20	0.047

DISCUSSION

The present study demonstrated dexamethasone given intravenously at a dose of 10 mg after induction of general anesthesia in rhinoplasty surgery delayed the reversal of neuromuscular blockage with sugammadex. In this study, a single dose of dexamethasone after induction of general anesthesia received for prophylaxis against postoperative nausea, vomiting, and edema.

It is well known that sugammadex is a modified cyclodextrin that exerts its action as a neuromuscular block reversal agent through encapsulation of the steroidal neuromuscular blocking agent molecules thereby preventing their action at the neuromuscular junction.[16] However, this structure may interact with other similar molecules to rocuronium as well, such as hormonic contraceptives, fucidic acid, flucloxacillin, toremifene, or steroids, leading to decreased availability of sugammadex to act with rocuronium when these substances are also available in plasma.[17]

The result of this effect might theoretically be a clinical decrease in sugammadex's action and a delay in the reversal process.

The early report by Zhang[18] documented that sugammadex affinity to rocuronium is very much higher than that to corticosteroids, however different doses of the drugs have not been well identified as well as the duration of exposure. Additionally, Zwiers et al.[17] have investigated in detail those interactions, between sugammadex and 300 commonly used drugs, using a pharmacokinetic–pharmacodynamic model. The study demonstrated that flucloxacillin, fucidic acid, and toremifene had a potential of displacement, whereas specifically for dexamethasone, no such effect was proven.

On the other hand, an in vitro study by Rezonja et al.[12] in innervated human muscle cells, showed that there was a dose-dependent inhibition of sugammadex's action by dexamethasone. Therefore, studies in clinical conditions under general anesthesia were necessary. Three clinical trial results to date have failed to prove an interaction between dexamethasone and sugammadex.

Buonanno et al.[13] investigated the interaction of dexamethasone and sugammadex in a retrospective manner, by analyzing data from 45 patients who received general anesthesia with rocuronium. Patients were divided into three groups, who received dexamethasone 8 mg shortly after induction, dexamethasone 8 mg just before reversal, or ondasentron 8 mg (control group). No significant difference was observed between the three groups as for time to reversal of rocuronium using sugammadex, 2 mg/kg at the end of the operation, at reappearance of T2.

Batisti and et al.[19] studied 44 patients who had undergone cholecystectomy. Patients were divided into two groups, who received dexamethasone 5 mg shortly after induction, or 5 ml plasebo (control group). Similarly, Gulec et al.[14] studied the effect of intravenous dexamethasone (0.5 mg/kg) versus placebo, on sugammadex's action, in 60 children (aged 3-8 years) undergoing elective tonsillectomy and/or adenoidectomy, in a prospective, randomized manner.

No significant difference was also observed regarding the time required from administration of sugammadex until reversal of neuromuscular function to TOF 0.9 the two in both articles. However, there are studies in the literature that say the opposite. Salih et al.[???] In their study on 80 patients who had undergone strabismus surgery, they divided the patients into two groups and gave 0.25 $mg.kg^{-1}$ metoclopramide to one group and 0.5 $mg.kg^{-1}$ dexamethasone to the other group after induction. They concluded that the interaction of sugammadex and dexamethasone in children aged 1-6 years may prolong the recovery time of rocuronium after general anesthesia.

Articles in the literature have produced different results. However, in our study, Rezonja et al.[12] A similar result was obtained in the in vitro experimental model of. One issue to be investigated is the dose and timing of administration of dexamethasone. Because there is no reliable way in the literature to determine what dose of dexamethasone is needed to prevent reversal of sugammadex.

One interesting result from our study was that patients who received dexamethasone at induction of anesthesia, required more rocuronium to maintain their deep neuromuscular blockade, and this was statistically significant. The possible explanation might be that dexamethasone may act by facilitating the impulse generating end of the motor end plate and also may act at the presynaptic membrane stimulating the release of acetylcholine.[20] Our study has a number of limitations. Concentration measurements of dexamethasone in at the time of sugammadex administration was not measured.

CONCLUSION

We can conclude from the present study that the interaction between sugammadex and dexamethasone may prolong the duration of reversal of rocuronium after general anesthesia in the rinoplasty. Further randomized clinical trials are needed to investigate the effects of the timing and dose of corticosteroids administered and their interactions with sugammadex.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Yeni Yüzyıl University Clinical Researches Ethics Committee (Date: 23.11.2016, Decision No:003).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Retrospective investigation of acute kidney injury in postoperative patients in ICU

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ABSTRACT

Aims: The development of acute kidney injury (AKI) in the postoperative period is associated with increased morbidity and mortality. This study aims to determine the incidence of postoperative acute kidney injury (AKI) and the factors affecting the development of AKI in the intensive care unit (ICU) and to evaluate the outcomes of the patients.

Methods: Postoperative patients hospitalized in the ICU between December 2021 and January 2023 were retrospectively analyzed, and 192 patients were included in the study. Kidney disease: patients with and without AKI were identified using the improving global outcomes (KDIGO) criteria.

Results: While 150 of the patients did not develop AKI (non-AKI group), 42 of them developed acute kidney injury (AKI group). The patients were operated on mostly by the orthopedics clinic (58.9%) and operated on at least by the urology clinic (2.1%) were taken to the intensive care unit. 39.6% of the patients underwent emergency surgery, and 60.4% underwent elective surgery. 57.1% of the AKI group and 34.7% of the non-AKI group had emergency surgery (p=0.008).

Conclusion: In our study, age, timing of surgery, use of diuretics, and use of vasopressors were found to be associated with the development of postoperative AKI. In addition, comorbid diseases such as diabetes mellitus, hypertension, coronary artery disease, and cerebrovascular disease have also been found to be associated with AKI. Mortality, length of stay in the intensive care unit, and need for mechanical ventilation (MV) were also higher in our postoperative intensive care patients who developed AKI than in patients who did not develop AKI.

Keywords: Intensive care, acute kidney injury, KDİGO, postoperative, mortality

INTRODUCTION

Acute kidney injury (AKI) is a common complication in patients undergoing major surgery and is associated with both short-term morbidity and mortality and long-term adverse outcomes such as the development of chronic kidney disease.[1] Kidney disease; defined as meeting the criteria for improving global outcomes (KDIGO) within seven days of surgical intervention.[2]

It is important to maintain hemodynamic stability in the perioperative period. All anesthesia techniques are associated with venodilation and intraoperative hypotension.[3,4] Not only blood loss but also preoperative fasting and systemic inflammation are associated with volume reduction and are important in the development of postoperative AKI.[5,6]

Comorbid diseases, and the surgery itself, especially emergency and major surgery in critical patients, are associated with a high incidence of AKI. Nephrotoxic drugs, contrast agents, and diuretics are widely used in the perioperative period and are responsible for a significant portion of AKI.[7]

Careful selection and use of perioperative fluids and vasopressors and appropriate blood management are important to prevent AKI. We aim to investigate the frequency incidence of postoperative AKI after major non-cardiac surgery and the affecting factors in patients with previously normal renal functions.

METHODS

The study was carried out with the permission of Afyonkarahisar Health Sciences University Faculty of Medicine Ethics Committee (Date: 04.11.2022, Decision No: 14). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.This study was conducted retrospectively between December 2021 - January 2023. The clinical data of patients ≥18 years of age who were admitted to the intensive care unit (ICU) postoperatively were analyzed

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retrospectively, and 192 patients were included in the study. Patients with missing data, patients with chronic kidney insufficiency and kidney transplant, patients with sepsis, multi-trauma, and crush syndrome, and patients who were in ICU for less than 48 hours were excluded. All postoperative patients in anaesthesia intensive care unit were included in the study. Since our hospital has branch intensive care units of general surgery, neurosurgery and cardiovascular surgery departments, patients hospitalised in these departments were not included. The flow chart of the patients included in the study is shown in Figure 1. Age, gender, comorbidities, type and timing of surgery (elective-emergency), length of stay in the intensive care unit, and mechanical ventilation (MV) data of all patients included in the study were recorded. We defined and staged AKI according to KDIGO serum creatinine criteria. According to KDIGO, AKI is defined as an increase of ≥0.3 mg/dl in serum creatinine within 48 hours or a 1.5-fold increase in serum creatinine from baseline within seven days or urine output of <0.5 ml/kg/h in the last 6 hours.[8] Postoperative acute kidney injury is defined as AKI occurring within 7 days of an operative intervention using the Kidney Disease Improving Global Outcomes (KDIGO) definition of AKI.[5] In our patients, no classification was made regarding the day on which AKI developed in the first 7 days. The baseline creatinine value was taken as the last value in the last year available in the pre-hospital system. The patients were divided into two groups, as developed AKI and not developed AKI (non-AKI), by the KDIGO classification. The patients were compared according to their clinical features, medications, comorbidities, intensive care and mechanical ventilation durations, and mortality.

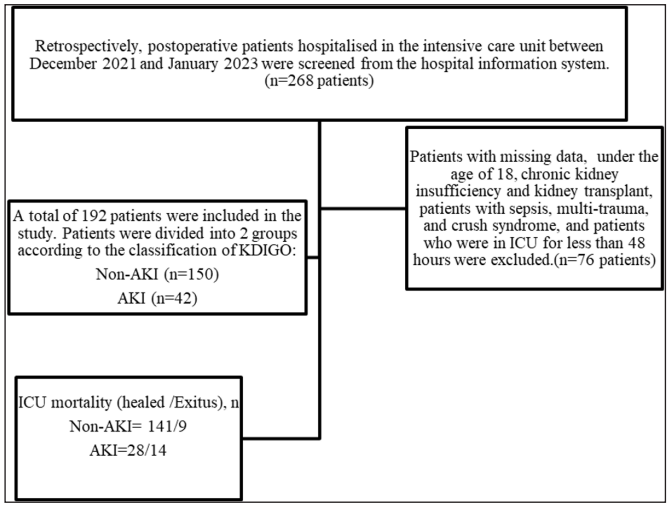

Figure 1. Flow chart shows the patient selection process

Statistical Analysis

IBM SPSS Statistics v.20 was used for statistical analysis. Data were expressed as a ratio, median (min-max), mean±SD, and conformity of variables to normal distribution was determined by visual (histogram) and analytical methods (Kolmogorov-Smirnov test). Student T or Mann-Whitney U tests were used to compare continuous variables, and the Chi-square test was used to compare categorical variables. P values of <0.05 were considered statistically significant.

RESULTS

A total of 192 patients, 92 women and 100 men, were included in the study. While 150 of the patients did not develop AKI (non-AKI group), 42 (21.9%) developed AKI (AKI group). While there was no statistically significant difference between the groups in terms of gender (p=0.149), a significant difference was found regarding age (p=0.006). If we evaluate the clinics where the patients were operated, they were operated mainly by the orthopedics clinic (58.9%) and the least operated by the urology clinic (2.1%). 39.6% of the patients underwent emergency surgery, and 60.4% underwent elective surgery. Emergency surgery was performed in 39.6% and elective surgery in 60.4% of the patients. While 57.1% of the group who developed AKI underwent emergency surgery, 42.9% had elective surgery, 34.7% of the non-AKI group underwent emergency surgery, and 65.3% had elective surgery, and there was a significant difference between the two groups (p=0.008) (Table 1).

Table 1. Demographic data of the patients

	AKI (n=42)	Non-AKI (n=150)	Total (192)	p
Gender F/M	16 (38.1) / 26 (61.9)	76 (50.7)/ 74 (49.3)	92 (47.9)/ 100 (52.1)	0.149*
Age, years, median (min-max)	72 (48-98)	67 (18-97)	68.50 (18-98)	0.006#
Surgical department, n(%)				
Orthopedics	26 (61.9)	87 (58)	113 (58.9)	
Thoracic surgery	3 (7.1)	21 (14)	24 (12.5)	
General surgery	7 (16.7)	18 (12)	25 (13)	
Ear, noseandthroat	3 (7.1)	12 (8)	15 (7.8)	0.072*
Plastic surgery	0	3 (2)	3 (1.6)	
Brain surgery	0	8 (5.3)	8 (4.2)	
Urology	3 (7.1)	1(0.7)	4 (2.1)	
Surgical status, emergency/elective	24 (57.1)/ 18 (42.9)	52 (34.7)/ 98 (65.3)	76 (39.6)/ 116 (60.4)	0.008#

Data are given as the number of patients (%), and median (minimum-maximum). #Mann Whitney U, *Chi-Square

The patients' additional disease and drug use histories are shown in Table 2. While there was a difference between the groups in terms of diabetes mellitus (DM), hypertension (HT), coronary artery disease (CAD), and cerebrovascular disease (CVD) history, there was no difference in terms of chronic obstructive pulmonary disease (COPD) history. No significant difference was observed between the patients in terms of the use of other drugs and erythrocyte suspension (ES) except for the use of diuretics and contrast agents.

Table 2. Comorbidities and drug use history

	AKI (n=42)	Non-AKI (n=150)	Total (n=192)	P*
Hypertension, yes/no	27 (64.3)/ 15 (35.7)	62 (41.3)/ 88 (58.7)	89 (46.4)/ 103 (53.6)	0.008
DM, yes/no	18 (42.9)/ 24 (5.,1)	38 (25.3)/ 112 (74.7)	56 (29.2)/ 136 (70.8)	0.027
CAD, yes/no	15 (35.7)/ 27 (64.3)	18 (12)/ 132 (88)	33 (17.2)/ 159 (82.8)	<0.001
CVD, yes/no	5 (11.9)/ 37 (88.1)	5 (3.3)/ 145 (96.7)	10 (5.2)/ 182 (94.8)	0.027
COPD, yes/no	6 (14.3)/ 36 (85.7)	13 (8.7)/ 137 (91.3	19 (9.9)/ 173 (90.1)	0.281
ACE use, yes/ no	11 (26.2)/ 31 (73.8)	21 (14)/ 129 (86)	32 (16.7)/ 160 (83.3)	0.061
Diuretic use, yes/no	19 (45.2)/ 23 (54.8)	41 (27.3)/ 109 (72.7)	60 (31.2)/ 132 (68.8)	0.027
ES usage, yes/ no	33 (78.6)/ 9 (2.,4)	114 (76)/ 36 (24)	147 (76.6)/ 45 (23.4)	0.728
Steroid use, yes/no	16 (3.1)/ 26 (61.9)	66 (44)/ 84 (56)	82 (42.7)/ 110 (57.3)	0.494
Ab use, yes/no	37 (88.1)/ 5 (11.9)	116 (77.3)/ 34 (22.7)	153 (79.7)/ 39 (20.3)	0.125
Contrast agent use, yes/no	17 (40.5)/ 25 (59.5)	40 (26.7)/ 110 (73.3)	57 (2.7)/ 135 (70.3)	0.083
Vasopressor use, yes/no	7 (16.7)/ 35 (83.3)	6 (4)/ 144 (96)	13 (6.8)/ 179 (93.2)	0.004

Data are given as the number of patients (%). *Chi-Square, DM: Diabetes mellitus, CAD: Coronary artery disease, CVD: Cerebrovascular disease, COPD: chronic obstructive pulmonary disease, ACE: Angiotensin-converting enzyme inhibitors, ES: Erythrocyte suspension, Ab: Antibiotics

There was a significant difference between the groups regarding the length of stay in the intensive care unit, discharge, need for dialysis, and need for mechanical ventilation and APACHE II scores. (Table 3, p<0.001).

The biochemical values of the patients by groups are shown in Table 4. Except for creatinine, no significant difference was observed between the groups regarding Na, leukocytes, Hb, and Hct values.

DISCUSSION

The incidence of AKI in postoperative intensive care patients varies between 10% and 56%.[9,10] The AKI rate of 21.9% in our postoperative intensive care patients is consistent with this reported range.

Risk indices [Simple Postoperative AKI Risk (SPARK) index, AKI prediction model] were used to predict the risk of developing AKI in non-cardiac surgeries in the postoperative period.[11,12] These predictive models can be used to identify high-risk postoperative patients and provide a scientific and effective basis for clinicians to identify AKI early. Seven parameters are used in the AKI prediction model used in intensive care: advanced age, emergency surgery, increased baseline creatinine level, chronic kidney disease, nephrotoxic drug use, diuretic use, and Sequential Organ Failure Assessment (SOFA) score. In our study, age, emergency surgery, use of diuretics, and use of contrast agents from nephrotoxic drugs were associated with AKI.

Although there is no definite consensus that blood loss is associated with the risk of AKI, it was shown in a study conducted on liver transplant recipients that each 1 liter of perioperative blood loss significantly increased the risk of continuous renal replacement therapy.[13] Although we do not know the amount of intraoperative blood loss in our patients, the rate of AKI was higher in patients who needed ES in the postoperative period.

A study involving 893 postoperative orthopedic surgery patients concluded that patients with risk factors for AKI should be followed up in the postoperative period. In the same study, DM was found to be associated with the incidence of AKI.[14] 58.9% of our patients were orthopedic patients, and AKI was more common in our patients with DM.

Table 3. Intensive care status of patients

	AKI (n=42)	Non-AKI (n=150)	Total (n=192)	P
Intensive care hospital stay (days), median (min-max)	5 (1-80)	3 (1-30)	3 (1-80)	<0.001#
Intensive care exit, service/exitus, n (%)	28 (66.7)/ 14 (33.3)	141(94)/ 9(6)	169 (88)/ 23 (12)	<0.001*
Dialysis, yes/no, n (%)	10 (23.8)/ 32 (76.2)	0 (0)/ 150 (100)	10 (5.2)/ 182 (94.8)	<0.001*
Need for mechanical ventilation, yes/no, n (%)	15 (35.7/ 27 (64.3)	22 (14.7)/ 128 (85.3)	37 (19.3)/ 155 (80.7)	<0.001*
Mechanical ventilation length of stay, median (min-max)	0 (0-61)	0 (0-10)	0(0-61)	0.001#
APACHE II, median (min-max)	10.50 (4-26)	6 (4-22)	6 (4-26)	<0.001#

Data are given as the number of patients (%), and median (minimum-maximum). #Mann Whitney U, *Chi-Square

Table 4. Biochemical values of patients

	AKI (n=42)	Non-AKI (n=150)	Total (n=192)	P
Creatinine mg/dl, entry, median (min-max)	1.05 (0.33-5.18)	0.74 (0.21-2.62)	0.78 (0.21-5.18)	<0.001
Creatinine, mg/dl, highest, median (Min-Max)	1.95 (1.10-8.22)	0.80 (0.21-2.62)	0.89 (0.21-8.22)	<0.001
Na, mEq/L, median (Min-Max)	139 (122-155)	138 (128-198)	138.5 (122-198)	0.715
Wbc, ($\times 10^3/\mu L$), median (Min-Max)	11.61 (4.63- 30.20)	11.24 (2.65-37.21)	11.32 (2.65-37.21)	0.841
Hb, gr/dl, median, IQR:	10.40 (7-15.10)	10.95 (3.32-16.60)	10.9 (3.32-1.60)	0.084
Hct,%, median, IQR:	31.4 (21.30-48.50)	34.15 (22.60-50)	34 (21.3-50)	0.155

Data are given as the number of patients (%), and median (minimum-maximum). #Mann Whitney U, *Chi-Square, Na: Sodium, Wbc: White blood cell count, Hb: Haemoglobin, Hct: Hematocrit

In the perioperative period, oliguria is common and is not always accompanied by an increase in creatinine. It can be seen physiologically with the effect of pain, nausea, and increased ADH in response to surgery.[15,16] Although some studies suggest that intraoperative oliguria and the incidence of postoperative AKI are unrelated, it was suggested that intraoperative oliguria, especially in cardiac and intra-abdominal surgeries, and vasopressor therapy initiated intraoperatively were associated with the incidence of postoperative AKI.[17-19] One of the limitations of our study was that it was retrospective, so we could not record the data of our patient's intraoperative period.

Postoperative Recovery (ERAS) guidelines cover surgical and anesthetic preoperative, perioperative, and postoperative care. The instructions in this guide cover all postoperative patients, including patients in the post-anesthesia intensive care unit. It was shown that protocols including early mobilization, nutrition, fluid status, and pain control of patients reduce the ICU length of stay and complications and improve surgical outcomes.[20] However, some studies have reported that the restrictive fluid therapy approach in these protocols increases the risk of AKI.[21,22] We do not have a restrictive protocol for fluid therapy. However, since our study was retrospective, we could not evaluate the amount of fluid given.

In a prospective study performed on 1200 postoperative patients excluding cardiovascular surgery, AKI was found to be associated with increased morbidity and mortality.[23] In a study conducted on general surgery patients, mortality increased eight times in those with perioperative AKI.[24] In another study conducted with general surgery patients, 30-day mortality was found as 1.9% in those who did not develop AKI, while it was 31% in patients with AKI.[25] While our overall mortality rate was 6% in patients who did not develop AKI, it was 33.3% in those who did. The reason for the high mortality rate in our patients who did not develop AKI may be due to different surgical groups, and different factors may be effective in mortality.

The limitations of our study are the inclusion of different surgical groups, being a retrospective study and not including the intraoperative period.

CONCLUSION

In the study we conducted in the intensive care unit, it was found that AKI developed in approximately one-fifth of the patients who were followed up in the postoperative intensive care unit after non-cardiac surgery. Our patients who developed AKI had higher mortality, longer intensive care unit stays, and higher mechanical ventilation (MV) needs. It was observed that the AKI development rate was higher after emergency operations. Hence, we think that the evaluation of risk factors that may cause the development of AKI and trying to correct them may be effective in preventing the development of acute kidney injury.However, for the management of postoperative AKI in the intensive care unit, multicentre studies involving the intraoperative period in the same surgical group are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Afyonkarahisar Health Sciences University Faculty of Medicine Ethics Committee (Date: 04.11.2022, Decision No: 14).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The efficacy of volumetric computed tomography histogram analysis in adrenal masses

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ABSTRACT

Aims: The rate of adrenal mass detection has increased due to the development of imaging modalities. It is vital to differentiate benign adrenal adenomas from other adrenal masses in order to establish whether an active management strategy is essential. Volumetric CT histogram analysis calculates the percentage of covered pixels in the negative attenuation region. The goal of this research was to evaluate the diagnostic utility of volume histogram analysis for adrenal tumors confirmed histopathologically as well as the ideal slice thickness for CT histogram analysis to differentiate between benign and malignant lesions with a density greater than 10 Hounsfield units (HU).

Methods: The research analyzed the CT images of 127 individuals with 136 adrenal masses that were verified histopathologically after resection (57 lipid-poor adenomas, 21 pheochromocytomas, 47 metastases, and 11 adrenocortical carcinomas). For imaging, a 40-row MDCT device (Siemens Medical Solution, Erlanger, Germany) was utilized. 1 mm and 5 mm unenhanced CT images were obtained. Two separate radiologists manually assessed the Hounsfield units (HU) of the masses. The 5th to 95th percentiles of HU values, as well as the minimum, mean, and maximum values, skewness, kurtosis, and variance, were calculated. Interobserver agreement was determined by means of the interclass correlation coefficient (ICC).

Results: The HU parameters for the malignant group were all higher than those of the benign group, and the difference in the 5 mm slice thickness was more significant than the 1 mm slice thickness. The difference between HUmin (P=0.007), HUmean and HUmedian (P <0.001), 5th to 50th (P <0.001), 75th (P=0.004), 90th (P=0.016), and 95th (P=0.049) percentiles was statistically significant. The malignant group had higher skewness and kurtosis than the benign group, while the benign group had higher variance. Statistically, the disparity between the variances was significant (P=0.046). The area under the curve (AUC) of the 25th percentile of the HU value was the highest (AUC=0.932; cut-off value=15; sensitivity=90.0%; specificity=85.7%).

Conclusion: Noninvasive volumetric CT histogram analysis can detect malignant adrenal masses from benign tumors before an operation. Histogram analysis benefits from thicker slices. HUmin, HUmean, HUmedian, percentile values, and variance can identify adrenal masses.

Keywords: Adenoma, adrenal mass, computed tomography, histogram analysis, pheochromocytoma

INTRODUCTION

As imaging techniques have advanced, the rate of adrenal mass identification has climbed to 10%.[1] It is important to distinguish adenomas from pheochromocytomas, carcinomas, and metastases to determine whether an active strategy for management is required.[2] A mean density greater than 10 Hounsfield units (HU) is a straightforward method for diagnosing about two-thirds of adenomas.[3-5] The others have a density greater than 10 HU in non-contrast CT images due to containing a small amount of intracellular lipid and should be regarded as indeterminate. This group of patients may also undergo computed tomography (CT) with percentage washout measurement,[6,7] chemical-shift MRI assessment,[8] and volumetric non-contrast histogram analysis.[9]

CT histogram analysis requires intracytoplasmic lipid concentrations below 0 HU. Histograms can show the attenuation values of each pixel in a region of interest over an adrenal adenoma. Negative and positive CT attenuation values are shown on the x-axis. Pixel frequency is on the y-axis. There is currently no consensus regarding the ideal CT monitoring criteria. One of the many adjusting factors is slice thickness, which increases CT image noise but improves clarity and spatial resolution.

This study aimed to determine the value of volumetric CT histogram analysis for the diagnosis of adrenal tumors confirmed histologically after surgery as well as the optimal slice thickness for CT histogram analysis to distinguish between benign (lipid-poor adenomas and pheochromocytomas) and malignant (adrenal

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carcinomas and metastases) masses with an unenhanced attenuation of greater than 10 HU.

METHODS

This retrospective study was carried out with the permission of University of Health Sciences, Bakırkoy Dr. Sadi Konuk Training and Research Hospital. Ethics Committee (Date: 09.01.2023, Decision No: 2023/06). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Study Population

We analyzed the patients for those who required operations at our facility between June 2015 and November 2022. These were the inclusion criteria for the research: (a) patients diagnosed postoperatively; (b) patients who underwent an unenhanced CT scan prior to surgery; (c) patients with histologically confirmed adrenal adenoma, pheochromocytoma, adrenal carcinomas, and metastases. CT scans were conducted to obtain contrast-enhanced series along with non-contrast series for the purpose of excluding alternative pathologies.

The database included a total of 361 patients. The exclusion criteria are summarized in Table 1. Lastly, 127 patients with 136 lesions were analyzed. The research group consisted of 49 men and 78 women. In 69 cases, the lesions were on the left side; in 49 cases, on the right side; and in nine patients, on both sides. There were 57 lipid-poor adenomas, 21 benign pheochromocytomas, 47 metastases, and 11 carcinomas in the study cohort.

CT Examination

Using a 40-row MDCT scanner, the images were obtained (Siemens Medical Solution, Erlanger, Germany). The imaging was performed utilizing a variety of protocols with standard settings. In a supine position, a standard CT protocol for the abdomen was executed with. The effective mAs was regulated by Siemens' "CARE dose" at 120 kV. The rotational speed of the gantry was 0.5 seconds, the collimation was 1 millimeter, and the pitch was 1.2. Individually adjusting the field of view (FOV) to encompass the body, and the matrix was 512 x 512. Image thickness of 5 mm was used for multiplanar reconstruction.

Image Analysis

The unprocessed CT raw data were transferred from the picture archiving and communication system (PACS) to a personal computer and analyzed with the open-source LIFEx 7.2.0 voxel tool (https://lifesoft.org). All images were separately evaluated by two radiologists, who were blind to the medical data and histologic results and had 11 and 8 years of abdominal imaging experience, respectively. In each segment, they manually drew the ROI encompassing the lesion (Figure 1). Each ROI was automatically combined into a volumetric ROI comprising voxel data for the entire tumor. Then, a volumetric HU map was created. The 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 95^{th} percentiles of HU values were determined, along with the minimum, mean, and maximum values, as well as the skewness, kurtosis, and variance. The area under the curve (AUC) was calculated for all parameters. The n^{th} percentile was the point on the histogram where n percent of the voxel values were detected on the left. Positive skewness reflects the deviation of the distribution's median from its mean value. Kurtosis represents the peakiness of the histogram distribution, with high kurtosis characterized by a noticeable peak near the mean, a quick drop, and heavy tails.

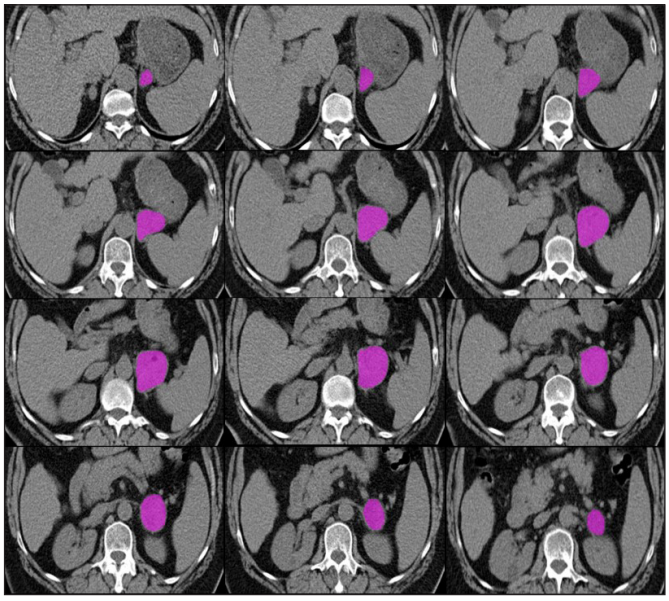

Figure 1. An example of volumetric CT histogram analysis. The whole lesion was manually evaluated as an area of interest (ROI) in each slice of the unenhanced CT images.

Statistical Analysis

IBM SPSS 25.0 was utilized to conduct the analysis (Chicago, IL, United States). From the data set obtained by combining the HU values of each patient, histograms of the groups were generated. Histograms revealed a difference in the distribution of all patient measurements. Using these measurements, statistical values such as minimum, median, mean, maximum, standard deviation, skewness, kurtosis, and percentiles were computed for each patient group, and the variations in the resulting descriptive statistics were depicted graphically. These group statistics were computed for individuals. Using the t-test for independent samples, it was determined whether these statistics derived from individuals differed between groups. On the basis of individual data, receiver operating characteristic (ROC) curves were generated, and the threshold value for the obtained statistics was computed. Sensitivity and specificity values for threshold values were calculated.

RESULTS

Demographic Information

A total of 75 cases with 78 adrenal masses (57 lipid-poor adenomas and 21 pheochromocytomas) were enrolled in the benign group (Figures 2 and 3), and 52 cases with 58 adrenal masses (47 metastases and 11 adrenocortical carcinomas) were included in the malignant group (Figure 4). In total, 49 men and 78 women, with a mean age of 55.44±12.14 years, were included in the study. The percentage of women in the benign group was higher than in the malignant group (p=0.010). The mean age of the patients in the malignant group was higher than that in the benign group (p=0.014) (Table 1).

Table 1. Data of the patients excluded from the study

Patients Excluded From the Study	**n**
Patients without an unenhanced CT scan	77
Patients whose unenhanced CT image was compatible with an adenoma	114
Poor image quality	12
Patients with direct adrenal gland infiltration by renal cell carcinomas	4
Pathologically confirmed other than adrenal adenoma, pheochromocytoma, and malignancies	
Myelolipoma	14
Granulomatous disease	5
Abscess	3
Hemangioma	1
Ganglioneuroma	1
Patients with malignant pheochromocytoma	3

CT: computed tomography

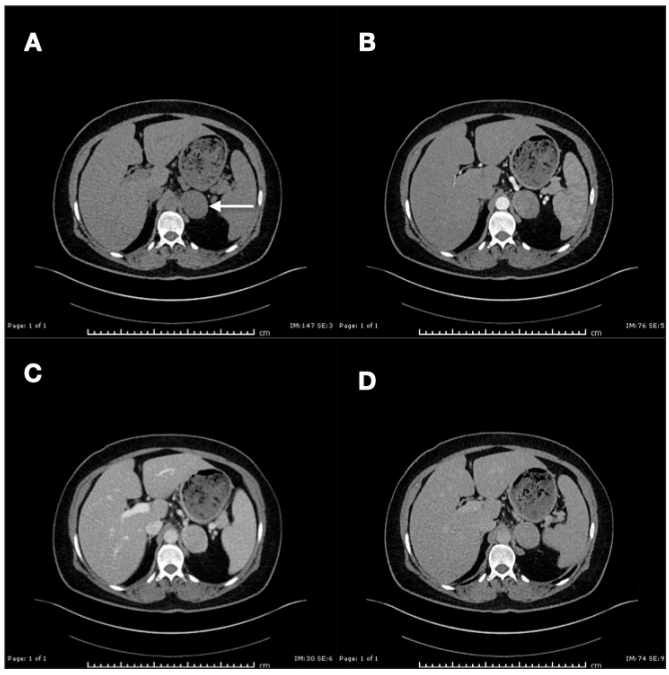

Figure 2. Lipid-poor adenoma in a 39-year-old male patient. On unenhanced CT images, a hyperdense mass was detected in the left adrenal gland (white arrow) (a). The contrast-enhanced series displayed substantial enhancement (b–d).

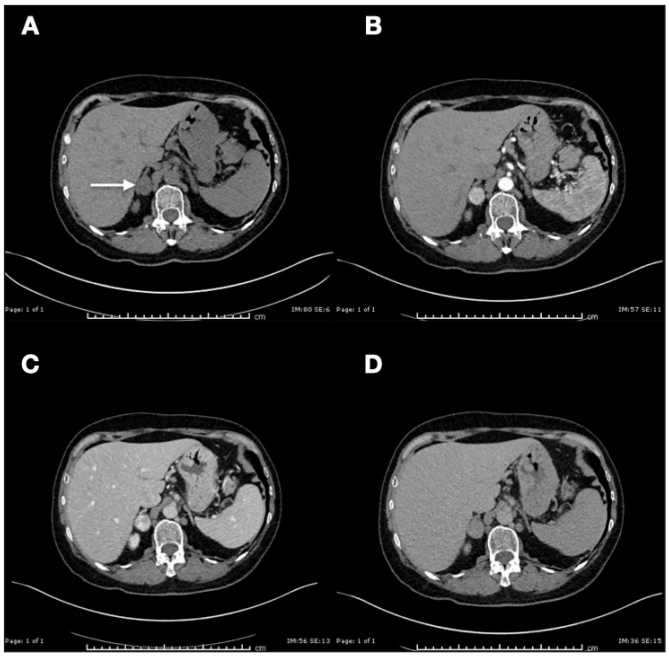

Figure 3. Pheochromocytoma in a 42-year-old female patient. A heterogeneous hyperdense mass was observed in the right adrenal gland (white arrow) on unenhanced CT images (a). In the contrast-enhanced series, the early phase (b,c) shows rapid enhancement of the mass and wash-out in the delayed phase (d).

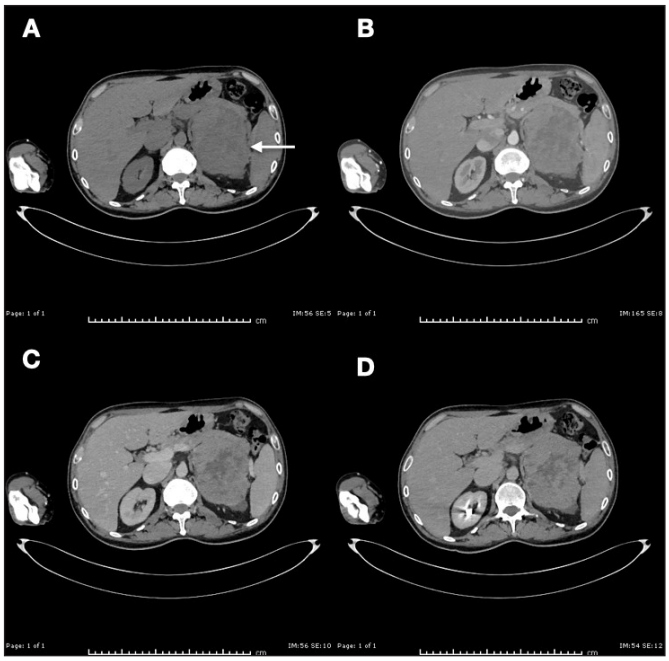

Figure 4. Adrenocortical carcinoma in a 54-year-old female patient. A massive heterogeneous hyperdense mass was observed in the left adrenal gland (white arrow) on unenhanced CT images (a). Heterogeneous progressive enhancement was seen in the contrast-enhanced series (b–d).

Interobserver Agreement

Using the interclass correlation coefficient (ICC), the agreement between the observers was assessed. The ICCs for each parameter exceeded 0.80, showing almost perfect agreement.

Results and Diagnostic Performance of CT Histogram Parameters Figures 5 and 6 display the histogram curves pertaining to the benign and malignant groups. HUmin,

HUmean, HUmedian, HUmax, 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles were all higher for the malignant group than for the benign group. The difference in the 5 mm slice thickness was more substantial than the 1 mm slice thickness (Tables 2 and 3). There was a significant difference in HU values between HUmin (p=0.007), HUmean (p <0.001), HUmedian (p <0.001), 5th (p <0.001), 10th (p <0.001), 25th (p <0.001), 50th (p <0.001), 75th (p=0.004), 90th (p=0.016), and 95th (p=0.049) percentiles in the 5 mm slice thickness (Table 3).

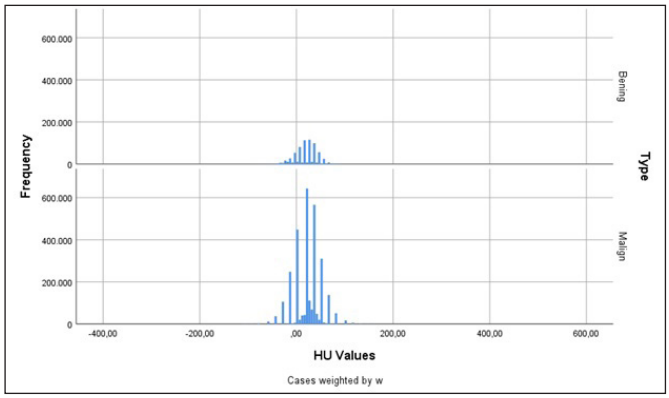

Figure 5. The histogram curve depicting the distribution of data for both the benign and malignant groups was generated based on a section thickness of 1 mm.

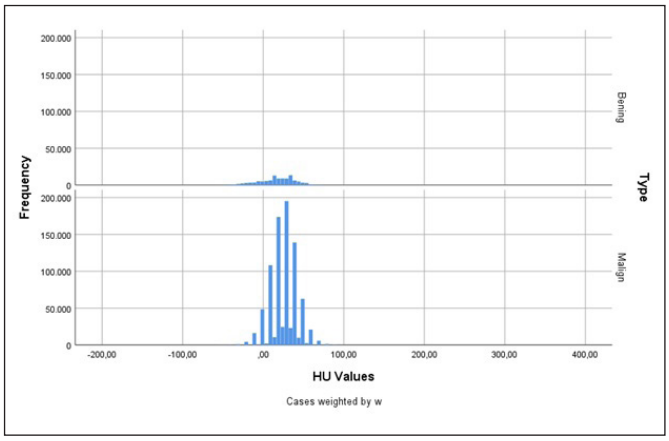

Figure 6. The histogram curve depicting the distribution of data for both the benign and malignant groups was generated based on a section thickness of 5 mm.

Table 2. Demographic data of patients

		Bening	Malign	p
		n (%) / mean±s.d.	n (%) / mean±s.d.	
Age		53.19±13.10	58.69±10.76	0.014[a]
Sex	Male	22 (29.3)	27 (51.9)	0.010[b]
	Female	53 (70.7)	25 (48.1)	

aMann Whitney U Test; bChi-squared test

Table 3. Comparisons of HU histogram parameters between benign and malignant adrenal masses in 1 mm slice thickness

HU	Malignant	Bening	Total	p	Significance Level
Mean	29.07±6.98	8.86±16.62	17.28±16.7	0.002	99%
Std. deviation	20.46±10.16	26.8±12.95	24.16±12.06	0.128	-
Median	29.0±7.48	9.21±16.83	17.46±16.77	0.003	99%
Minimum	-83.2±51.21	-131.86±55.14	-111.58±57.84	0.057	-
Maximum	152.5±158.34	146.21±105.54	148.83±126.95	0.930	-
Kurtosis	0.98±1.55	1.51±1.53	1.29±1.52	0.266	-
Skewness	-0.22±0.44	-0.18±0.35	-0.2±0.38	0.464	-
5th	-4.8±21.58	-34.36±25.61	-22.04±27.83	0.007	99%
10th	3.7±18.19	-24.0±22.53	-12.46±24.72	0.007	99%
25th	16.4±12.68	-8.43±18.66	1.92±20.4	0.003	99%
50th	29.0±7.48	9.21±16.83	17.46±16.77	0.003	99%
75th	41.6±6.1	26.36±20.04	32.71±17.34	0.037	95%
90th	53.6±9.61	41.57±24.34	46.58±20.19	0.177	-
95th	62.0±13.76	52.07±28.79	56.21±23.82	0.278	-

HU, Hounsfield unit

Table 4. Comparisons of HU histogram parameters between benign and malignant adrenal masses in 5 mm slice thickness

HU	Malignant	Bening	Total	p	Significance Level
Mean	29.54±7.24	7.81±16.50	16.86±17.15	<0.001	99%
Std. deviation	15.77±6.81	20.15±6.06	18.32±6.61	0.046	95%
Median	30.8±7.07	8.5±16.21	17.79±17.15	<0.001	99%
Minimum	-46.3±36.7	-87.81±32.8	-70.46±39.63	0.007	99%
Maximum	113.6±97.55	86.07±42.34	97.54±70.21	0.837	-
Kurtosis	1.22±1.74	1.19±1.44	1.20±1.53	0.447	-
Skewness	-0.24±0.49	-0.34±0.42	-0.3±0.44	0.412	-
5th	4.1±15.91	-26.07±18.5	-13.5±22.88	<0.001	99%
10th	9.1±13.81	-17.0±17.4	-6.13±20.46	<0.001	99%
25th	20.3±9.74	-4.71±16.63	5.71±18.77	<0.001	99%
50th	30.8±7.07	8.5±16.21	17.79±17.15	<0.001	99%
75th	39.6±6.36	20.71±17.69	28.58±16.83	0.004	99%
90th	48.6±6.47	32.36±18.82	39.13±16.84	0.016	95%
95th	53.96±9.72	38.63±19.38	45.01±17.57	0.049	95%

HU, Hounsfield unit

The skewness and the kurtosis were larger in the malignant group as compared to the benign group; however, there was no statistically significant difference. The variance was higher in the benign group, and the difference was statistically significant (p=0.046) (Table 3).

The ROC curve indicated the effectiveness of HU histogram parameters in the diagnosis of adrenal masses (Figure 7), with the AUC of the 25th percentile of the HU value being the greatest (0.932). Under the cut-off value of 15, the sensitivity and specificity were, respectively, 90.0% and 85.7%. Following diagnostic effectiveness was the 5th percentile of the HU value (AUC=0.925). Below the threshold value of 0.5, the sensitivity and specificity were 90% and 92%, respectively. AUC was also greater for the HUmedian and the 50th percentile (AUC=0.921) of the HU value. Under the threshold value of 27.5, the sensitivity and specificity were 80% and 92%, respectively. Table 4 summarizes the ROC results for every parameter.

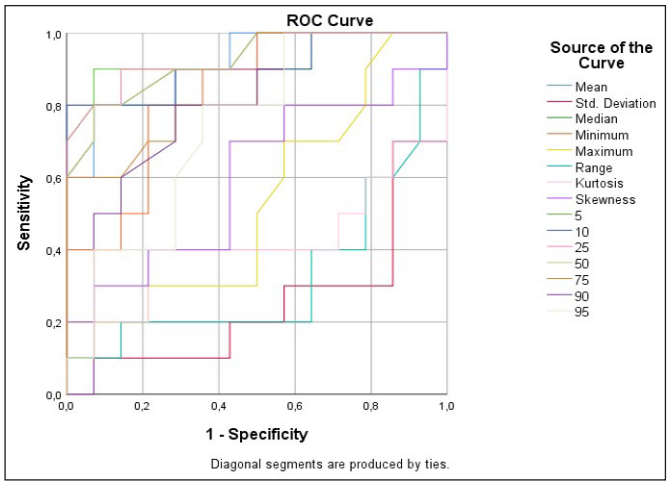

Figure 7. The ROC (receiver operating characteristic) curve (a) represents all histogram parameters. There was a significant difference in HU values between HUmin (P=0.007), HUmean (P <0.001), HUmedian (P <0.001), 5th (P <0.001), 10th (P <0.001), 25th (P <0.001), 50th (P <0.001), 75th (P=0.004), 90th (P=0.016), and 95th (P=0.049) percentiles. The AUC (area under the curve) was correspondingly 0.829, 0.914, 0.921, 0.925, 0.907, 0.932, 0.921, 0.854, 0.793, and 0.739.

DISCUSSION

Adrenal focal masses present a significant difficulty in the daily routine. If either identified incidentally or at the endocrinologist's request, all differential diagnosis options must be thoroughly investigated.[10] Each incidentaloma must always be evaluated for secretory hormonal activity and malignant malignancy.[3] The choice is crucial for individuals with confirmed extra-adrenal malignancies. The therapy and prognosis for adenomas and metastases are very different. The goal of the assessment is to correctly identify benign lesions without further study, saving the patient from ionizing radiation, contrast media, uncertainty, and follow-up exams.

A CT scan can help determine the biological nature of lesions and differentiate between common adrenal pathologies. The non-contrast density of adenomas is usually low due to the high lipid content, whereas the attenuation of carcinomas and metastases is higher. Commonly, 10 HU of unenhanced attenuation is recognized as the threshold.[4,5] The simplicity of this assessment is a major benefit, as it can be conducted even when the CT imaging was not intended to examine the adrenal masses. However, around one-third of the adenomas have mean density values higher than 10 HU, which are regarded "lipid-poor", and they pose a substantial dilemma for why they cannot be consistently distinguished from other adrenal tumors.

A CT histogram analysis is an alternative technique for analyzing the initial unenhanced CT image.[9] The volumetric CT histogram has been utilized in research including both malignant and benign processes.[11-15] This study represents a novel contribution to the existing literature, as it is the first to ascertain HUmin, HUmean, HUmedian, HUmax, and percentile values of HU, alongside standard deviation, skewness, and kurtosis with different slice thicknesses.

Table 5. ROC results of HU metrics histogram parameters in 5 mm slice thickness

Test Result Variable(s)	AUC	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% confidence interval		Cut off	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Mean	0.914	0.058	0.001	0.801	1.000	26.5	0.800	0.929
Std. deviation	0.257	0.112	0.046	0.038	0.477	32.5	0.100	1.000
Median	0.921	0.056	0.001	0.812	1.000	27.5	0.800	0.929
Minimum	0.829	0.084	0.007	0.664	0.993	-69.5	0.800	0.786
Maximum	0.525	0.123	0.838	0.285	0.765	75.5	0.700	0.429
Kurtosis	0.407	0.132	0.447	0.149	0.665	1.2	0.400	0.786
Skewness	0.600	0.124	0.412	0.357	0.843	-0.2	0.700	0.571
5th	0.925	0.065	0.000	0.798	1.000	0.5	0.900	0.929
10th	0.907	0.069	0.001	0.771	1.000	9.5	0.800	1.000
25th	0.932	0.054	0.000	0.826	1.000	15.0	0.900	0.857
50th	0.921	0.056	0.001	0.812	1.000	27.5	0.800	0.929
75th	0.854	0.079	0.004	0.699	1.000	34.5	0.800	0.714
90th	0.793	0.094	0.016	0.608	0.978	45.0	0.800	0.714
95th	0.739	0.102	0.0498	0.540	0.939	48.5	0.800	0.643

AUC, area under the curve

Many publications have verified the method; however, most of them involved a limited number of cases, only a fraction of the tumors were histopathologically validated, and only a negative pixel percentage was calculated.[16-19] Our work employed volumetric histogram analysis instead of single CT image histograms. Whole-lesion volumetric histogram evaluation is utilized to examine the distribution of HU values of the entire lesion and avoids the subjectivity of ROI placement to assure repeatability and calculation precision. This method records the HU values of the entire tumor and may eliminate sampling bias. Szász et al.[20] aimed to determine the appropriate threshold with the volumetric CT histogram analysis of adrenal lesions. However, they only calculated the percentage of negative pixels and concluded that a threshold of 10% of negative pixels yielded a sensitivity of 82.9% and a specificity of 98.2%. In our study, the significance of difference, sensitivity, and specificity increased with lower percentile values.

It is also vital to note that increasing CT image noise leads to a greater distribution of CT values.[21] CT images are reconstructed using a wide variety of slice widths; a thinner slice results in a reduced voxel size and a greater noise level.[22] Although slice thickness affects the HU values, the link between noise and histogram analysis is only hypothesized or addressed in general terms in the published studies. They utilized a wide variety of slice thicknesses from 1 to 8 mm, and it is unknown how these variations may have affected the histogram analysis results.[16-19,23,24] Just one study determined the appropriate slice thickness of CT images, but only for negative voxels. Utilizing a slice thickness of 5 mm and 10% negative voxels, they attained 53.0% sensitivity, 98.8% specificity, and the highest positive predictive value (PPV).[25] We found in our study that a thicker slice thickness is more substantial.

In a study with histologically verified adrenal masses using non-contrast density, at a threshold value of 5%, sensitivity was 78.0–81% and specificity was 67.1–76.3%; at a threshold value of 10%, sensitivity was 69.5–72.4% and specificity was 85.5–89.5%.[24]

Clark et al.[26] recommended using an algorithm based on noise correction to identify adrenal masses. Their formula estimates and eliminates noise using slice thickness, mean density, pitch, standard deviation, tube voltage, and tube current.

The histological verification of all masses is a key benefit over previous research, with the exception of Szász et al.[20] and Remer et al.[24] CT wash-out rates, chemical shift MRI, and PET/CT tracer accumulation were employed to diagnose previous CT histogram analysis studies. Consequently, wash-out rate measurements may produce false-positive results with these techniques for adrenal masses.[27-29] Likewise, the specificity of PET/CT and MRI may not be enough, making histology the only totally valid reference standard.[10] Additionally, we assessed various slice thicknesses and utilized volumetric histogram analysis to eliminate sampling bias. Unlike previous studies, numerous characteristics, including percentile values, minimum, mean, and maximum values, as well as variance, kurtosis, and skewness of histogram analysis, were assessed.

Our study had some limitations. Patient selection was based on a potentially biased retrospective analytic technique. A variety of CT protocols for the evaluation of abdominal organs were studied. Our study did not examine the patients with diagnostic doubt in their adrenal mass or the extent to which CT histogram analysis might resolve this uncertainty.

CONCLUSION

Before the surgical intervention, noninvasive volumetric CT histogram analysis may assist in distinguishing malignant adrenal masses from benign tumors. Greater slice thicknesses can yield more useful data for histogram analysis. For distinguishing adrenal masses, HUmin, HUmean, HUmedian, percentile values, and variance can be used as references.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences, Bakirkoy Dr. Sadi Konuk Training and Research Hospital. Ethics Committee (Date: 09.01.2023, Decision No: 2023/06).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Retrospective evaluation of the prevalence of endodontic-periodontal lesions on panoramic images in the latest classification of periodontal and peri-implant diseases

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ABSTRACT

Aims: This study aimed to determine the prevalence of endodontic-periodontal lesions (EPLs) and EPL grades 1–3 without root damage in patients with and without periodontitis according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases.

Methods: This study included 500 panoramic radiographs from the Faculty of Dentistry at the University of İstanbul Medipol. Each radiograph was evaluated by a calibrated investigator for diagnostic signs. Patients' age, sex, total number of teeth, total number of implants, number of filled teeth, number of missing teeth, number of caries, presence of fixed prosthesis, full mouth periodontal diagnosis and determination of the region with the highest radiographic bone loss without EPL were recorded in all patients. For patients with EPL, the presence of EPL and its grading according to the 2017 World Workshop on Classification of Periodontal and Peri-Implant Diseases, the presence of furcation involvement, degree of furcation involvement, the presence of caries in the related tooth, and restoration in the relevant tooth material were also evaluated.

Results: The mean age was higher for patients with EPL teeth than without EPL teeth ($p<0.05$). The mean number of teeth was higher for patients without EPL teeth than patients with EPL teeth ($p<0.05$). Patients with stage 1 or 2 bone loss mostly had EPL teeth, while patients with stage 3 or 4 mostly did not have EPL teeth ($p<0.05$). Patients with full mouth stage 1 or 2 diagnoses mostly had teeth with EPL. Patients with grade A or B bone loss mostly had teeth with EPL. Maxillary incisors, mandibular premolars, and mandibular incisors mostly had a "j" profile.

Conclusion: The presence of EPL is affected by age, number of teeth, and different periodontal conditions. EPLs were most frequently observed in molars. These results are unsurprising due to the difficulty in brushing the molar areas and periodontal treatment in this area. Because of the complexity of concurrent endodontic and periodontic treatments, the clinical treatment procedure is difficult, the sequence of procedures must be rigorous, and the selection of appropriate materials is critical for optimal and successful treatment in these EPL cases.

Keywords: Endo-perio lesions, panoramic radiographs, periodontal disease, j-shape, cone shape

INTRODUCTION

The prognosis and treatment of teeth with combined endodontic-periodontal lesions (EPLs) are challenging for dentists. Clinically, the prognosis of these teeth may be good, poor, and even hopeless. There is little evidence for guiding practitioners to decide which treatment should precede for the affected tooth, the endodontic or periodontic approach.[1] The factors affecting treatment success are not yet completely known.

Both anatomical and non-physiological pathways connecting endodontic and periodontal tissues can cause EPLs. The anatomical pathways are the root canal system's apical foramina, accessory canals, or dentinal tubules, often located in the apical third of the root. The non-physiological pathways may be iatrogenic perforations or vertical root fractures.[2]

Different classification systems have been established to describe and categorize EPLs. Older classifications focused on the lesion's history and origin. The new classification was proposed by the Working Group 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[1,3]

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This new classification system focuses on the current disease status and prognosis of the teeth. It has often been suggested to overcome the problem of unknown history and origin of the disease. It is based on the assumption that there is no meaningful outcome on the treatment method since treatment always comprises endodontic and periodontal methods.[1,4]

EPLs are categorized as with or without root damage. EPLs without root damage are differentiated into those in patients with and without periodontitis. Grades I to III are defined by the spread around the tooth. Epidemiological data supporting this classification are lacking.

Therefore, this study aimed to determine the prevalence of EPLs and EPL grades 1–3 without root damage in patients with and without periodontitis, according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases.

METHODS

The study was approved by the Non-invasive Clinical Studies Ethics Committee of İstanbul Medipol University (Date: 31.01.2023, Decision No: 64). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients' panoramic radiographs were evaluated in this study who applied to the Dental School Clinic of İstanbul Medipol University. It comprised patients who needed treatment by specialist dentists or who came for routine dental visits.

The sample size was calculated at a 95% confidence level using the G*Power programme (version 3.1.9.2).[5] Based on a previous study comparing two independent groups,[6] considering an α of 0.05, a standardized effect size of 0, and a theoretical power of 80%, the minimum size for each group was estimated to be 50.

Inclusion and Exclusion Criteria

This study retrospectively included 500 patients' panoramic radiographs were assessed from the Faculty of Dentistry. Radiographs belonging to patients who were over 18 years of age were evaluated. In our study, an experienced dentomaxillofacial radiologist (K.A.) and periodontist (C.A.), performed the initial examination of these teeth and performed both endodontic vitality tests and periodontal examination of the patients. X-rays of patients who underwent these tests and who actually had periodontal examinations were included in the study. But also, radiographs were excluded which had poor image quality (e.g., focus, artifacts, or orientation) or which were duplicates (e.g., second radiograph of an included patient). The radiographic archive was evaluated and the systemic status of the patients was not taken into account.

Radiograph Evaluation

Each radiograph was evaluated by a calibrated investigator (T.P.) in the same room on an approved monitor. For calibration, the investigator evaluated the diagnostic signs of 10 panoramic images. If there were differences, they were discussed with a second investigator.

Patients' age, sex, total number of teeth, total number of implants, number of filled teeth, number of missing teeth, number of caries, presence of fixed prosthesis, full mouth periodontal diagnosis and determination of the region with the highest radiographic bone loss without EPL were recorded in all patients. For patients with EPL, the presence of EPL and its grading according to the 2017 World Workshop on Classification of Periodontal and Peri-Implant Diseases; the presence of furcation involvement, degree of furcation involvement, the presence of caries in the related tooth, and restoration in the relevant tooth material were also evaluated.

Grades 1 and 2 were combined into a single group (later referred to as grade 1/2) since no critical width value was given in the classification to distinguish between narrow and wide pockets. Therefore, the rating was made as either grade 1/2 or grade 3.[1]

Additional recordings in patients with EPL were RCT, restorations, or caries in teeth with EPL. It included radiographic bone destruction and root length of teeth 13–17, 23–27, 33–37, and 43–47 (according to the FDI World Dental Federation notation) to provide periodontal diagnosis according to the staging recommended by the new classification.

Radiographic bone loss was categorized based on its extension into the coronal third (<15%; stage I), the upper coronal third (15%–33%; stage II), or the middle third and beyond (stage III/IV) of the root. The extension differed further by the number of teeth affected: ≥30% led to a generalized form and <30% to a localized form.

The radiographic profile of defects surrounding teeth with EPL was graded as cone-shaped or j-shaped. There are indications that vertical root fractures often cause j-shaped lesions on radiographs.[7] Teeth with and without RCT but with j-shaped lesions or significant signs of root damage (e.g., horizontal fractures) were excluded to enable the prevalence of EPLs without root damage to be determined as reliably as possible without clinical data. They were excluded to avoid over- or under-estimating results due to the indistinct representation of anterior teeth on panoramic images.

Teeth with EPL were excluded from staging and grading to achieve a higher variation in periodontal disease severity because they constituted their own periodontal disease entity in the 2017 classification.

Due to the lack of clinical information, stage III and IV periodontitis were combined as stage III/IV periodontitis.

Statistical Methods

This study reports descriptive statistics (number, percentage, minimum, maximum, mean, and standard deviation). The normal distribution assumption was checked with the Shapiro–Wilk test. The means of normally distributed variables between two groups were compared by using the independent samples t-test and The means of normally distributed variables among three or more groups were compared by using analyses of variance. The means of nonnormally distributed variables between two groups were compared by using the Mann–Whitney U test and the means of nonnormally distributed variables among three or more groups using the Kruskal–Wallis test. Fisher's exact test was used to assess the relationship between categorical variables All analyses were performed in using the IBM SPSS (version 25) software.

RESULTS

The patients' demographic and clinical characteristics are shown in Table 1. Thirty-two of the 500 examined panoramic radiographs were excluded from this study. The localization of bone loss (vertical vs. horizontal; data not shown) did not differ significantly by age (p=0.590), the number of missing teeth (p=0.923), or the total number of teeth (p=0.974).

The presence of furcation involvement (data not shown) did not differ significantly by age (p=0.626), the number of missing teeth (p=0.877), or the total number of teeth (p=0.957). Similarly, furcation grade (grades 1, 2, and 3/4; data not shown) did not differ significantly by age (p=0.631), the number of missing teeth (p=0.164), or the total number of teeth (p=0.171).

Table 1. Patients' characteristics

	n	Minimum	Maximum	Mean	Standard deviation
Age	468	18	80	43.32	14.20
Total number of teeth	468	4	32	25.22	5.66
Number of implants	468	0	12	0.18	0.89
Number of decayed teeth	468	0	14	4.03	2.60
Number of filled teeth	468	0	19	3.61	3.46
Number of missing teeth	468	0	28	6.81	5.69
			n		%
Sex	Male		209		44.7
	Female		259		55.3
Presence of fixed prosthesis	No		315		67.3
	Yes		153		32.7
Radiographic bone loss stage	Stage 1		63		13.5
	Stage 2		122		26.1
Extension	Stage 3/4		139		29.7
	<30%		62		13.2
	≥30%		261		55.8
Full mouth diagnosis	Stage 1		56		12.0
	Stage 2		122		26.1
	Stage 3		125		26.7
	Stage 4		19		4.1
Radiographic bone loss	Stage 1		173		37.0
	Stage 2		227		48.5
	Stage 3		68		14.5
EPL grade	Grade 1 or 2		74		50.0
	Grade 3		74		50.0
Radiographic bone loss localization	Vertical		79		53.4
	Horizontal		69		46.6
Radiographic profile	Cone-shaped		76		51.4
	j-shaped		72		48.6
Presence of furcation involvement	Yes		116		78.4
	No		32		21.6
Furcation involvement grade	Grade 1		6		5.2
	Grade 2		69		60.0
	Grade 3 or 4		40		34.8
Presence of restoration material in the related tooth	No		63		42.6
	Yes		85		57.4
Caries status of the related tooth	Yes		132		89.2
	No		16		10.8

The localization of bone loss (vertical vs. horizontal; p=0.503), furcation involvement status (p=0.931), and furcation grades (p=0.668) did not differ significantly by sex (Table 2). The diagnosis did not differ significantly by the localization of bone loss or its extent (p>0.05). However, the horizontal region mostly had stage 1 radiographic bone loss, while the vertical region mostly had stage 2 radiographic bone loss (p<0.05; Table 3).

The diagnosis did not differ significantly by furcation grade or extent (p>0.05). However, the patients with stage 1 or 2 bone loss mostly had grade 2 furcation levels, while those with stage 3 or 4 bone loss mostly had grade 3 or 4 furcation levels (p<0.05; Table 3). The localization of bone loss did not differ significantly by tooth group (p>0.05; Table 4).

The mean age was higher for patients with EPL teeth than patients having teeth without EPL (p<0.05). The mean number of teeth was higher for patients without EPLs than with teeth with EPL (p<0.05). The sex ratio did not differ significantly between patients with and without EPL (Table 5).

Table 2. Relationships between sex and the localization of bone loss, furcation involvement, and grade.

	Sex		Test statistic	p
	Male	Female		
Localization			0.449	0.503
Vertical				
n	41	38		
%	51.9	48.1		
%G	56.2	50.7		
Horizontal				
n	32	37		
%	46.4	53.6		
%G	43.8	49.3		
Presence of furcation involvement			0.007	0.931
Yes				
n	57	59		
%	49.1	50.9		
%G	78.1	78.7		
No				
n	16	16		
%	50.0	50.0		
%G	21.9	21.3		
Furcation grade			0.955**	0.668
Grade 1				
n	4	2		
%	66.7	33.3		
%G	7.1	3.4		
Grade 2				
n	32	37		
%	46.4	53.6		
%G	57.1	62.7		
Grade 3 or 4				
n	20	20		
%	50.0	50.0		
%G	35.7	33.9		

Key: G, sex; **, Fisher's exact test.

Table 4. Relationships between localization of bone loss and tooth groups

	Localization		Test statistic	p
	Vertical	Horizontal		
Groups			4.824**	0.433
Maxillary molars				
n	32	31		
%	50.8	49.2		
Maxillary premolars				
n	4	4		
%	50.0	50.0		
Maxillary incisors				
n	0	4		
%	0.0	100.0		
Mandibular molars				
n	29	30		
%	49.2	50.8		
Mandibular premolars				
n	8	2		
%	80.0	20.0		
Mandibular incisors				
n	3	1		
%	75.0	25.0		

Key: **, Fisher's exact test

The patients with stage 1 or 2 bone loss mostly had teeth with EPL, while those with stage 3 or 4 mostly did not have teeth with EPL (p<0.05). The patients with <30% localized extent mostly had teeth with EPL (p<0.05). The patients with full mouth stage 1 or 2 diagnoses mostly had teeth with EPL, while those with full mouth stage 3 or 4 diagnoses mostly did not have teeth with EPL (p<0.05). The patients with grade A or B bone loss mostly had teeth with EPL, while those with grade C bone loss mostly did not have teeth with EPL (p<0.05; Table 6). Maxillary incisors, mandibular premolars, and mandibular incisors mostly had a "j" profile (Table 7).

Table 6. Relationships between EPL tooth status and bone loss grade.

	Have an EPL		Test statistic	p
	Yes	No		
Bone loss grade			117.057	<0.001*
A				
n	164	9		
%	94.8	5.2		
B				
n	133	94		
%	58.6	41.4		
C				
n	23	45		
%	33.8	66.2		

Key: *, p<0.05

Table 3. Relationships between localization of bone loss, the furcation grade, EPL tooth status and radiographic bone loss stage, extent, and diagnosis.

	Localization		Furcation grade			Have an EPL	
	Vertical	Horizontal	Grade 1	Grade 2	Grades 3 and 4	Yes	No
Radiographic bone loss stage							
Stage 1							
n	4	8	1	6	0	51	12
%	33.3	66.7	14.3	85.7	0.0	81.0	19.0
Stage 2							
n	33	15	3	24	8	74	48
%	68.8	31.3	8.6	68.6	22.9	60.7	39.3
Stages 3 and 4							
n	41	45	2	38	31	53	86
%	47.7	52.3	2.8	53.5	43.7	38.1	61.9
Test statistic/p	7.839**/0.033*			11.762/0.040*		34,696/<0.001*	
Extent							
Localized form							
n	10	6	1	8	2	46	16
%	62.5	37.5	9.1	72.7	18.2	74.2	25.8
Generalized form							
n	68	61	5	60	37	132	129
%	52.7	47.3	4.9	58.8	36.3	50.6	49.4
Test statistic/p	1.107**/0.575			3.222**/0.503		11.297/<0.001*	
Full mouth diagnosis							
Stage 1							
n	4	7	1	6	2	45	11
%	36.4	63.6	11.1	66.7	22.2	80.4	19.6
Stage 2							
n	29	13	2	22	5	80	42
%	69.0	31.0	6.9	75.9	17.2	65.6	34.4
Stage 3							
n	39	38	3	37	24	48	77
%	50.6	49.4	4.7	57.8	37.5	38.4	61.6
Stage 4							
n	6	9	0	3	8	4	15
%	40.0	60.0	0.0	27.3	72.7	21.1	78.9
Test statistic/p	7.217/0.125			13.186**/0.066		42.818/<0.001*	

Key: *, p<0.05; **, Fisher's exact test

Table 5. Comparison of patients' age, sex, and number of teeth by EPL status.

	n	Mean	Standard deviation	Rank average	Test statistic	p
Age						
Without EPL	320	40.66	14.78	208.64	15403.5**	<0.001*
With EPL	148	49.08	10.83	290.42		
Total number of teeth						
Without EPL	320	25.43	5.81	244.00	20640.5**	0.025*
With EPL	148	24.77	5.32	213.96		

	Have an EPL		Test statistic	p
	Yes	No		
Sex				
Male			1.907	0.167
n	136	73		
%	65.1	34.9		
%S	42.5	49.3		
Female				
N	184	75		
%	71.0	29.0		
%S	57.5	50.7		

Key: **, Mann–Whitney U test; S, status.

Table 7. Relationships between radiographic profile and tooth number.

	Radiographic profile		Test statistic	p
	Cone-shaped	j-shaped		
Groups			11.324	0.031*
Maxillary molars				
n	33	30		
%	52.4	47.6		
%P	43.4	41.7		
Maxillary premolars				
n	4	4		
%	50.0	50.0		
%P	5.3	5.6		
Maxillary incisors				
n	1	3		
%	25.0	75.0		
%P	1.3	4.2		
Mandibular molars				
n	36	23		
%	61.0	39.0		
%P	47.4	31.9		
Mandibular premolars				
n	1	9		
%	10.0	90.0		
%P	1.3	12.5		
Mandibular incisors				
n	1	3		
%	25.0	75.0		
%P	1.3	4.2		

Key: *, p<0.05; P, profile.

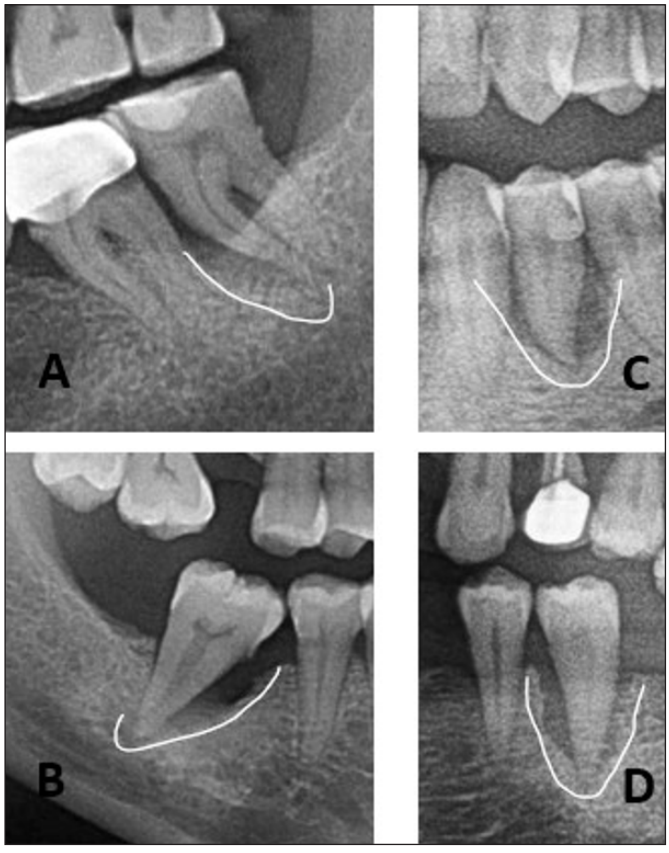

Figure 1. Different radiographic images: j-shape (A-B) and cone-shape (C-D)

DISCUSSION

This study explored the prevalence and radiographic characteristics of EPLs according to the 2017 World Workshop on Periodontal and Peri-Implant Disease Classification. Evaluation criteria include age, sex, the total number of teeth, the number of implants, the number of carious teeth, the number of filled teeth, the number of missing teeth, fixed prosthesis status, radiographic bone loss stage, extension status, full mouth diagnosis, radiographic bone loss, EPL grade, radiographic bone loss localization, furcation involvement, restorative material status of the relevant tooth, and the carious status of the relevant tooth. While previous classifications were used in studies on the presence of EPLs,[8-12] and there is only one study on the presence of EPL using the new classification has been published.[6] Therefore, our study is distinctive in detecting the presence of EPL according to the new classification. In addition, our study aimed to contribute to the literature by referring to the parameters not clarified in previous studies.

EPL was diagnosed in 148 of the 468 radiographs in this study, corresponding to 31.6% of this population. This rate varies widely in other studies,[8-10,12] possibly due to differences in the study population and the number of radiographs.

The EPL diagnosis should respond to tooth preservation or extraction. In evaluation, there are three types of EPL dental diagnoses: hopeless, classified for extraction; poor or good, classified for treatment.[1] EPLs have always been challenging to treat due to their lower success rate than endodontic or periodontal lesions alone. Periodontal involvement following endodontic lesions is complex because it is often accompanied by massive periodontal destruction that can compromise tooth viability.[13] Under the new classification in periodontology, EPLs are mainly classified by combining radiographic images with clinical findings, resulting in clearer classification concepts. In our study, importance was given to analyzing the shape of the lesions to reduce the impact of the lack of clinical data. It was found that mostly stage 1 radiographic bone loss was in the horizontal region and mostly stage 2 radiographic bone loss was in the vertical region. In addition, the patients with stage 1 or 2 bone loss mostly had grade 2 furcation involvements, while those with grade 3 or 4 mostly had grade 3 or 4. These results are consistent with the characteristics of the furcation lesions.[14]

In our study, the mean age was higher for patients with EPLs than teeth without EPL. These results are similar to the study by Walton,[6] suggesting that EPL development may be an age-related condition. A higher prevalence of EPLs in patients aged 31–40 years was shown in the

study by Prashaanthi et al.[10] That range is similar to the age of our study cohort. The mean number of teeth was higher for patients without EPL than teeth with EPL. The high number of teeth may also reduce the risk of EPL development by reducing the number of caries due to regular oral hygiene habits. Considering behavioral factors such as oral hygiene habits in future studies is valuable in determining the validity of this parameter.

In our study, the patients with stage 1 or 2 bone loss mostly had teeth with EPL, while those with stage 3 or 4 mostly did not have teeth with EPL. The patients with full mouth stage 1 or 2 diagnoses mostly had teeth with EPL, while those with full mouth stage 3 or 4 diagnoses mostly did not have teeth with EPL. The patients with grade A or B bone loss mostly had teeth with EPL, while those with grade C bone loss mostly did not have teeth with EPL. This finding suggests that more tooth extraction is preferred in cases with advanced periodontitis. This interpretation can be made for population reasons since age is more advanced in these patient groups.

Teeth with a j-shape or cone-shape in radiographic images are usually extracted because of the coexistence of EPLs and a hopeless prognosis. Historically, clinicians have attempted to save these teeth with various treatments, including scaling and root planning, periodontal regeneration techniques, and endodontic surgery. However, the prognosis is unfavorable.[15,16] Therefore, the prevalence of teeth with a j-shape or cone-shape in radiographic images was comparable and similar to the study by Ruetters et al.[6]

Similar to previous studies,[6,8] EPLs were most frequently observed in molars in our study. This result is unsurprising due to lack of access for brushing molar areas and the periodontal treatment of those areas.[1] Restorations (57.4%) or caries (89.2%) were detected in most teeth with EPL. Restorations with inadequate coronal closure may affect the development of EPL. The presence of caries may also increase the risk of EPL by causing pulp infections.

One limitation of this study was that only panoramic radiographs were used since periapical radiographs, cone beam computed tomography (CBCT) images, and additional clinical findings were unavailable. Clinical findings and CBCT may help for distinguishing patients with active and inactive periodontal disease states, which is impossible with two-dimensional radiographic images alone. In addition, staging by radiographs alone is not as valid as staging with additional clinical and anamnestic information, and grading is not possible. Nevertheless, each image should be examined for common or rare findings to provide a complete assessment of the radiographic anatomy. Panoramic radiographs have also some limitations in image clarity and reliability. Therefore, they were not used to measure precise radiographic bone loss for anterior teeth staging to avoid inaccurate estimates. In addition, visual disturbances due to superposition caused by imaging or improper positioning of the patient's head were also excluded from this study. Caries, periapical inflammation, periodontal bone loss (PBL), and EPLs were considered easily detectable on periapical radiographs, further justifying scientific research. CBCT is also increasingly used in daily practice.[17] A three-dimensional image is more precise than a two-dimensional image and allows multiple viewing layers to be displayed. A problem with CBCT use may be inexperience, leading to misreading the image for artifacts and grayscales.[18] In addition, our study population does not reflect an entire population since it comprised patients attending a university hospital-based clinic with many patients. Therefore, care should be taken when interpreting the study's results.

CONCLUSION

This prevalence study detecting caries, periodontal status, PBL, and EPLs using panoramic radiographs documented moderate to significant reliability data for the classifications proposed by Working Group 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Besides the complexity of concurrent endodontic and periodontic treatments, the clinical procedure is complex, the sequence of procedures must be rigorous, and selecting appropriate materials is critical for optimal and successful treatment in these EPL cases.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Medipol University Non-invasive Clinical Studies Ethics Committee (Date: 2023, Decision No: 64).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Analysis of the relationship between clinical features, treatment options and recurrence of patients diagnosed with anogenital warts

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ABSTRACT

Aims: Our study aimed to describe the demographic and clinical characteristics of patients with anogenital warts and to investigate the relationship between treatment options and recurrence.

Methods: The data of patients who were admitted to the dermatology, urology, and gynecology outpatient clinics between 2010 and 2021, and diagnosed with anogenital warts were retrospectively analyzed. Demographic characteristics of the patients, presence of other sexually transmitted diseases, anatomical distribution of warts, number of anatomical regions and warts, frequency of recurrence, type of treatment before the first recurrence, and follow-up periods were documented. Statistical analysis was performed and the results were evaluated at a 95% confidence interval and $p<0.05$.

Results: A total of 201 patients, 181 (90%) male and 20 (10%) female, who met the study criteria, were included in the study. The ages of the patients vary between 20-67 years; the median was 31 years. The rate of the number of warts of 10 or more in patients with recurrence was found to be statistically significantly higher than in cases without recurrence (p=0.013). The recurrence rate was statistically significantly higher in patients with pubic localization (p=0.001). There was a significant difference between the number of localization regions according to recurrence status (p=0.003). The recurrence rate of patients who received cryotherapy was statistically significantly higher (p=0.002). According to the logistic regression analysis; the number of 10 or more warts increases the risk of recurrence to 2.665 times (95% CI: 1.225-5.799) (p=0.013). Cryotherapy increases the risk of recurrence to 6.243 times (95% CI: 1.786-21.828) (p=0.004). Male sex increases the risk of recurrence to 3.034 times (95% CI: 1.029-8.940) (p=0.044).

Conclusion: Anogenital warts often recur even if they disappear completely after treatment. It has been observed that the recurrence is more common when the number of warts is more than 10. Recurrence may be observed more frequently in the male gender. Recurrence occurs more frequently with cryotherapy than electrocauterization. The importance of prophylactic human papillomavirus vaccination in preventing anogenital warts is emerging once again due to the high recurrence rate and prolonged treatment period.

Keywords: Anogenital warts, clinical features, recurrence

INTRODUCTION

Anogenital warts are the most common sexually transmitted diseases and the causative agent is human papillomavirus (HPV). There are more than 170 subtypes of HPV, and genital warts are most commonly caused by type 6 and type 11. The incubation period of the disease varies between 2 and 50 months, and the quality of life of patients is negatively affected. Anogenital warts are more common in men than in women. It plays a role in the etiology of penile, oropharyngeal and anal cancer in men, and cervical, anal and oropharyngeal cancers in women. About 30% of genital warts disappear within four months after it is formed. However, most of the patients require treatment. Treatment options include topical agents, cryotherapy, electrocauterization, surgical excision and ablative laser treatments, but recurrence after treatment is frequent. Although treatment is administered, recurrence occurs within 3 months in 25-67% of cases. Therefore the treatment takes a long time.[1-3] Our study aimed to describe the demographic and clinical characteristics of patients with anogenital warts and to investigate the relationship between treatment options and recurrence.

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METHODS

The records of patients who were admitted to the dermatology, urology and gynecology outpatient clinics of the private hospital between 2010 and 2021, and diagnosed with anogenital warts were retrospectively scanned. Before the study, ethics committee approval was obtained from the Medicana Hospital Ethics Committee (Date: 31.03.2021, Decision No: 09). All procedures were carried out in accordance with the ethical rules ant the principles of the Declaration of Helsinki. Patients who presented for the first time with the diagnosis of anogenital warts and were followed up for at least 12 months were included in the study. Demographic characteristics of the patients, comorbidities, presence of other sexually transmitted diseases (STIDs) or infections (including hepatitis B and hepatitis C viruses, HIV, herpes simplex virus type 2, *treponema pallidum, ureaplasma urealyticum, chlamydia trachomatis and mycoplasma hominis* infections), anatomical distribution of warts, number of anatomical regions, number of warts, types of treatment, frequency of recurrence, type of treatment before the first recurrence, and follow-up periods were documented. Recurrence was considered to be the reappearance of lesions after their clearance with at least one method of treatment. Histopathological examination was performed in the majority of cases but HPV type determination could not be performed due to the lack of insurance coverage in most of the patients.

Patients who had previously been treated for anogenital wart, who had a clinical follow-up of less than 12 months and who had more than 3 years between recurrences (due to the risk of infection with different HPV types) were excluded from the study.

Statistical Analysis

While evaluating the findings obtained in the study, NCSS (Number Cruncher Statistical System) 2020 Statistical Software (NCSS LLC, Kaysville, Utah, USA) program was used for statistical analysis. When evaluating the study data, quantitative variables were determined by mean, standard deviation, median, minimum and maximum values; qualitative variables were indicated by descriptive statistical methods such as frequency and percentage. Shapiro Wilks test and Box Plot plots were used to evaluate the suitability of the data to the normal distribution. Student's t-test was used for two quantitative group evaluations with normal distribution. Logistic regression modeling was performed in further evaluation of the relationships between the variables. In the comparison of qualitative data, the Chi-Square test, Fisher's Exact test and Fisher Freeman Halton test were used. The results were evaluated at a 95% confidence interval and $p<0.05$.

RESULTS

The records of 830 patients diagnosed with anogenital warts were retrospectively analyzed. Of these patients, a total of 201 patients, 181 (90%) male and 20 (10%) female, who met the study criteria, were included in the study. The ages of the patients vary between 20-67 years; the median was 31 years (mean age: 33.63±8.88 years). 45.8% of the patients were 30 years of age or younger, 34.8% were between 31-40 years old, and 19.4% were over 40 years old. While 55.7% (n=112) of the cases participating in the study were single; 44.3% (n=89) were married. It was determined that 48.1% of the patients were smokers. When their alcohol use was examined, it was seen that 48.1% used alcohol occasionally, 3.1% used regular alcohol, and 48.8% did not use alcohol. Detailed demographic characteristics of the patients are given in Table 1.

Table 1. Distributions of descriptive features

		n (%)
Gender	Female	20 (10.0)
	Male	181 (90.0)
Age (year)	Mean±Sd	33.63±8.88
	Median (Min-Max)	31 (20-67)
	≤30	92 (45.8)
	31-40	70 (34.8)
	>40	39 (19.4)
Marital status	Single	112 (55.7)
	Married	89 (443)
Smoking	No	98 (51.9)
	Yes	91 (48.1)
Alcohol use (n=162)	No	79 (48.8)
	Occasionally	78 (48.1)
	Regularly	5 (3.1)

n: number of patients

Disease onset ranged from 0.5 months to 12 months, and the mean disease duration was 2 months. When the anatomical distribution of warts is examined; the warts were most commonly located together in two anatomical regions with a rate of 45.3%. The most common anatomical localization of the warts was the penis region with a rate of 75.6% and the second was the pubis region with a rate of 38.7% (there were multiple locations in the same patient). At the time of first admission, 41.3% of the patients had a wart number of less than 10; 58.7% had a wart number of 10 or more.

When the treatments were examined, it was determined that 38.3% of the patients received only electrocauterization treatment, 18.9% received cryotherapy, electrocauterization and topical imiquimod treatment, 12.4% received electrocauterization and topical imiquimod treatment, and 10% underwent only cryotherapy during the follow-up period. Those who use topical imiquimod alone constitute 5% of patients. At least one or more recurrences were observed in 82.6% of patients. The mean duration of the first recurrence was 8.0±7.4 months. When the number of recurrences

occurs is examined; 38% of the patients with recurrence had recurrence 1 time, 15.7% had 2 times, 21.7% had 3 times, and 24.7% had 4 or more recurrences.

When the treatments given before the first recurrence are examined; 61.7% of the patients received electrocauterization, 27.9% received cryotherapy, 6.4% received topical imiquimod, 2% received cryotherapy and topical imiquimod, 1.5% received electrocauterization and topical imiquimod, and one patient received podophyllotoxin. Other STIDs were present in 33.9% of the patients. The most common concomitant infection was ureaplasma infection with a rate of 15.1%. The mean follow-up period of the patients was 28.3±19.7 months, ranging from 12 to 120 months. The clinical and therapeutic characteristics of the patients are detailed in Table 2.

When the relationship between the demographic characteristics of the patients and recurrence was examined, the frequency of recurrence according to the gender, age, marital status, smoking and alcohol use of the patients did not show a statistically significant difference (Table 3) (p>0.05).

Table 3. Evaluation of the relationship between recurrence and demographic characteristics

		Recurrence		p value
		No (n=35) (%)	Yes(n=166) (%)	
Gender	Female	7 (20.0)	13 (7.8)	[a]0.055
	Male	28 (80.0)	153 (92.2)	
Age (year)	Mean±Sd	34.00±9.63	33.56±8.75	[b]0.791
	Median (Min-Max)	32 (20-55)	31 (20-67)	
	≤30	14 (40.0)	78 (47.0)	
	31-40	13 (37.1)	57 (34.3)	
	>40	8 (22.9)	31 (18.7)	
Marital status	Single	18 (51.4)	94 (56.6)	[c]0.574
	Married	17 (48.6)	72 (43.4)	
Smoking	No	20 (57.1)	78 (50.6)	[c]0.488
	Yes	15 (42.9)	76 (49.4)	
Alcohol use (n=162)	No	20 (66.7)	59 (44.7)	[d]0.201
	Occasionally	10 (33.3)	68 (51.5)	
	Regularly	0 (0)	5 (3.8)	

[a]Fisher Exact Test, [b]Student-t Test, [c]Pearson Chi-Square Test, [d]Fisher Freeman Halton Test

When non-recurrence cases and recurrent cases were compared, there was no statistically significant difference in the incidence of other STIDs (p>0.05).

The rate of the number of warts of 10 or more in patients with recurrence was found to be statistically significantly higher than in cases without recurrence (p=0.013; p<0.05). The recurrence rate was statistically significantly higher in patients with pubic localization (p=0.001; p<0.01). The rate of recurrence was significantly higher in patients with penile localization (p=0.018; p<0.05). Scrotum, perineum/perianal, vulva and vagina localizations did not differ significantly in terms of recurrence (p>0.05).

Table 2. Distribution of clinical and treatment features of patients

	n (%)
Onset duration of disease (month)	
Mean±Sd	2.99±2.49
Median (Min-Max)	2 (0.5-12)
Distributions of anatomical region ▪	
Pubis	138 (38.7)
Penis	152 (75.6)
Scrotum	38 (18.4)
Perine/perianal	15 (7.5)
Vulva	14 (7.0)
Vagina	5 (2.5)
Number of anatomical regions	
1	76 (37.8)
2	91 (45.3)
≥3	34 (16.9)
Number of warts	
<10 lesion	83 (41.3)
≥10 lesion	118 (58.7)
All treatments during the follow-up	
Cryotherapy	20 (10.0)
Electrocauterization	77 (38.3)
Topical imiquimod	5 (2.5)
Cryotherapy-Topical imiquimod	9 (4.5)
Electrocauterization-Topical imiquimod	25 (12.4)
Cryotherapy-Electrocauterization-Topical imiquimod	38 (18.9)
Electrocauterization-Cryotherapy	25 (12.4)
Other	2 (1.0)
Recurrence	
No	35 (17.4)
Yes	166 (82.6)
Number of recurrences (n=166)	
1	63 (38.0)
2	26 (15.7)
3	36 (21.7)
≥4	41 (24.7)
Time of the first recurrence (month)	
Mean±Sd	8.0±7.4
Median (Min-Max)	5(1-34)
Follow-up duration (month)	
Mean±Sd	28.3±19.7
Median (Min-Max)	20 (12-120)
Treatment before the first recurrence	
Cryotherapy-Topical imiquimod	56 (27.9)
Electrocauterization	124 (61.7)
Topical imiquimod	63 (6.4)
Cryotherapy-Topical imiquimod	4 (2.0)
Electrocauterization-Topical imiquimod	3 (1.5)
Other	1 (0.5)
Other STIDs (n=186)	
No	123 (66.1)
Yes	63 (33.9)
Herpes genitalis	10 (5.4)
Hepatitis B	6 (3.2)
Molluscum contagiosum	10 (5.4)
Ureaplasma	28 (15.1)
Ureaplasma-Mycoplasma	4 (2.2)
Herpes genitalis-Molluscum contagiosum	2 (1.1)
Hepatitis B-Molluscum contagiosum	1 (0.5)
Mycoplasma	1(0.5)
Gonorrhea	1 (0.5)
Co-morbidities (n=199)	
No	158 (79.4)
Yes	41 (20.6)
Arterial hypertension	10 (24.3)
Diabetes	5 (12.2)
Psychiatric disorders	3 (7.3)
Cancer	3 (7.3)
Atopic dermatitis/Psoriasis	3 (7.3)
Rheumatoid disease	7 (17.1)
Thyroid disease	4 (9.8)
Epilepsy	2 (4.9)
Other	4 (9.8)

▪ When more than one anatomical region was detected in the same patient, multiple options were selected in these patients.

There was a significant difference between the number of localization regions according to recurrence status (p=0.003; p<0.01); As the number of localization regions increases, the incidence of recurrence increases.

The recurrence rate of patients who received cryotherapy was statistically significantly higher (p=0.002; p<0.01). While the recurrence rate did not show significant differences in those who received electrocauterization treatment (p>0.05); the recurrence rates of those treated with topical imiquimod were not statistically significant (p>0.05). These data are detailed in Table 4.

Table 4. Evaluation of the relationship between recurrence and clinical features

		Recurrence		p value
		No (n=35) (%)	Yes (n=166) (%)	
Other STIDs	No	18 (52,9)	105 (69,1)	[c]0,072
	Yes	16 (47,1)	47 (30,9)	
Number of warts	<10	21 (60,0)	62 (37,3)	[c]0,013*
	≥10	14 (40,0)	104 (62,7)	
Distributions of anatomical region ▪	Pubis	16 (45,7)	122 (73,5)	[c]0,001**
	Penis	21 (60,0)	131 (78,9)	[c]0,018*
	Scrotum	8 (22,9)	30 (18,2)	[c]0,511
	Perine/perianal	5 (14,3)	10 (6,0)	[c]0,091
	Vulva	4 (11,4)	10 (6,0)	[c]0,254
	Vagina	1 (2,9)	5 (3,0)	[c]1,000
Number of anatomical regions	1	22 (62,9)	54 (32,5)	[c]0,003**
	2	8 (22,9)	83 (50,0)	
	≥ 3	5 (14,3)	29 (17,5)	
Treatment before the first recurrence ▪	Cryotherapy	3 (8,6)	57 (34,3)	[d]0,002**
	Electrocauterization	27 (77,1)	100 (60,2)	[d]0,060
	Topical imiquimod	5 (14,3)	15 (9,0)	[d]0,346

▪When more than one anatomical region or treatment option was detected in the same patient, multiple options were selected in these patients. [c]Pearson Chi-Square Test, [d]Fisher Freeman Halton Test, **p<0,01 *p<0,05

Logistic Regression Analysis of Risk Factors Affecting Recurrence

Gender, number of lesions, localization pubis, penis, perineum/perianal localizations and treatments were evaluated by Backward Stepwise Logistic regression analysis from the risk factors affecting recurrence. The 6-step model for risk factors affecting recurrence is shown in Table 5.

Table 5. Logistic regression analysis of risk factors affecting recurrence

	P value	ODDS	%95 CI	
			Lower	Upper
Gender (male)	0,044*	3,034	1,029	8,940
Penile localization (+)	0,659	1,271	0,438	3,693
Perine/perianal localization (+)	0,893	1,139	0,170	7,651
First treatment electrocauterization (+)	0,541	1,597	0,355	7,181
First treatment topical imiquimod (+)	0,789	1,293	0,197	8,475
Pubic localization (+)	0,273	1,639	0,677	3,966
Number of warts (≥10)	0,013*	2,665	1,225	5,799
First treatment cryotherapy (+)	0,004**	6,243	1,786	21,828

*p<0,05 **p<0,01

The variables included in the study were evaluated by Backward Stepwise Logistic regression analysis. In this study, it is seen that gender, number of warts and cryotherapy constitute a significant model of risk factors that have an effect on recurrence at the end of step 6 (p=0.003; p<0.01). The explanatory coefficient of the model is 83.1%.

According to the model; the number of 10 or more warts increases the risk of recurrence to 2.665 times (95% CI: 1.225-5.799) (p=0.013; p<0.05). Cryotherapy increases the risk of recurrence to 6.243 times (95% CI: 1.786-21.828) (p=0.004; p<0.01). Male sex increases the risk of recurrence to 3.034 times (95% CI: 1.029-8.940) (p=0.044; p<0.05). Gender, number of warts and cryotherapy are independent risk factors for the presence of recurrence.

DISCUSSION

In our study, the demographic and clinical characteristics of patients with anogenital warts were examined and the conditions affecting recurrence were tried to be analyzed. Known risk factors for anogenital warts are male gender, being under 30 years of age, smoking history, a high number of sexual partners, unprotected sexual intercourse, HIV positivity, presence of other STIDs, and immunosuppression.[4-6] In our study, male gender and under 30 years of age were observed at a high rate in patients with these risk factors. In another study conducted in our country, it was seen that 88% of the patients with anogenital warts were male and 65% were under 35 years of age.[6] The reason why there are fewer female patients in our study can be explained by the fact that female patients apply to gynecology more frequently due to genital warts.

While 48.1% of our patients smoked, alcohol consumption was 52.2%. In the study of Tamer et al.[6] from our country, the smoking rate was 61% in patients. It has been shown in studies that the risk of HPV increases as smoking increases, and it has also been found to be a factor in recurrence.[7] In a meta-analysis examining studies on anogenital warts from the Sub-Saharan Africa region, it was found that smoking was a risk factor for anogenital warts in female patients.[8] When other risk factors are examined; since our study was retrospective, there was no data on the sexual behavior of most patients. Approximately half of the patients were married. In the study of Tamer et al.[6] it was shown that 43.5% of the patients were married and this rate was lower than the control group.

Other STIDs were observed in 33.9% of cases. In other

studies, this rate was found to be 11.1%, 10.2%, 25.6% and 31.2%.[2,9-11] In a meta-analysis conducted in Sub-Saharan Africa, it was shown that the risk of anogenital wart was increased in HIV-positive women and men; and that bacterial vaginosis in women was a risk factor for anogenital wart.[8] Recurrence is the most important problem of anogenital warts and is common despite treatment. In clinical and observational studies, the recurrence rate varies between 26% and 67% depending on the treatment used in anogenital warts. Recurrence is usually seen within three months after clearance. This leads to deterioration of the quality of life in patients, negative effects on relationships and an increase in the cost burden of treatment.[9]

In our study, the recurrence rate was as high as 82.6%, and 24.7% of the patients required at least four or more treatments. In a retrospective study conducted in Canada, at least 1 recurrence was observed in 48.5% of men who mostly underwent cryotherapy, and four or more recurrences were observed in 5.3%.[9] In a study conducted in Brazil, Mexico and the USA, 1 or more recurrences were seen in 44.3% of patients, while 4 or more recurrences were seen in 6.5% of patients.[12] In a study evaluating women with anogenital warts treated with laser, the recurrence rate was found to be approximately 30% at least 1 time after follow-up.[13] In the study conducted by Demir and Güder from Turkey, the recurrence rate was observed at a rate of 29.9% in patients.[14]

Although in other studies, the rate of patients decreased as the number of recurrences increased; there is no tendency to decrease in the rate of our patients, especially those with 4 or more recurrences. The difference in the factors affecting recurrence in the studies and the fact that the same treatment model was not applied to the patients may have caused different rates to be obtained. In our study, the mean duration of initial recurrence was 8 months. In other studies, the mean initial recurrence time was 53.7 months, 4.52 years and 14.6 weeks.[9,12,13]

The effect of age, marital status, smoking, alcohol use and the presence of other STIDs on recurrence could not be demonstrated. Although studies are showing that the recurrence rate decreases with increasing age, there was no difference between age and recurrence rate in our study. In another study, although the frequency of anogenital warts decreased with age, there was no relationship between recurrence and age.[12,13] The female sex was higher in the non-recurrence group, and regression analysis showed that the male sex was an independent factor that tripled the risk of recurrence. It was thought that the high number of male patients could cause this difference. Other studies have not shown an effect of gender on recurrence.[11] Although smoking for more than 10 years has been shown to affect recurrence; another study did not show the effect of smoking on recurrence as in our study.[10,11]

In our study, the frequency of other STIDs was not different in patients with recurrence. Similarly, in the study of Habel et al.[10] although the frequency of other STIDs was more frequent in the anogenital wart group than in the control, it was not found to affect recurrence. In the study conducted by Zhan et al.[11] it was shown that urogenital diseases are one of the independent factors affecting recurrence in patients with anogenital warts. In our study, the number of warts more than 10 was shown as an independent factor affecting recurrence. Similarly, in Zhan et al.[11] the wart number was found to be an independent factor in recurrence. In the study of Demir et al.[14] from Turkey, the effect of the number of lesions on recurrence could not be shown.

In addition to the number of warts, the location of the lesions in more than one anatomical region, the location of the pubic and penile region and cryotherapy t were found to affect recurrence, and in the regression analysis, it was shown that the number of warts was more than 10, male gender and cryotherapy were independent factors affecting recurrence.

It has been previously shown that if the location of the warts is multifocal, recurrence is more frequent (3 times more) and the frequency of recurrence increases as the number of affected areas increases.[13] In our study, the risk of recurrence increases as the number of sites increases. Although recurrence in the pubic and penile regions was more frequent when the affected areas were examined, they were not identified as an independent factor in regression analysis. Similarly, in another study, no relationship between the anatomical region and recurrence could be shown.[13]

Recurrence of 20-30% or more can be seen in anogenital warts with all types of treatment.[15] Although cryotherapy is a relatively easy and non-time-consuming procedure, rarely causes scarring and depigmentation and can be applied in pregnant women, the recurrence rate within 1-3 months after clearance has been reported as 21-42%. In electrocauterization, the risk of recurrence has been reported as 19-29%. With topical imiquimod treatment, recurrence rates (6-26%) are relatively low.[15] In a study comparing treatment types, complete clearance of warts was observed in 41%, 79% and 94% of patients receiving podophylline, cryotherapy and electrocautery therapy, respectively. Recurrence occurred in 25% of all patients and 3-month clearance rates of 17%, 55% and 71% were given for podophylline, cryotherapy and electrocautery, respectively.[16] In other studies, the effects of treatment on recurrence were not evaluated because patients

received same treatment.[9,11]

In our study, when the relationship between treatment and recurrence was examined, it was found that the risk of recurrence was higher in patients who underwent cryotherapy. There was no significant difference in electrocauterization and topical imiquimod treatments. Studies have shown that cryotherapy has lower clearance rates than electrocauterization; this may explain the fact that the recurrence is higher in the cryotherapy group. Topical imiquimod treatment was used especially at the warts of the perianal region in our study. In a randomized controlled trial, the efficacy of topical imiquimod in this region is shown that high in female patients.[17] Accordingly in our study, it was thought that there was no significant relationship between topical imiquimod and recurrence.

The limitations of our study are that retrospective design and the small sample size, as well as the inability to perform HPV typing in patients and the absence of sexual behavior data.

CONCLUSION

As a result, anogenital warts often recur even if they disappear completely after treatment. It has been observed that the recurrence is more common when the number of affected anatomic sites increases and the number of warts is more than 10. Recurrence may be observed more frequently in the male gender. Recurrence occurs more frequently with cryotherapy than electrocautery treatment.

The importance of prophylactic HPV vaccination in preventing anogenital warts is emerging once again due to the high recurrence rate and prolonged treatment period.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Medicana Hospital Ethics Committee (Date: 31.03.2021, Decision No: 09).

Informed Consent: Because the study was designed retrospectively, no written informed consent from was obtained from the patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Original Article

HEALTH SCIENCES
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Perceptions of finger-amputated hand appearance and its effects on social life from the perspectives of affected and unaffected individuals

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ABSTRACT

Aims: Traumatic finger amputation leads to dysfunction and disfigurement of the hand. Hand disfigurements can affect the social lives of affected individuals. The purpose of this study was to investigate affected and unaffected individuals' perceptions of finger-amputated hand appearance and its effects on social life.

Methods: A group of 26 finger-amputated individuals completed a questionnaire assessing the impact of their hand appearance on their social lives and rated their hand appearance using a visual analogue scale (VAS). Hand photographs of members of this group were shown to 50 unaffected individuals, who were asked to rate their appearance using the VAS and complete a questionnaire assessing their reactions to similar hands encountered during daily life.

Results: In the patient group, the mean VAS and questionnaire scores were 5.1±2 (range: 1–10), and 60.3±17 (range: 31–94), respectively. In the assessor group, the mean VAS score for all 26 hand photographs was 7.1±2 (range: 3–9) and the mean questionnaire score was 83.6±11 (range: 56–100). While the patients' and assessors' VAS scores were significantly correlated for each hand photograph (r=0.511, p=0.008), in total, 21 of the 26 VAS scores were lower for the patient than assessor group. The VAS and questionnaire scores in the patient group improved significantly with time since amputation (p =.00 and p=0.011, respectively).

Conclusion: The self-evaluation results of the affected group were worse than those of the unaffected group in terms of perceptions of appearance and the resultant effects on their social lives. We conclude that individuals without finger-amputated hands perceived hand disfigurements less negatively than affected patients. This knowledge may aid the psychosocial support of patients with hand anomalies.

Keywords: Finger-amputated, hand appearance, perception, psychosocial impact

INTRODUCTION

Finger amputation is one of the most frequent injuries affecting the upper extremities.[1,2] Finger replantation is required surgical procedures for amputations. Despite technological advancements, the outcome of replantation is dependent on both immutable and modifiable factors (e.g., type of injury, patient age, medical condition, and risk factors).[3,4] Therefore, after these injuries, individuals may be forced to live the rest of their lives with a finger-amputated hand.

The appearance of the hand is important in social life.[5-9] After the face, the hand is the most visible part of the body and is also used as a means of expression. Traditionally, functional results were prioritized when resolving hand-related problems. However, hand appearance is also important in the evaluation of treatment results in hand-related problems.[9-13] Few studies have dealt with the relationship between hand appearance and social life.[7,9-17] Nevertheless, in all these mentioned studies, the appearance of the hand has been evaluated by the patient, the parent, or the researchers.[7,9-13] In this study, we evaluated the effects of finger-amputated hand appearance on social life from the perspectives of affected and unaffected individuals.

METHODS

The study was carried out with the permission of Mersin University Medical Faculty Clinical Researches Ethics Committee (Date: 04/09/2019, Decision No: 2019/366). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of

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Helsinki. Two study groups were formed. The first (patient group) consisted of individuals with amputated fingers after hand injuries. The second (assessor group) consisted of unaffected participants who were asked to assess the patients' hand photographs and complete a related survey. The number of individuals in the patient and assessor groups was set to minimum 25 participants because these would provide us with adequate power (1-β=0.9, α=0.05). Informed consent was obtained from all study participants.

Patient Group

We included patients who had undergone single or multiple finger stump revision surgery after traumatic amputation, had fully healed wounds, were aged 18–65 years, and agreed to participate in the study. Patients with a history of receiving any form of psychiatric support or treatment were excluded from the study. Patients were invited to participate in the study on presenting to the orthopedic outpatient clinic with a complaint other than their injured hand. A total of 26 patients (26 males) were included. The mean age of the patient group was 36.2±14 years (range: 18–65 years), and the mean time after injury was 51.1±103 months (range: 3–400 months).

Information regarding the level of the amputations, involved rays, and number of amputated fingers was collected. Regarding the level of the amputations, 9 amputations were distal to the distal interphalangeal (DIP) joint, 7 were between the proximal interphalangeal (PIP) and DIP joints, and 15 were between the metacarpophalangeal and PIP joints. Regarding the number of involved rays, there were 22 single ray (3 thumbs and 8 index, 6 middle, 4 ring, and 1 little finger), and 4 multiple ray (3 index and middle, and 1 index, middle and ring finger) amputations.

Photographs were taken of both hands, all using the same camera (PL170; Samsung, Seoul, South Korea), light settings, distance, and platform. Both palmar- and dorsal-side photographs were taken (Figure 1). The patients were asked to complete a questionnaire consisting of 10 items (Table 1). After completion, each patient was asked to rate the appearance of their own injured hand using a 10-point visual analogue scale (VAS). Point 0 represented an "always disturbing" appearance and point 10 a "never disturbing" one.

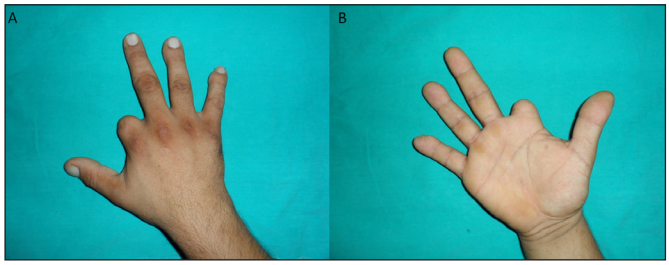

Figure 1. Example hand photographs shown to the assessors: dorsal (A) and palmar (B) views.

Table 1. The questionnaire developed for the patient group

1. Do you need to hide your hand during daily life? (for example; with gloves or with long sleeves)			
Yes, always	1	Seldom	3
Yes, often	2	No, never	4
2. How often do your first-time contacts ask the question "what happened to your hand"?			
At least once every day	1	Seldom	3
Often, even if not every day	2	I don't remember being asked at all	4
3. Do you use your affected hand in activities (shaking hands, giving/receiving items, etc.) that may come into contact with the hands of other individuals of the community?			
I never use	1	Sometimes I hesitate, but use it often	3
I try not to use as much as possible	2	I always use without hesitation	4
4. Do you have the feeling that other individuals in the community are disturbed by the appearance of your hand in daily activities (handshaking, giving/receiving items, etc.) that make your hand visible?			
Yes, always	1	Seldom	3
Yes, often	2	No, never	4
5. Are you currently working in a job?			
No	0	Yes	4
6. If "No", why are you not working?			
I hesitate of people seeing my hand			1
Because of both the dysfunction and appearance of my hand			2
Because of dysfunction of my hand			3
The cause is not about my hand (for example; I got laid off and couldn't find a job again)			4
7. If you have returned to your job, how did your hand problem affect you in terms of your profession?			
I have serious problems because of the appearance of my hand rather than its dysfunction.			1
I have problems because of both the dysfunction and appearance of my hand.			2
I have problems because of the dysfunction of my hand rather than its appearance.			3
Neither the appearance nor the dysfunction of my hand is a problem.			4
8. Has there been a change in your job after amputation?			
Yes	0	No	4
9. If "Yes", which one was the most influential in your decision?			
Appearance of my hand			1
Appearance and dysfunction of my hand			2
Dysfunction rather than the appearance of my hand			3
It is not about my hand			4
10. Do you have the feeling that you get different reactions from other individuals (customer, colleagues, boss, etc.) due to the appearance of your hand in your workplace?			
Yes, always	1	I don't realize much	3
Yes, often	2	Never before	4

***maximum score is 32.**
****Final point= (total score/32)x100**

Assessor Group

Participants were selected for the assessor group from among the companions of patients who presented to the outpatient clinic with lower extremity complaints. Healthcare workers, individuals aged < 18 or > 65 years, and first-degree relatives with a previous history of hand

injury or traumatic finger amputation were excluded from the study. On every day during a 50-day period, patient companions with these characteristics were invited to participate in the study. If they refused to take part, another companion meeting the same criteria was invited to participate.

The assessor group consisted of 50 participants (29 males and 21 females) with a mean age of 39.4±13 years (range: 18–64 years). The album of hand photographs of the patient group was shown to each participant, and they were asked to rate the appearance of each injured hand using the VAS. On this scale, 0 represented a "very disturbing" appearance and 10 an appearance that was "not disturbing". Then, the participants were asked to complete a questionnaire consisting of five items (Table 2). For each injured hand, the mean VAS score for the 50 assessors was calculated.

Table 2. The questionnaire developed for the assessor group

Please answer the following questions, taking into account the photo album of the hands you have looked at.			
1. How did it make you feel to look at the photos?			
I was so emotionally affected that I couldn't look at them all.	1		
I was uncomfortable and had a hard time looking.	2		
I felt a little uncomfortable but I kept looking atthem all.	3		
It was like I was looking at a normal hand and I did not feel uncomfortable.	4		
2. In social activities where hands can touch (shaking hands, giving/receiving items, etc.) does it bother you to encounter a hand similar to the photographs?			
I would be emotionally affected and do not know how to react.	1		
I would be disturbed and would not want to look.	2		
I would be uncomfortable, but I would try not to show my discomfort.	3		
I would take it completely normal and not be bothered	4		
3. When you first meet someone who has a hand similar to the photos, do you ask the question "What happened to your hand?"?			
I surely ask.	1		
Even if I force myself not to ask, I ask.	2		
I wonder what happened, but I don't ask.	3		
I think it's a personal and natural situation and I never ask.	4		
4. Do you avoid touching a hand similar to the photos in your social life? (for example; handshaking, giving/receiving items etc.)			
I definitely avoid.	1		
I avoid as much as possible.	2		
I touch but I feel disturbed.	3		
I never hesitate to touch.	4		
5. If one of your co-workers had a hand similar to that in the photos, would you treat him/her differently from your other colleagues? (if you are not working please assume that you are working)			
Yes	1	Seldom	3
Maybe	2	Never	4
***maximum score is 32. ****Final point= (total score/32)x100**			

Assessed Parameters

The VAS is a psychometric scale that can be used to assess the appearance of the hand.[9-11,13] The VAS scale completed by the patients in this study captured their usual perceptions of the appearance of their injured hands. The VAS scale for the assessor group was designed to provide a "snapshot" of their perceptions of the appearance of the injured hands.

To assess the effect of hand appearance on the participants' social lives, two questionnaires were developed. On the questionnaire for the patient group (Table 1), a score of 100 indicated no effect of hand appearance on the patient's social life. On the assessor group questionnaire (Table 2), a score of 100 indicated that the appearance of the injured hands was not perceived as abnormal, i.e., that the participant did not react negatively to hand abnormalities encountered in daily life similar to those in the photo album.

To allow correlation analysis between the patient and assessor group scores, "cross-questions" were included in the questionnaires. Both groups completed the survey only once, to ensure that there was no practice effect.

Statistical Analysis

To measure the reliability and internal consistency of the questionnaires, Cronbach's alpha values were calculated. After analyzing the normality of the data, Pearson correlation analysis (r= 0.1-0.3 weak; r=0.3-0.5 moderate; r=0.5-1.0 strong correlation), the chi-square test, and cross-tables were used to analyze the data. The t-test was used for comparison of two independent groups. A p-value of < 0.05 was taken to indicate statistical significance.

RESULTS

The Cronbach's alpha values of the patient and assessor questionnaires were 0.73 and 0.74, respectively.

The mean VAS and questionnaire scores of the patient group were 5.1±2 (range: 1–10), and 60.3±17 (range: 31–94), respectively (Figures 2 and 3); these scores were significantly correlated (r=0.434, p=0.027).

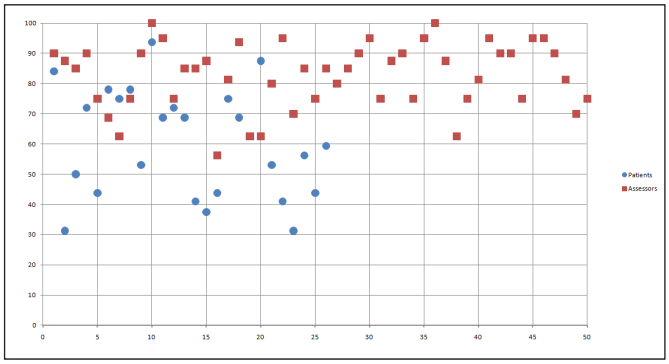

Figure 2. Comparison of the questionnaire results of the patients and assessors for each hand photograph. Red squares: mean questionnaire scores of the assessors; blue circles: questionnaire scores of each patient.

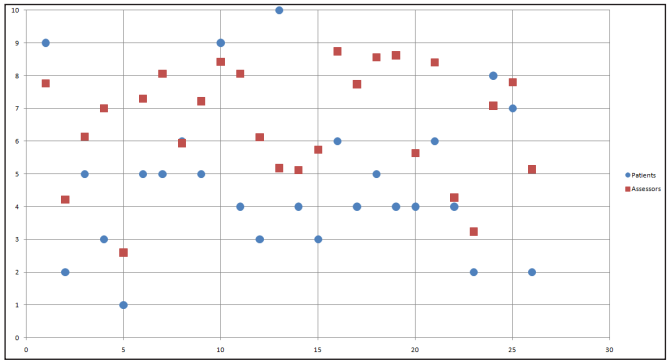

Figure 3. Comparison of the visual analogue scale (VAS) scores of the patients and assessors for each hand photograph. Red squares: mean VAS scores of the assessors; blue circles: VAS scores of each patient.

In the assessor group, the mean VAS score for all 26 hand photographs was 7.1±2 (range: 3–9) and the mean questionnaire score was 83.6±11 (range: 56–100) (**Figures 2** and **3**). In this group, the mean VAS and questionnaire scores also showed a significant correlation (r=0.302, p=0.033).

For each hand photo, the patients' and assessors' VAS scores were significantly correlated (r=0.511, p=0.008). In total, 21 of the 26 VAS scores were lower for the patient than assessor group.

There was no statistically significant correlation between the questionnaire scores of the two groups (p=0.887).

In the patient group, both the VAS and questionnaire scores improved significantly according to the time since the amputation (p=.00 and p=0.011). Patient age was not significant related to the VAS or questionnaire scores (p=0.995).

In the assessor group, age and gender showed no significant relationships with the VAS or questionnaire scores (p=0.08 and p=0.28).

DISCUSSION

Traditionally, the primary goal of treatment for patients with hand injuries is to restore functionality; therefore, hand appearance is often neglected when evaluating treatment outcomes.[6,18] However, aesthetic appearance is an important consideration because the hands are uncovered in daily life and play a role in non-verbal communication.[6,19] If hand appearance is compromised, body image may worsen, which has psychological and social consequences.[6,14,17,18,20] The influence of body image on psychological wellbeing can be explained by an "external view", where reactions in the social environment hold up a mirror to the self, and by an "internal view", i.e., the impact of body image on self-perceptions.[8] We assessed both factors in our patients with traumatic finger amputations.

In the literature, there are studies assessing social impact or social participation after loss of upper limb or hand injury but none of them simulated the perspective of the community.[14,16,17] Rumsey et al.[15] stated that most people with hand injuries were experiencing psychosocial problems in addition to physical and functional difficulties. In this study, the VAS and questionnaire scores of the patients were worse than those of the unaffected group, in terms of the perceived appearance of their hands and the reservations caused by this perception in social life effects of the abnormalities (5.1 versus 7.1; 60.3 versus 83.6 respectively). The VAS scores of the patient and unaffected groups were significantly correlated (r=0.511, p=0.008). We interpreted this situation as the patient's and society's similar perceptual reactions to the abnormal hand appearance. However, the correlation between the VAS scores and ratings for social life problems were stronger in the patient group. Also, the questionnaire scores of the unaffected group reflected a much more optimistic outlook regarding social interactions.

According to the results of our study, we think that the patient perspective perceives the appearance of the hand slightly worse than the community. If we interpret the impact of social life from both perspectives; we think that patients are negatively affected in social life, but community is not disturbed as patients.

Our findings suggested that the above-mentioned internal view may exert more influence on the interaction between the hand appearance and social lives of patients than the external view. This accords with a previous study, in which emotional well-being and quality of life in the context of general body disfigurement were mainly determined by the internal view.[8]

In case of any disability in the body, it is well-known that as time passes after the injury, a person's ability to adapt to the situation increases.[12,14,16,21,22] In the present study we observed a significant improvement in our patients' VAS and questionnaire scores with increasing time since the trauma and resulting finger amputation. This finding, which is in line with previous studies, shows that patients cope better with their altered hand appearance over time.

The main limitation of our study was that the results were pertain to a specific sociocultural environment; validation in other social environments is thus required. The validity of survey-based research also depends on the clarity, length, and content thereof, and the level of comprehension of the participants.[23] We believe that our questionnaire was favorable in these respects. Another potential limitation of survey studies relates to the reliability of the questionnaires, although the Cronbach's alphas in this study indicated high reliability and internal consistency.[24] Nevertheless, similar studies in different social settings are required to confirm the validity and reliability of our results.

CONCLUSION

The results of this study could inform therapy and psychosocial support for patients with hand trauma. Interventions could be developed to inform patients about the external and internal perspectives of hand disfigurements discussed above, i.e., that others' perceptions of hand abnormalities tend to be more favorable than self-perceptions, and that coping improves over time. With greater awareness of these issues, patients may be motivated to participate in social life earlier and more fully.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Mersin University Medical Faculty Clinical Researches Ethics Committee (Date: 04/09/2019, Decision No: 2019/366).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

HEALTH SCIENCES
MEDICINE

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The impact of COVID-19 pandemic on surveillance of influenza and influenza-like viruses: a single center experience

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ABSTRACT

Aims: Influenza and the agents responsible for influenza-like illness (ILI) are a significant cause of upper and lower respiratory tract infections, with a notable pattern of seasonal incidence. The surveillance of influenza and agents causing ILI is important for the developmentof a prophylaxis approach and infection control. In the present study, we utilize the Reverse transcription-polymerase chain reaction (RT-PCR) method to identify the presence of influenza virus and other agents associated with ILI in nasopharyngeal smears obtained from patients exhibiting symptoms of influenza and ILI. The study sample included patients who were admitted to multiple clinics and intensive care units (ICU) of the Health Sciences University Ankara Training and Research Hospital in the period preceding, and in the initial months and first year following the coronavirus disease 2019 (COVID-19) outbreak. Throughthe comparisons made in the study, the intention is to gain a better understanding of the influence of the COVID-19 pandemic on other viral infectious agents transmitted via the respiratory tract.

Methods: Included in the study were 257 admitted to different wards and ICUs of our hospital due to symptoms of upper or lower respiratory tract infection and ILI between 2015 and 2021 (excluding data from 2017–2018). The study was conducted prospectively within the scope of the Global Influenza Hospital Surveillance Network project. Using sterile swabs, nasopharyngeal swab samples were collected from inpatients who provided informed consent for their participation in the study, and the samples were placed in a viral transport medium. The presence of influenza viruses (Influenza A, subtypes of Influenza A and Influenza B) and agents causing ILI (i.e. respiratory syncytial virus, coronavirus) was investigated with an RT-PCR approach.

Results: The most common symptoms among the 257 patients included in the study with pre-diagnoses of influenza and ILI were cough (82.2%), fever (67.7%), shortness of breath (66.1%) and myalgia (40%). The RT-PCR detected a viral agent in 60 (23.3%) of the 257 patients, whereas no agent could be detected in 197 (76.6%) patients. Furthermore, 51 (18.5%) tested positive for influenza virus, five (1.9%) for respiratory syncytial virus (RSV), and four (1.5%) for SARS-CoV-2. An analysis of the results within two distinct time frames, namely prior to the COVID-19 pandemic (2016–2017, 2019–prior to December 10, 2020) and during the COVID-19 pandemic (between December 11, 2020 and 2021), influenza viruses (influenza A H1N1 and influenza A H3N2), RSV and influenza type B were identified as the dominant viruses before the COVID-19 pandemic, while the predominant viruses were a single influenza strain and four SARS-CoV-2 variants during the COVID-19 pandemic.A significant difference was noted in the distribution of viruses between the two time frames – prior to the pandemic and during the pandemic. Of the patients, 199 (77.4%) were discharged with full recovery while 58 patients died (22.6%). Of the 58 patients that died, 25 were female (25/131 females) and 33 were male (33/126 males), 11 had tested positive for influenza virus andone for RSV, while no infectious agent could be identified in 46 patients.

Conclusion: The implementation of molecular testing methods for the identification of viral infectious agents among inpatients during influenza and ILI outbreaks, administering antiviral and prophylactic treatments targeting influenza, RSV and SARS-CoV-2 infections, and adopting infection control measures, could significantly decrease mortality and morbidity rates while mitigating the complications associated with these infectious agents.

Keywords: Influenza, influenza-like viruses, SARS-CoV-2, pandemic

INTRODUCTION

Influenza and influenza-like viruses are the most common causes of upper and lower respiratory tract infections in both adults and children.[1–3] Influenza, caused by highly contagious influenza viruses, is an acute respiratory tract infection that constitutes a substantial global threat, with an estimated 3–5 million people developing severe infections caused by seasonal flu viruses annually, leading to the death of 290,000–650,000 people around the world.[4]

The influenza-like viruses that are often mistaken for influenza viruses include rhinoviruses, respiratory syncytial virus (RSV), adenovirus, human parainfluenza viruses, human metapneumovirus, human bocavirus, coronaviruses and SARS-CoV-2, that last of which triggered the recent global pandemic.[1–5] These agents are significant causes of mortality and morbidity, particularly in children under the age of 5, older adults (65 years and above), immunocompromised patients and those with underlying chronic conditions.2,6–10 Influenza viruses

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undergo frequent antigenic mutations, and such changes lead to annual epidemics, prompting the production of new vaccines targeting specific virus strains and the regular vaccination of high-risk people each year. The surveillance of influenza viruses and influenza-like viruses is crucial for the identification of the predominant virus strain and for the planning of disease prevention and control activities, the early detection of outbreaks, and the planning of patient care and vaccination regimes.

The COVID-19 pandemic has had a profound impact on the lives of people around the world, prompting the implementation of comprehensive public health control measures, especially during the initial months of the pandemic, such as the avoidance of crowded places, lockdowns, restrictions on travel, emphasized hand hygiene practices, obligatory facemask use and social distancing. It is believed that these measures have had a comparable impact also on the influenza virus and other influenza-like viruses.

In the present study,an RT-PCR method is applied to investigate the presence of the influenza virus and other agents responsible for influenza-like illness (ILI) in nasopharyngeal smears obtained from patients exhibiting symptoms of influenza and ILI. Included in the study werepatients who were admitted to multiple clinics and ICUs of the Health Sciences University . Hospital during the period preceding, as well as in the initial months and the first year following the COVID-19 pandemic. The study results were examined within two distinct time frames, namely prior to the COVID-19 pandemic and during the COVID-19 pandemic, to gain a better understanding of the impact of the COVID-19 pandemic on other infectious agents transmitted via the respiratory tract.

METHOD

The study was carried out with the permission of Hacettepe University Faculty of Medicine Ethics Committee (Date: 21.12.2021, Decision No: 2021/21-48) as was authorized by the head physician, while written informed consent for their inclusion in the study was obtained from all patients. The study was conducted in accordance with good clinical practice guidelines. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study group comprised 257 patients who were admitted to different wards and ICUs of our hospital with symptoms of upper or lower respiratory tract infection and ILIs during the flu seasons (November–May) of 2015 and 2021 (excluding data from 2017–2018). ILI is defined as the presence of symptoms including fever (≥38°C), cough and sore throat.[11]

The study was conducted prospectively within the scope of the Global Influenza Hospital Surveillance Network project. The hospital records were examined daily and patients meeting the inclusion criteria were subjected to a bedside examination. The presence of flu-like symptoms was first enquired, followed by the demographic characteristics of the patients, clinical and laboratory findings, underlying conditions and the clinical course of the disease, and the forms displaying the vaccination status were completed.

Nasopharyngeal and/or pharyngeal smears were collected from the patients and sent to the contracted laboratory daily after placement in a viral transport medium (Virocult, Medical Ware& Equipment, UK) for the investigation of the influenza virus. The samples were stored at -20°C if not to be tested on the same day. An RT-PCR test was used for the identification and subtyping of influenza viruses. The nucleic acid extraction from the samples was carried out using an E21 Virus Mini Kit V2.0 (Qiagen, Germany). The samples were tested for the presence of influenza A, B and C viruses and other respiratory tract viruses (Enterovirus, Human coronavirus [229E/NL63], Human coronavirus (OC43), Human parainfluenza virus [1-4], Human metapneumovirus, Human bocavirus [types 1–4], Human respiratory syncytial virus A and B, Human adenovirus) using a multiplex influenza A, B and C kit (TibMolbiol, Germany) and a Bio-Rad CFX96 device (Bio-Rad, USA). Samples that tested positive for influenza viruses were further tested for the presence of influenza A H1 and H3 strains, and the influenza B Yamagata and Victoria subtypes using the CDC RT-PCR protocol. Analyses of SARS-CoV-2 were added to the study in 2020.

Statistical Analysis

IBM SPSS Statistics (Version 20.0. Armonk, NY: IBM Corp.) was used for the statistical analysis.A Chi-square test was used to determine the significance of the relationships between categorical variables. A p-value of ≤0.01 was considered statistically significant.

RESULTS

Nasopharyngeal smear samples were collected from 313 inpatients for the detection of influenza virus and other infectious viral agents. Of the patients, 132 (51.3%) were female and 125 (48.6%) were male, and the median age was 65.67 years. The most common symptoms in the study patients were cough (n=213, 82.8%), fever (n=174, 67.7%), shortness of breath (n=170, 67.7%) and myalgia (n=103, 40%).

The symptoms of the patients that reported flu-like complaints are presented in **Table 1**.

Of the 313 patients that underwent RT-PCR testing in the present study, 80 (26%) tested positive for influenza virus, five (1.9%) for RSV and four (1.5%) for SARS-CoV-2, while no viral agent could be identified in 224 patients

(70.6%). The distribution of PCR-positive and PCR-negative patient results by year presented in Table 2.

Table 1. Symptoms of patients reporting flu-like complaints

Symptoms	Number	%
Cough	213	82.8
Shortness of Breath	170	66.1
Fever	174	67.7
Myalgia	103	40

Table 2. Distribution of PCR-positive and PCR-negative patient results by year.

	PCR -positive patients n (%)	PCR -negative patients n (%)	Total
2015	11 (68.8)	5 (31.2)	16 (100)
2016	111 (79.9)	28 (20.1)	139 (100)
2019	2 (100)	0 (0)	2 (100)
2020	47 (63.5)	27 (36.5)	74 (100)
2021	26 (100)	0 (0)	26 (100)
Total	197	60	257 (100)

An examination of the monthly distribution of viral infectious agents showed that influenza virus, influenza A and B and RSV were the most common in the winter months (January–March), while SARS-CoV-2 was most commonly observed in April and May after March 11, 2020, when the first case of COVID-19 was identified in Turkey. The monthly distribution of viral agents is presented in Table 3.

The yearly distribution of viral agents is shown in Figure. The study period was divided into two distinct time frames: prior to the pandemic (2015-2016, 2016-2017, 2019 and before March 10, 2020) and during the pandemic (between March 11, 2020 and 2021).

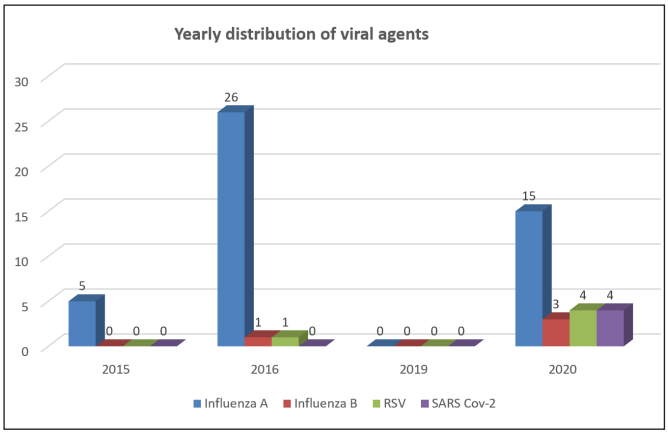

Figure. Most common viral agents by year

Influenza virus (influenza A H1N1 [n=16] and influenza A H3N2 [n= 12], RSV [n=5] and influenza B virus [n=4]) were predominant in the pre-pandemic period.

Only a single influenza virus was identified during the COVID-19 pandemic while SARS-CoV-2 was identified in four patients.

There was a significant difference in the distribution of viral agents between the pre- and peri-pandemic periods (Chi-square test; p=0.0001).

Of the sample, 199 (77.4%) were discharged with full recovery, whereas 58 patients (22.6%) died. Of the non-survivors, 25 were female (25/131 females) and 33 were male (33/126 males). Of those that died, 11 had tested positive for influenza virus and one for RSV, while no infectious agent could be identified in 46 patients. The median age was 77.46 years in the 58 non-survivors, 52 of whom (89.6%) had at least one comorbidity (i.e. diabetes mellitus, chronic kidney disease).

Of the total 257 patients (6.2%), 16 had received an influenza vaccination, among whom three were identified with the influenza virus based on the PCR testing of upper respiratory tract samples, and one of these died. The relationship between cause of death and influenza could not be identified due to a lack of detailed clinical data.

The clinical outcomes (death or discharge) did not differ significantly between the pre- and peri-pandemic periods (Chi-square test: p>0.05).

DISCUSSION

The objective of the present study was to analyze the yearly distribution of seasonal influenza viruses and other viral agents responsible for influenza-like illnesses (ILIs), and to evaluate the impact of the COVID-19 pandemic on the frequency of viral infectious agents following the first confirmed case of COVID-19 in Turkey on March 11, 2020.

The study examined the frequency of influenza and other viruses causing ILI in inpatients through the multiplex PCR testing of nasopharyngeal smear samples collected as part of the Global Influenza Hospital Surveillance Network project. The analysis was conducted for two distinct periods: before the COVID-19 pandemic and during the pandemic. An analysis of the two distinct

Table 3. Monthly distribution of viral agents

Agents	Months								Total (n)
	January	February	March	April	May	June	July	December	
Influenza A	33	1	4	0	0	0	0	9	47
Influenza B	0	1	2	0	1	0	0	0	4
RSV	1	3	0	0	0	0	0	1	5
SARS CoV-2	0	0	0	2	2	0	0	0	4
Total (n)	34	5	6	2	3	0	0	10	60

study periods (pre-pandemic, based on data from the 2015–2016, 2016–2017 2018–2019 and up to December 10, 2020, andthe peri-COVID-19 pandemic period, based on data from between December 11, 2020 and 2021) revealed influenza virus (influenza A H1N1 and influenza A H3N2, respectively), RSV and influenza B were to be predominant in the pre-pandemic period, whereas only a single influenza virus and four SARS-CoV-2 variants were the prevailing agents during the COVID-19 pandemic. A significant difference was noted in the distribution of viral agents between the two time frames.

The decrease in the frequency of the identification of the influenza virus and other agents responsible for ILIs may be attributable to the strict measures and restrictions imposed during the pandemic (the closure of schools, crowded workplaces and social establishments such as cafeterias, cinemas and restaurants), compliance with social distancing rules, wearing facemasks and hand hygiene, vaccinations against influenza and viral interference.

Studies conducted in the United States and Australia reported a decreased prevalence of the influenza virus during the COVID-19 pandemic. Although both SARS-CoV-2 and influenza viruses are transmitted through respiratory droplets, the lower transmission coefficient of the influenza virus compared to that of SARS-CoV-2 was suggested as the cause of the decrease in cases of influenza. Other factors contributing to the decrease in influenza cases during the pandemic include the reduced frequency of influenza testing compared to SARS-CoV-2 testing, the effectiveness of infection control measures implemented to combat COVID-19 in mitigating influenza transmission, and the potential influence of viral interference.[12–14]

Respiratory viruses are transmitted primarily through droplets, close contact, and through touching the mouth, nose or eyes with the hand after coming into contact with surfaces contaminated with the virus. Several studies have reported a decrease in the prevalence of other respiratory viruses that are transmitted primarily through droplets, such as influenza, RSV, coronaviruses, parainfluenza, adenoviruses and bocaviruses, attributing the decline to the restrictions and infection control measures implemented during the COVID-19 pandemic.[12,15]

Agca et al.[12] examined a total of 319 nasopharyngeal smear samples to investigate the epidemiological shift in prevalence of influenza and other respiratory viruses among patients during the COVID-19 pandemic, with positive tests recorded in 101 of the samples (31.7%). In all age groups, rhinoviruses and enteroviruses were identified as the most frequently encountered viral agents. During the initial years of the pandemic, a significant decrease was reported in the prevalence of influenza, declining from 17.3% to 2.3%, while no significant change was observed in the prevalence of the metapneumovirus during the same period. The authors suggested testing for other respiratory viruses, such as rhinoviruses/enteroviruses and metapneumovirus, in addition to SARS-CoV-2, for differential diagnoses, considering that the clinical presentation of such viruses can resemble COVID-19 and other respiratory tract infections.

In their study, Appak et al.16 analyzed a total of 4,770 respiratory samples to explore the monthly distribution of respiratory viruses and Mycoplasma pneumoniae from 2018 to 2021, and to assess the influence of the COVID-19 pandemic on the prevalence of respiratory viruses. The multiplex PCR method yielded a positive result in 2,603 out of the 4,770 samples analyzed (54.6%), coinfection in 474 samples (9.9%) and no specific agent in 2,167 samples (45.4%). In the same study, there were no identified cases of influenza A, influenza B or metapneumovirus during the COVID-19 pandemic, which were commonly encountered in previous years. On the other hand, rhinovirus was consistently identified as the most frequently encountered viral agent both before and during the pandemic. The study also identified respiratory syncytial virus as a coinfection in the last one month of the study period, which concurs with the findings of the present study, in which an influenza virus was identified in only one patient during the pandemic.

Seasonal influenza places a substantial burden on healthcare systems and can result in significant mortality and morbidity.[10] The implementation of national influenza prevention programs, along with the targeted vaccination of high-risk groups, has reduced the mortality rates associated with seasonal influenza significantly.[6,9,10]

A global influenza hospital surveillance network study carried out by Başaranoğlu et al.[1] in the Ankara province of Türkiye from 2016 to 2017 reported positive PCR test results for RSV in 145 out of the 917 patients (15.8%) analyzed. Among the cases with a positive RSV result, 1,322 were aged below 5 years, while 13 were aged above 5 years. Among those aged above 5 years, mortality was found to be associated with RSV in one out of two patients aged above 65 years. In the present study, influenza A was identified in 47 cases (18.5%) and influenza B in four cases (1.5%). Among the agents responsible for ILIs, RSV was detected in five cases (1.9%) and SARS-CoV-2 in four cases (1.5%). No specific agent could be identified in 197 of the 257 cases (76.6%). In the present study, the median age was 70.4 years among the five patients who tested positive for RSV using the PCR method (minimum 26 years, maximum 95 years). The median age of the 47

patients who tested positive for influenza A was 66.4 years (minimum 26 years, maximum 94 years). SARS-CoV-2 was also detected in a number of nasopharyngeal smear samples due to the study period (December 2019-April 2020), December 2020-May 2021) based on the date of the initial outbreak of the COVID-19 pandemic caused by SARS-CoV-2, in the city of Wuhan in China (December 31, 2019), and the date on which the first case of COVID-19 was identified in Türkiye (March 11, 2020). The clinical symptoms of COVID-19 resemble those of the influenza virus (i.e. fever, cough, shortness of breath, myalgia), and it is not possible to differentiate between the two infections based on the clinical manifestations, althoughthe lung involvement pattern observed in radiological imaging studies may sometimes be helpful in this regard – COVID-19 pneumonia is characterized by peripheral involvement in radiological imaging studies, whereas peribronchial involvement is common in influenza pneumonia.[17] That said, the isolation of the agent from respiratory samples is required for definitive diagnosis.

The clinical differentiation of viral infectious agents can be challenging due to the similarity of the associated symptoms in respiratory tract infections. To address this, various molecular diagnostic methods have been developed, including multiplex PCR and real-time PCR, and molecular tests based on respiratory tract syndromic panels.[18–21]

The main advantages of these tests include their ability to provide a rapid diagnosis, enabling the early initiation of treatment and prompt patient care, while their main disadvantages and challenges include their high cost, the lack of specified strategies for their optimum use and the challenges in the interpretation of the test results.[18]

In the present study, patients who tested positive for influenza were placed on antiviral therapy and droplet isolation measures were implemented. Those who tested positive for SARS-CoV-2 were started on COVID-19-focused therapies and transferred to wards designated for the care and follow-up of COVID-19 patients where appropriate infection control measures, including droplet and contact isolation precautions, were implemented. Patients who tested positive for other viral agents were started on symptomatic therapy and the appropriate infection control measures were implemented.

It has been reported that influenza vaccine scan reduce the prevalence of influenza infection by 75–80% in healthy patients, and the efficacy of the influenza vaccine in older adults, particularly those residing in nursing homes, in immunocompromised patients with underlying conditions or in those on immunosuppressive medication ranges from 40 to 60%.[22] Vaccination is considered the most effective preventive approach to the reductionof mortality and morbidity, and for the control of infections in older patients and those at high risk due to such underlying conditions as diabetes, chronic obstructive pulmonary disease (COPD), kidney and heart failure, malignancy and chronic collagen tissue disease.[6,22,23]

Of the 257 patients included in the present study, 16 (6.2%) had received the influenza vaccine, andof these, three tested positive for influenza virus during the PCR testing of upper respiratory tract samples, one of whom died. Due to a lack of detailed clinical data, the precise relationship between the cause of death and influenza could not be accurately determined in the present study.

The rate of patients that had received the influenza vaccine can be considered low in the present study (6.22%) when compared toprevious studies detailing vaccination rates in different risk groups. In a study carried out in Greece, Papaioannou et al.[23] reported an influenza vaccination rate of 34.8% among high-risk patients with such underlying conditions as chronic lung disease, chronic kidney disease, cardiovascular disease, diabetes, malignancy and neurological disease. The influenza vaccination rate was reported to be 57% among the older adult population in Japan, while in a Chinese study, a rate of 3.8% was reported among those aged 60 years and older. In Europe, an influenza vaccination rate of 45.5% has been reported for older adults, and 49.8% among those with a chronic condition.[24]

In a study carried out in Türkiye involving 155 patients, Özışık et al.[25] reported an influenza vaccination rate of 29.7% among 145 patients who were considered sufficiently high-risk for influenza vaccination. An influenza vaccination rate of 7.2% was reported in a study by Tanriover et al.[2] Given the low influenza vaccination rate (6.2%) observed in the present study, the authors recommend that individuals in high-risk groups should be actively encouraged to vaccinate for influenza, and that efforts should be made to convince those who are reluctant.

Demicheli et al.[26] reported that the influenza vaccine reduced the risk of influenza infection from 6% to 2.4%, and the risk of infection by influenza-like viruses from 6% to 3.5% in older adult patients.

Randomized and controlled studies as well as observational studies have suggested that vaccinating against influenza reduces the mortality and morbidity rates of adult patients with cardiovascular diseases,[5] although there have been several studies reporting that the influenza vaccine is effective in reducing influenza-

related morbidity, but has no effect on all-cause mortality.[7]

Influenza outbreaks are associated with increased mortality in winter months. The presence of a chronic disease and residing in a nursing home were identified as significant risk factors for the development of complications and increased mortality associated with influenza.[8]

In a study involving 774 inpatients carried out within the scope of the Global Influenza Hospital Surveillance Network, Tanrıover et al.[2] reported influenza positivity identified through the PCR testing of nasopharyngeal or oropharyngeal smear samples in 142 patients (18.4%), with the most commonly isolated influenza serotype being influenza A H1N1 PDM 09. Within the study, all older patients were reported to have at least one chronic underlying condition, the most common being cardiovascular disease, followed by chronic obstructive pulmonary disease. The rate of influenza vaccination among the study patients was reported to be 7.2%, and the same study reported that, regardless of the presence of influenza virus positivity, the clinical course was poorer in older patients, that half of the patients aged above 65 years required admission to the ICU, that one-third of the patients required the use of a mechanical ventilator, and that one-quarter of the patients died. The same study also reported ILIs to be associated with poorer clinical outcomes in older inpatients. The authors concluded the study with the suggestion that an influenza vaccination strategy could provide an opportunity to prevent deaths associated with ILI in older adults, and in adults with chronic conditions.

The present study has some limitations, including its retrospective design and the lack of long-term follow-up of the participants, which posed limitations on access to detailed clinical data, thereby hindering the assessment of the relationship between the cause of death and influenza and ILI. Furthermore, the patients were not tested for influenza or agents responsible for ILI between 2017 and 2018. Another limitation of the study, related to the assessment of the prevalence of viral agents, is the difference in the established periods before the pandemic (2015–2016, 2016–2017, 2019–December 10, 2020) and after the COVID-19 outbreak (between December 11, 2020 and 2021).

No significant difference was noted in the death and discharge rates recorded in the pre-pandemic period and after the COVID-19 outbreak.

The median age was 77.46 years in the 58 non-survivors, and 52 of these patients (89.6%) had at least one comorbidity (i.e. diabetes mellitus, chronic kidney disease).

CONCLUSION

The authors believe that the molecular testing for viral infectious agents of patients presenting with clinical symptoms suggestive of influenza and ILI, along with the administration of appropriate antiviral and prophylactic treatments targeting the specific agent, and the adoption of infection control measures to counter these agents, could decrease the mortality and morbidity rates associated with these infectious agents significantly.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hacettepe University Faculty of Medicine Ethics Committee (Date: 21.12.2021, Decision No: 2021/21-48).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of mesiobuccal root canal morphology and interorifice distance in maxillary first molar teeth: a CBCT study on Southeast Anatolian population

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ABSTRACT

Aims: The aim of this study was to evaluate the mesiobuccal root canal morphologies and interorifice distances of maxillary first molars using cone-beam computed tomography (CBCT).

Methods: CBCT images of 477 patients who had undergone CBCT for various reasons were examined and 654 maxillary first molars were included in the study according to the study criteria.

Results: The most common canal configuration observed in mesiobuccal roots was type II ($p < 0.05$). The median interorifice distance was 2.28 mm in roots with type II canal configuration, while it was measured as 2.55 mm in roots with type IV canals, and a significant difference was found depending on the presence of canal in the apical third of the root ($p < 0.001$). The median interorifice distance was found to be 2.58 mm in males and 2.34 mm in females, and a significant difference was detected ($p < 0.001$).

Conclusion: Various canal variations can be observed in the mesiobuccal roots of maxillary first molars, and the length of interorifice distance can predict the canal type. This information can increase the success rate of endodontic treatment.

Keywords: Maxillary first molar, cone-beam computed tomography, MB2, interorifice distance

INTRODUCTION

Root canal treatment depends on the dentist's knowledge of root canal morphology. The presence of an untreated root canal space is strongly related to apical periodontitis as any part of the root canal system that is not properly cleaned carries the risk of harboring microorganisms.[1]

Maxillary first molars have the highest clinical failure rate during canal treatment due to the particularly elusive mesiobuccal second Root canal, which is often undetected.[2] Studies investigating the incidence of the mesiobuccal second (MB2) canal in maxillary molars have reported detection rates ranging from 17.8% to 95.2%.[3-8]

The ethnic origin of the population under investigation may have a strong impact on the incidence of canal variations. Clinicians often encounter difficulties in localizing the MB2 canal in maxillary molars due to excessive dentin accumulation at the canal orifice during cavity access opening.[9] In the presence of undetectable extra canals, root canal treatment cannot be completed properly, leading to failed treatment and persistent pain.[10]

Conventional radiography is still commonly used for accurate diagnosis and treatment planning. However, the use of cone-beam computed tomography (CBCT) has enabled clinicians to examine teeth in three dimensions, as periapical radiographs only provide two-dimensional images. In their study evaluating the relationship between canal orifice configuration and MB2 canal morphology in maxillary second molars with fused roots, Keskin et al.[11] reported that the distance between orifices was a strong indicator of the presence of the MB2 canal.

The Aim of our study is to examine the incidence and morphology of the MB2 canal in maxillary first molar teeth using CBCT images obtained for general dental examinations.

METHODS

After obtaining approval from the Local Ethics Committee of Dicle University Faculty of Dentistry (Date: 29.09.2021, Decision No: 2021-48), 477 CBCT

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scans (253 female / 224 male) of patients who met the study criteria were selected from a total of 1271 CBCT scans taken for various reasons (implant planning, trauma, orthodontic treatment planning, etc.) between January 2018 and December 2019 at Dicle University Faculty of Dentistry. A total of 654 (338 female / 316 male) maxillary first molar teeth were analyzed and included in the study. Criteria for teeth not included in the study were (a) excessive crown destruction in the tooth, (b) presence of internal-external resorption in the root canals of the tooth, (c) the tooth having undergone endodontic treatment or having periapical pathology, (d) the tooth being an immature tooth with an open apex, (e) the root canals of the tooth being calcified, (f) the presence of root fracture, (g) the presence of deep restorations, crown-bridge restorations, and dental implants that impair image quality in the examination area and (h) CBCT scans containing artifacts.

In our study, CBCT images obtained with the I-Cat (Imaging Sciences International, Hatfield, PA) CBCT system, which can image the entire upper and lower jaws, were used. The images were obtained at 120 kVp, 5 mA, 9 seconds scanning time, and 0.3 mm voxel size. Measurements and evaluations were performed by two experienced independent researchers.

The three-dimensional images of the upper first molar teeth were examined in three planes (Sagittal, Axial, and Coronal) using I-CAT Vision software (Imaging Sciences International, Hatfield, PA). The presence of the MB2 canal and the canal configuration in the MB root were carefully evaluated in every section from the pulp chamber to the apex in the horizontal (MPR images) plane. The the interorifice distance at the pulp chamber floor level was measured using the distance measurement feature of I-CAT Vision software and recorded (Figure 1).

The canals of the mesiobuccal roots evaluated in the scans were classified according to the Vertucci classification (Figure 2).

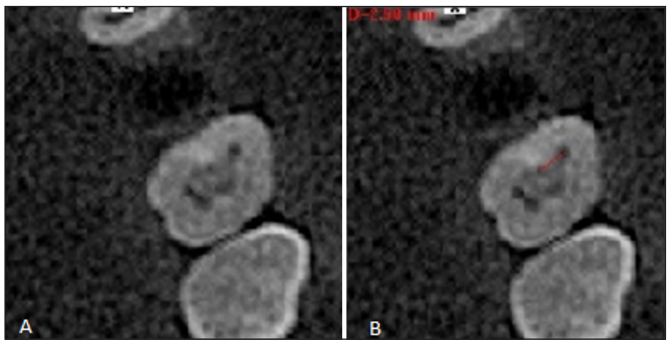

Figure 1. The image of MB2 canal in the axial section and the measurement of the interorifice distance. (A. Pre-measurement image, B. Post-measurement image).

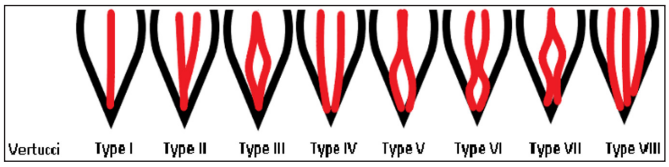

Figure 2. Root canal types according to the Vertucci classification.

Statistical Analysis

The data were analyzed using Rstudio (version 3.6.2 (2019-12-12) – CRAN). Normal distribution suitability was examined with Lilliefors (Kolmogorov-Smirnov) test. Mann Whitney U test was used for comparison of non-normally distributed data between two groups. The analysis results were presented as mean±standard deviation and median (minimum – maximum) for quantitative data, and as frequency and percentage for categorical data. The significance level was set at $p<0.050$.

RESULTS

A total of 654 (338 female / 316 male) maxillary first molars were analyzed from 477 CBCT images. Of these, it was determined that 108 (57 female / 51 male) had Type-I (16.5%) and one (male) had Type-III (0.1%) canal configuration in their mesiobuccal canals, while the remaining 545 (279 female / 266 male) teeth had MB2 canal configurations with two separate canal orifices in the mesial root, detected as Type-II and Type-IV canal configurations.

Of the 546 teeth with an MB2 canal detected in the mesiobuccal root, 177 had Type-IV (27%) canals that extend to the apical trio, while 369 teeth had Type-II (56.4%) and Type III (0.1%) canal types that end with an isthmus in the middle or apical trio of the mesiobuccal canal.

The median interorifice distance was 2.55 mm for teeth with MB2 canals in the apical trio (Type IV) and 2.28 mm for teeth without MB2 canals (Type II), and the median distance values obtained differed depending on the presence of MB2 canal in the apical trio ($p<0.001$) (Table 1).

When the interorifice distance was evaluated by gender, a statistically significant difference was found between the median distance values of the two genders ($p<0.001$). The median distance value was 2.34 for females and 2.58 for males (Table 2).

When the interorifice distance was evaluated independently of gender, the median distance value was determined as 2.42 and the mean distance value was determined as 2.44 (Table 3). Of the scanned teeth, 51.7% were from female patients and 48.3% were from male patients.

Table 1. Comparison of distances according to the presence of MB2 canal in the root apical third

	Present (Tip IV)		Absent (Tip-II)		Test Stats.*	p
	Average±SS	Median (Min.-Maks.)	Average±SS	Median (Min.-Maks.)		
Distance	2,51±0,55	2,55 (1,20-4,50)	2,31±0,54	2,28 (0,95-3,62)	26443,5	<0,001

*Mann Whitney U testi

Table 2. Comparison of interorifice distances by gender.

	Female		Male		Test Stats.*	p
	Average±SS	Median (Min.-Maks.)	Average±SS	Median (Min.-Maks.)		
Distance	2,33±0,54	2,34 (0,95-3,62)	2,56±0,54	2,58 (1,24-4,50)	28378	<0,001

*Mann Whitney U testi

Table 3. Frequency distributions, Vertucci configuration percentages and interorifice distance data

	Frequency (n=654)	Percentage (Total%)
Middle trio		
Canal present	546	83,5 (Type II, III, IV)
Canal absent	108	16,5 (Type I)
Apical trio		
Canal present	177	27 (Type IV)
Canal absent	477	73 (Type I-II-III)
Gender		
Female	338	51,7
Male	316	48,3
	Average±SS	Median (Min.-Maks.)
Interorifice distance	2,44±0,55	2,42 (0,95-4,50)

DISCUSSION

In routine dental practice, visual evaluation, dental operating microscope (DOM), and conservative X-ray are the most commonly used options for detecting the second root canal. The use of CBCT imaging in endodontic diagnosis and treatment planning has enabled the three-dimensional evaluation of root canals before treatment.[12] In this study, the mesial root canal morphologies of the first molars could be examined, and the distance between the mesiobuccal canal and the MB2 canal orifices could be measured clearly.

In our study, the root canal images of 654 maxillary first molars obtained from CBCT scans of 477 patients from the Southeast Anatolian population were examined. The results show that the existence rate of the mesiobuccal second canal in maxillary first molars in this population is 81%. This finding is consistent with the results of various international scientific studies, which show a prevalence between 66% and 87%.[13-15]

Al Mheiri et al.[16] reported in their CBCT studies of maxillary first molar canal morphologies that they most commonly encountered Vertucci Type II (59%) in the mesiobuccal canal, followed by Type I (19.9%), Type IV (15.3%), and Type III (5.7%). Mufadhal et al.[17] reported in a similar study that Type II (25%), Type III (23.1%), and Type I (17.7%) canal configurations were found most frequently. Induja et al.[18] who examined the mesiobuccal canals of maxillary first molars in vitro, detected the presence of Type II (44%) and Type IV (33.3%) canals most frequently.

The reasons for the different rates and configurations of the MB2 canal in maxillary first molars in different studies are ethnic differences, as well as the structural differences of the CBCT system and software used for the examination, the experience level of the researcher examining the images, and the age range of the patient groups being examined. Kiefner et al.[19] reported in their studies that secondary dentin accumulation in elderly patients can significantly narrow the root canal space and lead to canal calcification. Reis et al.[20] reported in their studies of maxillary molars that the prevalence of the MB2 canal decreases with increasing age and the canal progressing towards the apical third. They noted that this may be due to the increased dentin apposition on the root canal walls with advancing age.

Keskin et al.[11] reported in their studies that a distance between the mesiobuccal and palatal canal orifices of more than 4 mm is a strong indicator of the presence of the MB2 canal. Zhang et al.[21] reported in their studies that the ratio of the distance between the mesiobuccal and palatal canal orifices to the distance between the distobuccal and palatal canal orifices has high diagnostic accuracy in predicting the presence of an MB2 canal. Cimilli et al.[22] The distance between the mesiobuccal and mesiolingual canal orifices of the mandibular first molars is an important clinical parameter in predicting the formation of Vertucci Type IV canals, and teeth with a distance greater than 3 mm between the orifices tend to have separate canals, as reported by previous studies. Similarly, in our current study, teeth with Vertucci Type IV canal configuration had a greater average distance between the mesiobuccal canal orifices (2.51±0.55 mm) compared to teeth with Vertucci Type II configuration (2.31±0.54 mm).

In a study by Su et al.[23] on maxillary first molars, they found that the distance between the mesiobuccal and MB2 canal orifices was significantly longer in males than in females. Our study also found that the median distance between orifices was 2.58 mm in males and 2.34 mm in females.

CONCLUSION

The incidence of a second mesiobuccal canal in maxillary first molars in the Southeast Anatolian population was found to be 81%. When evaluating the interorifice distance, it was found to be significantly larger in males than females and in Type IV canals compared to Type II canals. Obtaining information about canal morphology through CBCT scans before endodontic treatment can increase the success rate of root canal treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dicle University Faculty of Dentistry Clinical Researches Ethics Committee (Date: 29.09.2021, Decision No: 2021-48).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The success of volumetric means ADC in predicting MGMT promoter hypermethylation in glioblastomas

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ABSTRACT

Aims: This study aimed to investigate the relationship between volumetric mean ADC values and MGMT promoter hypermethylation status in glioblastoma (GB) patients segmented into perilesional edema area, solid tumor area, and necrosis area.

Methods: The 212 GB patients in the University of California San Francisco Preoperative Diffuse Glioma MRI (UCSF-PDGM) dataset were retrieved from the Cancer Imaging Archive (TCIA). The mean volumetric ADC value was calculated in patients with shared segmentation data in the UCSF-PDGM dataset. The difference in mean volumetric ADC value was investigated in patients divided into groups based on MGMT promoter hypermethylation (MGMT+/ MGMT-).

Results: Of the patients in our study, 125 (59.0%) were male. The median age of the patients was 62 years (26-94). MGMT promoter hypermethylation was observed in 152 (71.7%) patients. Mean Survival was calculated as 574.14±345.57 days in the MGMT+ group and 484.68±301.71 days in MGMT- group. According to volumetric mean ADC values, a difference was observed in the solid tumor and perilesional edema areas according to MGMT promoter hypermethylation ($p<0.001$). In the ROC analysis, the AUC value was calculated as 0.897 for the edema area and 0.812 for the solid tumor area. MGMT+ group could be identified with a cut-off value of >1.14 in ADC measurements from the edema area with 72% sensitivity and 90% specificity. MGMT+ group could be determined with a sensitivity of 88% and specificity of 69% with a cut-off value of >1.01 in ADC measurements from the solid tumor area.

Conclusion: Volumetric ADC measurements from the perilesional edema and solid tumor areas revealed higher ADC values in the MGMT+ group.

Keywords: Glioblastoma, diffusion magnetic resonance imaging, MGMT hypermethylation, apparent diffusion coefficient (ADC)

INTRODUCTION

Glioblastoma (GB) is the most common and aggressive primary brain neoplasm. GB has a very poor prognosis, which is often explained by the molecular heterogeneity of its genome, leading to an unpredictable clinical course with unpredictable treatment response.[1] The 2016 WHO classification of central nervous system tumors added molecular features to the diagnostic criteria for gliomas, which were previously based on histological diagnosis, and with the 2021 edition, the term glioblastoma has been used only for isocitrate dehydrogenase (IDH) wild-type glial tumors.[2] O6-methylguanine-DNA methyltransferase (MGMT) repairs DNA damage induced by temozolomide, and therefore higher MGMT levels lead to temozolomide resistance. MGMT promoter hypermethylation (MGMT+) reduces MGMT protein expression, thereby increasing sensitivity to temozolomide.[3] Previous studies have shown that MGMT hypermethylated patients with grade II or III glioma or glioblastoma have a longer overall survival time than those whose MGMT promoter is not methylated (MGMT).[4]

Magnetic resonance imaging (MRI) is widely accepted as the preferred method for diagnosing and evaluating treatment response.[5] GB has an infiltrative growth pattern and may expand beyond the conventional radiologic margin into normal-appearing brain tissue. Standard MRI tests underestimate the true tumor size.[6] Diffusion-weighted imaging (DAG) is combined with other sequences to assess brain tissue function and physiology. Apparent diffusion coefficient (ADC) maps/ values, a subset of DAG, represent Brownian motion in water molecules at a sub-voxel level.[7] ADC maps and

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DAG are technically robust and can be obtained without administering a contrast agent. Since the extracellular volume fraction is linked to water diffusion and is highly correlated with tissue cellularity, tissue edema, and tissue necrosis, DAG and ADC maps are helpful in the initial evaluation and post-treatment assessment of GBs.[8,9] Studies have shown that low ADC values before treatment are associated with high cellularity and overall Survival of glioma patients.[10] Furthermore, mean ADC values have been found to correlate with overall Survival.[9] ADC values have been used in differentiation in the challenging diagnostic dilemma of pseudoprogression and radiation necrosis.[11]

This study aimed to investigate the relationship between volumetric mean ADC values and MGMT promoter hypermethylation status in glioblastoma patients segmented into perilesional edema area, solid tumor area, and necrosis area.

METHODS

Ethics

With the decision of Afyonkarahisar University of Health Sciences Medical Ethics Committee dated 13.04.2023 and numbered 2023/4, it was decided that there was no need to obtain ethics committee approval for this study. All procedures were carried out in accordance with the ethical rules and the principles.

Patient Selection

The 374 GB patients in the University of California San Francisco Preoperative Diffuse Glioma MRI (UCSF-PDGM) dataset[12] were retrieved from the Cancer Imaging Archive (TCIA).[13] Patient characteristics, including age, sex, pathologic grade, and genomic profile, were obtained from the TCIA. The TCIA data did not contain any personal identifying information; ethics committee approval and informed consent were obtained in the reference study of the open-source dataset.

Inclusion criteria were determined as follows: (a) patients with a pathologic diagnosis of primary (de novo) IDH wild type (glioblastoma), (b) patients with preoperative imaging DAG MR imaging data, (c) patients with gross total resectability of the lesion. Exclusion criteria were defined as (a) patients without MGMT mutation data; (b) cranial MR images with poor quality and artifacts. We included 212 glioblastoma patients who met the selection criteria.

Segmentation and Average ADC Value Measurement

For patients with shared segmentation data in the UCSF-PDGM dataset, a series of operations were applied to the images of all patients before the mean volumetric ADC value was measured. Advanced Normalization tools for Python, intensity normalization package, bias field correction, and Z-score normalization tools were used. Resampling of the images to 1 × 1 voxel spacing and resizing to 256 × 256 pixels was performed. Afterward, the mean ADC value was calculated in the areas of necrosis, solid tumor area, and perilesional edema. All these processes were performed with Slicer v 13 (http://www.slicer.org) (**Figure 1**).

Ethics Committee

With the decision of Afyonkarahisar University of Health Sciences Medical Ethics Committee dated 13.04.2023 and numbered 2023/4, it was decided that there was no need to obtain ethics committee approval for this study.

Statistical Analysis

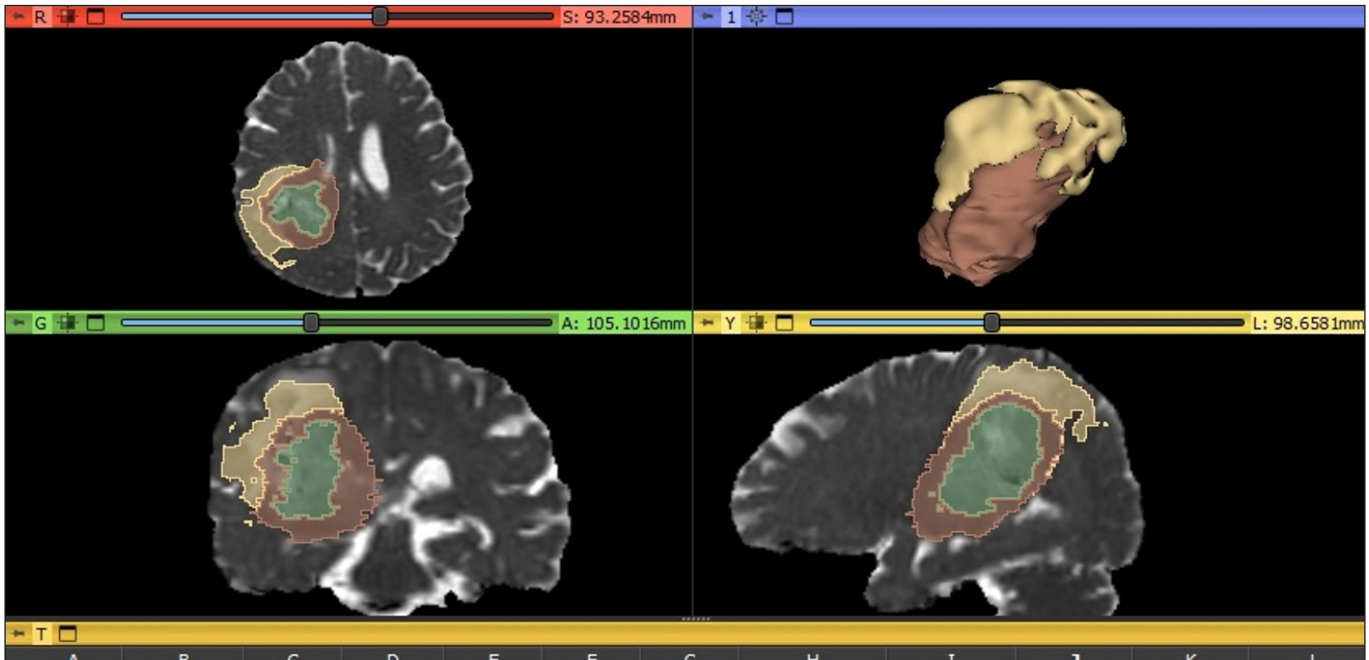
Statistical analysis was conducted using R (R Core Team v. 3.6.1). The Shapiro-Wilk test and the one-sample Kolmogorov-Smirnov test were used to assess the normality of the data, which are presented in quantile-comparison plots and histograms. The data were normally distributed. Student's t-test was used to compare between groups. Overall p-values <0.05 were considered statistically significant. Receiver-operating characteristic (ROC) curves were generated for significant areas, and the area under the curve (AUC) was calculated.

RESULTS

Of the patients included in our study, 125 (59.0%) were male. The median age of the patients was 62 years (26-94). MGMT promoter hypermethylation was observed in 152 (71.7%) patients. Mean Survival was calculated as 574.14±345.57 days in the MGMT+ group and 484.68±301.71 days in MGMT- group. Based on volumetric mean ADC values, a difference was observed in the solid tumor and perilesional edema areas according to MGMT promoter hypermethylation (p<0.001). No significant difference was observed between the two groups in the measurements made from the necrosis area. A more detailed evaluation can be seen in **Table**.

Table. MGMT promoter hypermethylation (MGMT+/ MGMT-) glioblastomas patients data

	MGMT+mean (standard deviation)	MGMT–mean (standard deviation)	P value
Age (years)	61.90 (12.07)	61.93 (12.07)	0.985
Gender (male)	84 (55%)	41(68%)	0.081
Frequency (percentage)	574.14 (345.57)	484.68 (301.71)	0.05
Mean survival (days)	1.201 (0.114)	1.013 (0.094)	<0.001
Average ADC in the edema area	1.102 (0.101)	0.971 (0.110)	<0.001
Average ADC in solid area	1.28 (0.216)	1.31 (0.234)	0.289



	A	B	C	D	E	F	G	H	I	J	K	L
1	Segment	Number of voxels ...	Volume [mm3] ...	Volume [cm3] (1)	Number of vox...	Volume [mm3] ...	Volume [cm3] ...	Minimum	Maximum	Mean	Median	Standard Deviation
2	necrosis	23110	23110	23.11	23110	23110	23.11	-0.000255512	0.00294501	0.000979736	0.00094575	0.000269469
3	oedema	47475	47475	47.475	47475	47475	47.475	-3.68573e-05	0.00250672	0.00103861	0.000953387	0.000264316
4	solid	52152	52152	52.152	52152	52152	52.152	-0.000256	0.00325223	0.000850679	0.000792179	0.000273206

Figure 1. Measurement screen of 1 patient in Slicer 3D program. In the figure, green represents the necrosis area, brown represents the solid tumor area, and yellow represents the perilesional edema area.

In the ROC analysis, the AUC value for edema area was 0.897 (Figure 2) and 0.812 for solid tumor area (Figure 3). MGMT+ group could be identified with a cut-off value of >1.14 in ADC measurements from the edema area with 72% sensitivity and 90% specificity. MGMT+ group could be determined with a sensitivity of 88% and specificity of 69% with a cut-off value of 1.01 in ADC measurements made from the solid tumor area.

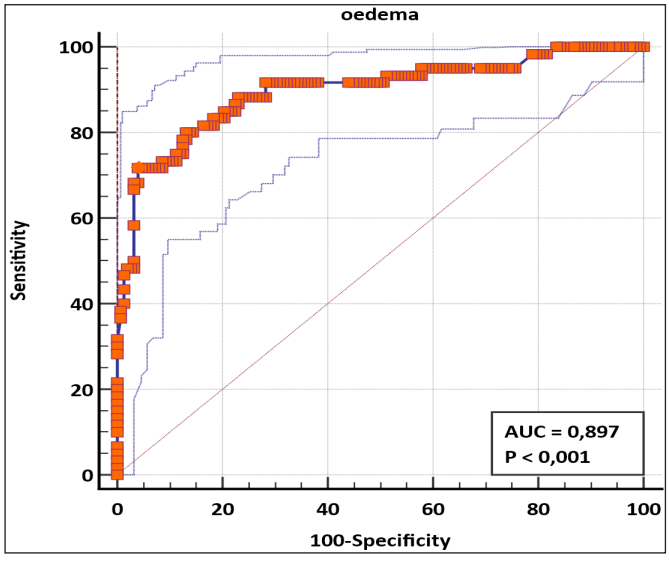


Figure 2. ROC analysis with volumetric mean ADC value from the perilesional edema area to determine MGMT status

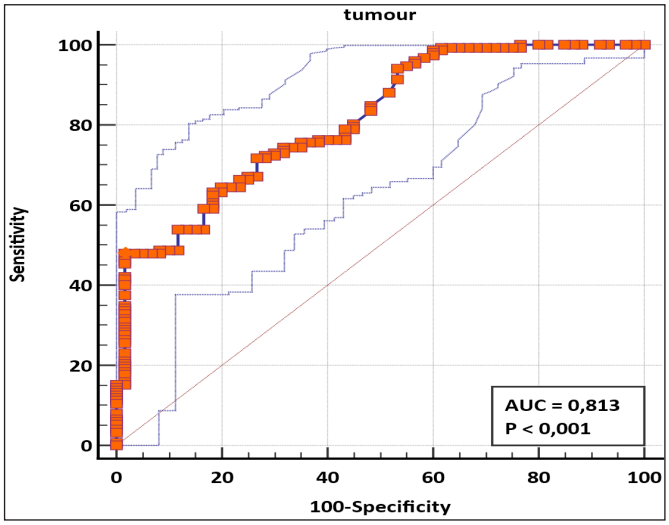


Figure 3. ROC analysis with volumetric mean ADC value from solid tumor area to determine MGMT status

DISCUSSION

Our results showed that there were significant differences in the ADC values obtained from the perilesional edema area and solid tumor area of patients with GB, which correlated with the genetic profiles of MGMT methylation status.

MGMT methylation status is an important prognostic factor because the methylation level of MGMT corresponds to the therapeutic effects of chemotherapeutic alkylating agents such as temozolomide.[14] Although many studies have been conducted on the correlation of visual parameters (tumor location and laterality, enhancement features such as ring enhancement) and MGMT methylation status, there is still no generally accepted consensus.[15] Advances in radiologic diagnostics may lead to a development where molecular profiles can be predicted based on an initial MRI study, which could have major implications for treatment options prior to any surgical intervention (biopsy or resection).

In studies, lower ADC values have been associated with more malignant tumors and tumors with higher cellularity.[16] It has been suggested that radiologic reports of gliomas should include the locations of areas with the lowest ADC values. These low ADC areas and other imaging indices and features are considered the parts of these tumors with the most malignant potential.[17,18]

Our results did not find a significant difference based on the MGMT methylation status of the centrally located necrosis area. We think that this is predictable because the necrosis area does not contain significant cellularity.

Higher ADC values were measured in the area of perilesional edema in the MGMT+ group. We used a cut-off value of >1.14 to determine MGMT+ status. The perilesional T2-hyperintense white matter in GB also contains a significant amount of tumor invasion area, unlike the vasogenic edema areas found in other brain mass lesions with metastases.[19] Ladenhauf et al.[20] found lower ADC values in the peri-tumoral edema area in the MGMT+ group in their ROI-based measurement from the peritumoral area, which differs from our study. Unlike our study, ROI-based normalized ADC value was used, and a relatively small sample group of 42 patients was studied. We think this may be the reason for the difference between the results.

Higher ADC values were measured in the solid tumor area in the MGMT+ group. The MGMT+ group could be identified with a sensitivity of 88% and specificity of 69% with a cut-off value of >1.01 in ADC measurements. In a recent study by Xie et al.[21] IDH mutant and MGMT+ patients were compared with patients with other IDH and MGMT conditions. They found higher ADC values in the IDH mutant and MGMT+ group according to the results of ADC analysis and ADC histogram analysis. Although the study group was quite heterogeneous, similar results with our study are noteworthy. Choi et al.[22] found no correlation between ADC histogram analysis and MGMT status.

Diffusion imaging, one of the main neuroimaging examinations, both tumor imaging and neuroimaging obtained in other conditions, stores much more information than routine use. Without clear results, there is still much uncertainty about the relationship between ADC values and MGMT profile. The results of our study with the largest sample group in the literature on this subject are promising. Although we only evaluated the mean ADC value in our study, it can be evaluated more accurately in future studies with refined texture analysis-based artificial intelligence models,[23] of which there are many examples in glial tumors.

Our study has some important limitations. Since the study was planned retrospectively, no evaluation could be made regarding data such as performance status and other mutations, not in the patient's available data set.

CONCLUSION

As a result, volumetric ADC measurements from the perilesional edema and solid tumor areas showed higher values in the MGMT+ group. It is promising that MGMT promoter hypermethylation, an important prognostic marker for glioblastoma, can be predicted preoperatively non-invasively with ADC maps. Future prospective studies, which may include more comprehensive texture analyses, will solidify the place of ADC analysis.

ETHICAL DECLARATIONS

Ethics Committee Approval: With the decision of Afyonkarahisar University of Health Sciences Medical Ethics Committee dated 13.04.2023 and numbered 2023/4, it was decided that there was no need to obtain ethics committee approval for this study.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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Original Article

HEALTH SCIENCES
MEDICINE

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Effects of chronic urticaria on ovarian reserve

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ABSTRACT

Aims: To evaluate the ovarian reserve in women with chronic urticaria (CU).

Methods: Thirty women with CU and twenty nine healthy controls were enrolled in this study. Serum levels of anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), estradiol (E2), and luteinizing hormone (LH) were measured on cycle day 2. All patients underwent transvaginal ultrasonographic examination on the second day of their menstrual cycle to assess Antral Follicle Count (AFC) and ovarian volume (OV). The disease activity of CU was measured with the urticaria control test (UCT) and urticaria activity score (UAS).

Results: Women with CU had significantly lower concentrations of AMH, AFC, and E2 than controls. Additionally, total AFC and ovarian volume were significantly lower in women with CU than in the control group. UAS and UCT were positively associated with FSH and negatively associated with AMH, AFC, and OV.

Conclusion: This is the first prospective controlled study to examine ovarian reserve in women with CU. In this study, we found that ovarian reserve was significantly reduced in women with CU. CU may negatively affect the ovarian reserve but the mechanism of this effect is unclear.

Keywords: Antral follicle count, ovarian volume, anti-Müllerian hormone, chronic urticaria

INTRODUCTION

Chronic urticaria (CU) is a chronic disease characterized by itchy wheals and flare-type skin reactions lasting more than six weeks.[1] The causes remain unknown, so it is considered idiopathic and occurs in about 0,75% of patients.[2,3] Generally, CU has an immunological and inflammatory process and may affect all organs.[4] There have been studies that found an association of CU with thyroid autoimmunity, which further supports its autoimmune origin.[5] In the literature, no study has examined the effects of CU on the ovary. The reproductive potential of women can be estimated with ovarian reserve tests consisting of basal levels of follicle-stimulating hormone (FSH), estrogen (E2), anti-Müllerian hormone (AMH), as well as antral follicle count (AFC) and ovarian volume (OV).[6] The use of multiple ovarian reserve markers suggests that no test is ideal. In fact, the biggest determinant of fertility potential in a regularly cycling woman is age.[7] However, the most widely used and reliable tests measuring ovarian reserve are AMH and AFC. A diminished ovarian reserve is defined as a reduced ability to produce eggs in the ovaries of a woman at any age.[8] Thus far, only one case report found an association between CU and ovarian reserve.[9] Chronic diseases negatively affect the ovarian reserve of women and decrease their reproduction potential. The most commonly used tests, including AFC and AMH, may measure this effect of chronic diseases on ovarian reserve. In the literature, this is the first study to evaluate the effects of CU on ovarian reserve using both ultrasonographic and biological markers. The present study investigated the efficacy of the regularly used markers for predicting ovarian reserve in reproductive-age women with CU.

METHODS

The study was carried out with the permission of Acıbadem University Ethics Committee (Date: 22.11.2018, Decision No: 2018-18/29). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was conducted in the Department of Obstetrics and Gynecology and Dermatology at Kayseri City Training and Research Hospital. Thirty women with

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CU and 29 healthy women as controls were admitted to this study. Female patients with a confirmed diagnosis of CU were asked to participate. After written consent was obtained, patients were screened for eligibility. The inclusion criteria were:

1. Reproductive age (20-30 years),
2. Regular menstruation cycle (interval of 21–35 days),
3. Less than 4 days of variation in cycle length, and
4. The presence of both ovaries.

The exclusion criteria were chronic kidney or liver failure, any known malignancy, smoking history, infertility, gynecological abnormalities including dysfunctional uterine bleeding or menorrhagia, previous ovarian surgery, use within the last 3 months of a hormonal preparation or unregulated herbal product, or a polycystic ovary syndrome (PCOS) diagnosis. The control group was selected from women attending their routine gynecological exam without any complaints (for example, yearly pap smear screening). Patients with CU were diagnosed in the Department of Dermatology. In the early follicular phase of the menstrual cycle (days 2–4), all patients had a venous blood sample taken between 08:00 am and 09:00 am from the antecubital region. The enzymelinked immunosorbent assay (ELISA) method was used to measure the levels of folliclestimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and AMH. The AMH Chemiluminescent Microparticle Immunoassay Model 602 (Cobas®, Roche, Manheim, Germany) was used to assess the AMH levels. The Photometric Model 702 (Cobas®, Roche, Manheim, Germany) was used to analyze FSH, LH, E2, and PRL levels. A pelvic ultrasound was used to measure ovarian volume and AFC on the same day as the blood draw. The same operator blinded to all patient information performed a pelvic ultrasound with a 7.5 MHz transvaginal probe to count the total number of antral follicles measuring 2–10 mm in diameter. Ovarian volume was calculated using the equation of an ellipsoid (0.526 x length x height x width) (10). Participants' height, weight, body mass index (BMI), disease duration, and age were noted. After the venous blood samples were taken, they were stored at -20±8 °C and assayed for LH, FSH, E2, and AMH. The disease activity of CU was measured using the urticaria control test (UCT) and urticaria activity score (UAS). The same author, R.E., evaluated all participants and the severity of the disease was scored from 0 (no symptoms) to 10 (highest symptoms) using a visual analog scale (11).

Statistical Analysis

Mean±standard deviation (SD) was used to report numerical variables. The Kruskal-Wallis test and Mann-Whitney U test were used to analyze non-normally distributed variables. For normally distributed data, paired t-tests were used to compare the groups. Relationships between parameters were assessed using Spearman correlation coefficients. $p<0.05$ was considered statistically significant. The Statistical Package for the Social Sciences, version 15.0 (SPSS, Chicago, IL, USA), was used to perform all analyses.

RESULTS

Table 1 shows the summary of demographic measurements of the CU and control groups. No significant differences were found between the groups for age, BMI, and LH. FSH was significantly higher in the CU group than in the control group (8.8±4.06 vs. 8.0±0.84, p=0.001).

Table 1. Demographic characteristics and hormonal levels of the participants

	Patient (n:30)	Control (n:29)	p-value
Age	23.26±7.89	24.24±2.01	0.44
BMI (kg/m²)	23.96±3.78	23.07±0.84	0.216
FSH (mIU/mL)	8.8±4.06	8.0±0.84	0.001
LH (mIU/mL)	7.27±3.62	7.0±0.88	0.09
E2 (pg/mL)	45.93±21.37	59.62±3.26	<0.001
Total AFC	9.2±3.5	12.5±1.5	<0.001
Total OV (cm³)	7.7±4.21	14.37±1.49	<0.001
AMH (ng/ml)	1.62±1.13	2.51±0.50	<0.001

BMI: Body mass index, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, AFC: Antral follicle count, OV: Ovarian volume, AMH: Anti Müllerian hormone.

The values of E2, total AFC, OV, and AMH were significantly lower in the CU group than in the control. When we looked for correlations, UAS and UCT were positively associated with FSH and negatively associated with AMH, AFC, and OV (Table 2).

Table 2. Association of urticaria activity score (UAS) and urticaria control test (UCT) with hormonal levels.

	UAS	UCT	p-value
FSH	0.44	0.32	0.03
AMH	-0.27	-0.21	0.05
AFC	-0.28	-0.18	0.04
OV	-0.35	-0.27	0.66

FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count, OV: Ovarian volume. Statistical analysis was done by Spearman correlation coefficient.

DISCUSSION

Ovarian reserve is important to achieve pregnancy for reproductive-age women. Recently, the measurement of ovarian reserve has been used to predict the reproductive capacity of women. The most widely used tests are AFC and AMH.[12] This is the first study to evaluate the effects of CU on ovarian reserve and evaluate the associated hormones and AFC. Decreased levels of AMH, which is produced by granulosa cells, are considered a sensitive marker of diminished ovarian reserve. Decreased AFC is another sensitive marker to assess diminished ovarian

reserve.[13] In this study, we used the most predictive and widely used tests to predict ovarian reserve in women with CU. The values of AMH and AFC were significantly decreased in our study. Additionally, UAS and UCT are sensitive tests to measure the severity of CU. In our study, we first evaluated the correlation between the severity of CU and ovarian reserve tests. UAS and UCT were positively associated with FSH and negatively associated with AMH, AFC, and OV. Recent studies indicate that lower AMH levels may show an increased risk of earlier menopause.[14] Autoimmune chronic diseases such as systemic lupus erythematosus(SLE), Behcet, and others may negatively affect ovarian reserves.[15-17] Although the etiology is not clear for CU, the most common reason is autoimmune conditions. CU is also associated with chronic inflammation and the disease activity affects the organs.[18] In this study, we evaluated effects on reproductive health in women with CU compared to women without CU. In the present study, the activity of CU negatively affected the ovarian reserve. Therefore, treatment and duration of CU may be used to evaluate this negative effect of CU on ovarian reserve. The most important factors for fertility are the number and quality of antral follicles. The number of oocytes is positively correlated to pregnancy in infertility treatment.[19] Age is one of the most pivotal factors for oocyte quality.[20] In this study, the diminished values of AMH and AFC may prompt a discussion of fertility preservation for women with CU such as oocyte freezing.

CONCLUSION

This is the first study to suggest that women with CU are more likely to have diminished ovarian reserves in their reproductive years than control women. Additional studies of women with CU using a larger sample size are needed to confirm that the disease activity negatively affects the ovarian reserve.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Acıbadem University Ethics Committee (Date: 22.11.2018, Decision No: 2018-18/29).

Informed Consent: All patients signed and free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

HEALTH SCIENCES
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The effect of age on the severity of dry mouth occurring in patients receiving high dose radioactive iodine treatment

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ABSTRACT

Aims: Dry mouth of individuals causes many oral discomforts and undesirable conditions. In order to prevent such an undesirable situation from occurring, it is aimed to complete the Radioactive Iodine Therapy (RAI) treatment with less damage by mastering all possible factors. In our study, we aimed to find the level of the effect of the age factor.

Methods: In this study, we included patients who received high-dose radioactive iodine treatment after total thyroidectomy for differentiated thyroid cancer and were hospitalized in our clinic. These patients were selected among the patients who applied to our hospital between 2021-2022. We specifically focused on patients who reported dry mouth and obtained salivary gland scintigraphies of their submandibular glands. Afterwards, we looked at the ratio of the age of the patients with the level of dry mouth. We analyzed the collected data using statistical methods.

Results: The data analysis was performed using the Chi-square ($\chi 2$) test and the Spearman correlation test to examine the relationship between age and the severity of dry mouth in patients undergoing high-dose RAI treatment for thyroid cancer. Our hypotheses were two-sided, and statistical significance was considered at p-values ≤ 0.05. The results of our analysis revealed that there was no significant direct correlation between age and the severity of dry mouth in the patients. This suggests that age is not a determining factor for the occurrence or severity of dry mouth in individuals receiving high-dose RAI treatment.

Conclusion: In our study, it was concluded that the age factor alone was not a determining factor in terms of the severity of dry mouth. Therefore, it is important to consider additional factors and potential underlying causes when evaluating and managing dry mouth in patients receiving high-dose RAI therapy for thyroid cancer.

Keywords: Dentistry, dry mouth, age

INTRODUCTION

High-dose radioactive iodine treatment is a medical procedure used to destroy cancer cells or reduce the size of a thyroid nodule by administering a high dose of radioactive iodine. This type of treatment is most commonly used for people with thyroid cancer or an overactive thyroid (hyperthyroidism). The radioactive iodine accumulates in the thyroid gland and destroys the gland cells or reduces the size of the nodule.[1,2] During treatment, a high dose of radioactive iodine is given to the patient to destroy the thyroid tissue. While this treatment can be effective in controlling the disease, it can also cause a side effect known as dry mouth.[3] This is because the treatment can damage the salivary glands that produce saliva. The degree and duration of dry mouth after radioiodine treatment depends on several factors, including the dose of radioactive iodine administered, the patient's general health, and the presence of other medical conditions.[4]

Apart from radioactive iodine treatment, there are various factors that cause dry mouth. It is important to know these factors in the studies conducted and to form the study group. Some of these factors are:

Medications, medical conditions, dehydration, aging, tobacco and alcohol use, nerve damage and radiation therapy.[7]

Knowing these factors is important in terms of eliminating external factors in the research to be done. There are many methods to determine the presence

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of dry mouth in patients. These investigating changes in parotid gland function caused by dry mouth such as salivary flow rate test, salivary gland scintigraphy, sialometry, biopsy, medical history and physical examination procedures to determine the cause and extent of parotid gland dysfunction due to dry mouth and to establish an appropriate treatment plan.[8]

Saliva plays a crucial role in maintaining oral health and comfort by moisturizing the mouth, neutralizing acids produced by bacteria, and aiding in the digestion of food.[5] Oral health in patients with dry mouth may encounter many problems, for example; tooth decay, oral infections, It can have a negative effect, such as difficulty speaking and swallowing, taste changes, mouth discomfort, and poor quality of life.[9]

Dry mouth can be an important problem in patients receiving high-dose radioactive iodine therapy and may negatively affect the overall quality of life.[10] Therefore, it is important that healthcare professionals and patients are aware of the potential for dry mouth after high-dose radioiodine treatment and take steps to manage and prevent its effects. Therefore, it is important for healthcare providers and patients to be aware of the potential for dry mouth after high-dose radioactive iodine treatment and to take steps to manage and prevent its effects.

The body's reactions to external factors or diseases change with age changes. The human body's defense mechanism and cell renewal rate differ with age. Investigating the effects of age, which affects many mechanisms in humans, or the responses to diseases is an important step in planning many treatments. As in many diseases, it is important to know the relationship between age and high-dose RAI treatment when determining the appropriate treatment plan for Thyroid patients. Age may affect the efficacy and potential side effects of treatment.[11]

It is an important issue whether the salivary glands of patients of different ages will be at higher risk for radiation-related side effects. In our study, we will investigate how the salivary glands are affected in patients who received different doses of radioactive iodine at different ages.

METHODS

The study was carried out with the permission of Batman University Clinical Researches Ethics Committee (Date: 16.02.2022, Decision No: 2022-02-08). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We conducted a retrospective study on patients with thyroid cancer who applied to our thyroid polyclinic in the nuclear medicine department of the training and research hospital between 2021-2022. The study included patients who developed dry mouth after receiving high-dose radioactive iodine (RAI) 131 treatment for thyroid cancer. Patients who underwent total thyroidectomy for differentiated thyroid cancer and received high-dose (>30 mCI) RAI from patients hospitalized in our clinic were included in the study. For the study, salivary gland scintigraphy was examined.

Patients who had previously had head and neck cancer and received chemotherapy or radiotherapy in their treatment and patients who developed dry mouth after using any drug were not included in the study. We examined the salivary gland scintigraphy of these patients.

Patient Preparation

When the patient arrived, a paper cup with a straw and a lemon were provided.

Shooting Protocol

The patient was positioned in a horizontal position, and the neck was hyperextended. Low-energy and high-resolution SPECT imaging was performed using a parallel collimator, with a peak of 140 keV and a 20% window width adjustment. Dynamic imaging was performed for 30 minutes. For imaging of the salivary glands, 10 mCi (370 MBq) of 99m Tc pertechnetate was injected intravenously through the cubital vein. Imaging was conducted for 30 minutes, and after 15 minutes, the patient drank 5 ml of freshly squeezed lemon juice from the paper cup using a pipette. During the imaging, the patient was instructed not to move or speak.

Evaluation Criteria

All images were evaluated by the same 2 nuclear medicine specialists. ROI (Regions of Interest) was drawn to penetrate the parotid and submandibular glands in bilateral patients. We performed a semi-quantitative analysis of salivary gland scintigraphy using the Salivary Gland Scintigraphy with Quantitative Analysis program located on the workstation. The program allowed us to measure the filling and emptying functions of the salivary glands, which are classified according to the curves as mild, moderate or severe impairment. The results were evaluated with the scoring method.

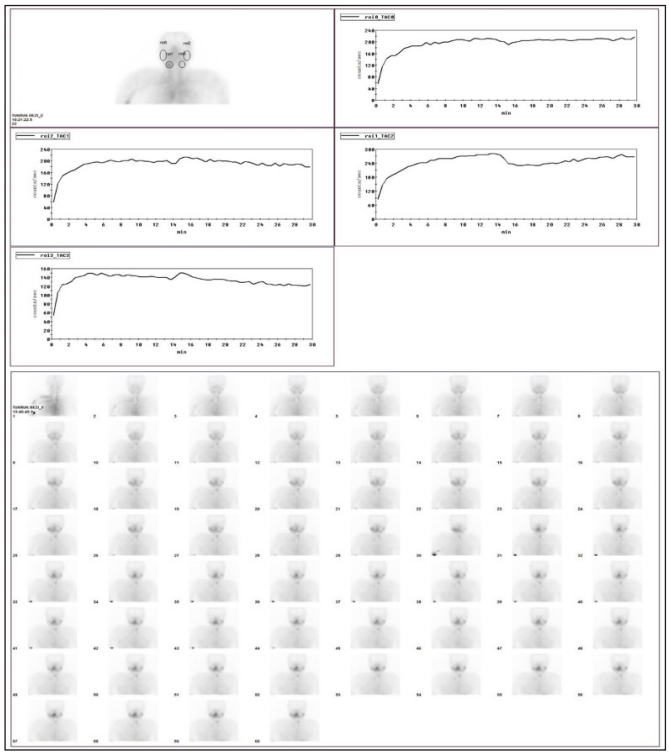

Figure 1: A 66-year-old patient received RAI. It was observed that the functions of the parotid gland and left submandibular gland were severely reduced (roi0, roi2 and roi3), and the right submandibular gland function was mildly affected (roi1)

RESULTS

Table 1: Data table of severity of dry mouth occurring in patients with scintigraphy

	Age	Right Parotis	Left Parotis	Right Submandibular	Left Submandibular
Patient 1	44	0	0	0	0
Patient 2	62	0	0	0	0
Patient 3	43	0	0	0	0
Patient 4	44	0	0	0	0
Patient 5	66	3	3	1	3
Patient 6	36	0	0	0	0
Patient 7	73	1	1	0	1
Patient 8	64	0	0	0	0
Patient 9	43	0	0	0	0
Patient 10	56	1	1	2	3
Patient 11	57	0	0	1	1
Patient 12	50	0	0	0	0
Patient 13	47	1	1	1	1
Patient 14	53	0	0	0	0
Patient 15	40	1	1	0	0
Patient 16	27	0	0	0	0
Patient 17	25	0	2	3	3
Patient 18	42	0	0	0	0
Patient 19	54	0	0	0	0
Patient 20	47	0	0	0	0
Patient 21	71	0	0	0	0
Patient 22	44	0	0	0	0

The following dial indicates the degree of degradation:

0= Normal
1= mild degradation
2= Moderate Degradation
3= Severe Degradation

The statistical analysis of our research data was carried out using the IBM SPSS 21.0 for Windows statistical package program. Mean±standard deviation (SD) was used to present the measured variables, while numbers and percentages (%) were used for categorical variables. To compare qualitative variables between groups, we employed the Chi-square ($\chi 2$) test analysis. Additionally, the Spearman correlation test was used to determine the relationships between variables. Our hypotheses were two-sided, and we considered results statistically significant if $p \leq 0.05$.

Table 2: Statistical analysis results

AGE	R	P
Right Parotis	0.293	0.186
Left Parotis	0.085	0.708
Right Submandibular	0.089	0.694
Left Submandibular	0.265	0.233

R: Correlation coefficient P: Significance value

There was no correlation found between age and the right parotid, left parotid, right submandibular, and left submandibular glands. This means that age did not have an effect on these glands. In our study, it was observed that dry mouth, which occurs after radioactive iodine treatment, did not increase proportionally with age. As age increased, dry mouth did not increase at the same rate.

DISCUSSION

In 2001, Solans R, et al.[12] In a study called salivary and lacrimal gland dysfunction after radioiodine treatment Salivary gland scintigraphy can be used to determine the functionality of the salivary glands, and scintigraphy from the high-activity RAI treatment is a method that allows early detection of glandular dysfunction. we also did our research by using salivary gland scintigraphy in our study.

In their 2018 publication, Park KW et al.[13] noted that the use of radioactive iodine (RAI) treatment in patients with thyroid cancer dates back to the 1940s. The authors specifically included patients who had undergone RAI treatment in their study.

In their 2020 publication, Singer MC et al.[14] emphasized the importance of acknowledging the

potential negative consequences of RAI treatment in patients with thyroid cancer. While it is widely accepted that RAI may have an adverse impact on salivary glands, the relationship between the degree of salivary gland dysfunction and its incidence, as well as the impact of patient age, has not been extensively studied. In our study, we aim to address this knowledge gap by observing the impact of age on salivary gland dysfunction in RAI treatment, with the goal of eliminating this deficiency.

In the Conclusion section of the same study,[14] while investigating the factors affecting RAI treatment, it was found that age, interval between RAI treatments, secondary tumors, pre-existing salivary gland diseases, systemic diseases, and medications used, among other factors, affect the risk of complications associated with RAI. The risk-benefit calculation of RAI treatment for individual patients generally takes all these factors into account together, and age alone should not be the sole consideration.

A 2013 study by Jeong SY et al.[15,16] found that serous salivary cells are better at concentrating iodide than mucinous acini, and the highest concentration of serous cells is found in the parotid glands. As a result, although all salivary glands transport RAI into saliva, the parotid glands are most negatively affected by RAI due to their high concentration of serous cells. Additionally, while sodium iodide symporters in submandibular glands continuously transport RAI from parenchymal cells to ducts, symporter function in the parotid glands is less consistent. This results in a longer transit time in the parotid glands, which exposes the parotid parenchyma to greater amounts of RAI. Consequently, these glands are particularly susceptible to the adverse effects of RAI. We included the two most sensitive salivary glands, the parotid and submandibular glands.

In a 2015 study, Kim et al.[15] noted that the parotid glands have the highest concentration of serous cells among all salivary glands. Due to this, they are particularly susceptible to the adverse effects of radioactive iodine (RAI) transport. The authors also included analysis of both the parotid and submandibular glands to better observe the effects of RAI on these glands.

In the study Eratilla[5] performed on 15 patients who received RAI treatment in 2021The rate of involvement of the parotid glands was examined by the scintigraphy method. Author reported that Dry mouth complication was observed in all of these patients. In our study, we found that patients were affected.

In a study conducted by Caglar et al.[17] in 2002, it was reported that age and gender had an impact on salivary gland function. They also noted an increased risk of salivary gland dysfunction after RAI treatment in women and individuals over the age of 45. However, in our study, when examining a wide range of age groups, we found that age alone did not significantly affect salivary gland dysfunction, and it should be considered in conjunction with other factors.

In their 2011 study, Van Nostrand et al.[18] did not include age as a variable when listing the factors contributing to the incidence and severity of sialadenitis resulting from RAI. In our study, we found that age did not have a significant effect on the rates of involvement of the parotid and submandibular glands. In a study titled "Early sialadenitis after radioactive iodine treatment for differentiated thyroid cancer: Prevalence and determinants," conducted by Riachy et al.[19] the occurrence of salivary gland infection (sialadenitis) following treatment was investigated. The results of their study showed that age alone is not associated with the development of salivary gland infection after radioactive iodine treatment, which supports our findings.[19]

CONCLUSION

Age may be a factor in terms of the severity and frequency of dry mouth, but it is not considered to be the sole or even the most important factor. Other factors that can contribute to dry mouth after RAI treatment include the dose of radioactive iodine received, the time elapsed since the treatment, the individual's overall health, and any pre-existing medical conditions or medications being taken.

Additionally, some individuals may be more prone to dry mouth due to poor habits or inadequate oral hygiene. While these factors may be associated with age, they are not solely dependent on age.

Overall, age is just one of several factors that can contribute to dry mouth after RAI treatment, and the severity and duration of dry mouth can vary significantly from person to person.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Batman University Clinical Researches Ethics Committee (Date: 16.02.2022, Decision No: 2022-02-08).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The comparison of success status and complications in peyronie disease patients: penile plication versus plaque incision and grating techniques

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ABSTRACT

Aims: This study aimed to evaluate and compare the outcomes of tunical lengthening and shortening techniques for the surgical management of Peyronie's disease (PD) in terms of penile length, patient satisfaction, and complications.

Methods: A retrospective analysis was conducted on PD patients who underwent surgical procedures between January 2017 and May 2023 at a single institute. Patient demographics, comorbidities, penile curvature, penile length, and surgical techniques were evaluated. The surgical procedures included plaque incision and grafting, and tunical plication. Post-operative data, including penile length, residual curvature, patient satisfaction, adverse events, and erectile function, were collected at 1-12 months of surgery.

Results: A total of 36 patients (mean follow-up period: 432.25±121.14 days) underwent surgical procedures for PD. Penile length in the tunical lengthening group (13.04±1.62 cm) was significantly longer than in the tunical shortening group (10.85±1.24 cm, p=0.001). Tunical shortening procedures were associated with a higher rate of penile shortening (57.1% vs. 6.7%, p=0.001) and suture-related discomfort (38.1% vs. 6.7%, p=0.031) compared to tunical lengthening procedures. Patient satisfaction was higher in the tunical lengthening group (80% vs. 66.7%, p=0.378). There was no significant difference in painful erection during penetrations or incapability of intercourse between the groups. The average length of hospital stay was longer for tunical shortening surgery compared to tunical lengthening surgery (1.27±0.27 vs. 1.79±0.39, p=0.023).

Conclusion: Tunical lengthening procedures demonstrated superior outcomes in terms of penile length and suture-related discomfort compared to tunical shortening procedures for the surgical management of PD. Patient satisfaction was also higher in the tunical lengthening group. Clinicians should consider various factors when selecting the appropriate surgical technique for PD, including penile length, the degree of penile curvature, and surgeon experience. Prospective randomized studies are needed to further validate these findings and assess long-term outcomes.

Keywords: Peyronie, plicatation, grafting, penile length, satisfaction

INTRODUCTION

Peyronie's disease (PD) is a connective tissue disorder of the tunica albuginea, often caused by repeated microvascular trauma during intercourse. Itsprevalence rates have been observed up to 20.3% in epidemiological studies.[1] In 1743, François Gigot de La Peyronie, a French surgeon, provided the first detailed description of PD. It was described as a disorder that is characterized by penile curvature and hardening, leading to pain during erection and sexual intercourse.[2] This progressive fibrotic process can cause anatomical and functional changes, including narrowing of the penis diameter, shortening of penis length, and difficulty of maintaining erectile function.[3] Shortening of the penis due to PD caused a significant physiologic stress on the patients.[4,5]

Many treatment modalities including intralesional injections and surgical procedures have been performed for the chronic phase of PD. Conservative treatment modalities for PD were associated with poor outcomes and current guidelines recommended that it can be only performed to the patients who are not eligible for the surgery.[1] For this reason surgery is the main treatment modality for clinically significant PD. There are various surgical procedures including tunical lengthening techniques such as plaque incision and

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grafting, as well as tunical shortening techniques like tunical plication. The type of surgery depends on several factors including the degree of curvature, presence of complex deformity, plaque size, penis length, and side of penile deformity. There is currently no worldwide-accepted standard surgical procedure for PD, and these surgical procedures may carry risks of serious adverse events. For example, tunical plication techniques may lead to penile shortening, while plaque incision and grafting procedures may lead to reduced penile sensitivity and erectile dysfunction. Careful evaluation and individualized treatment planning are necessary to minimize risks and optimize outcomes in the surgical management of PD.

Deciding the best surgical technique is the main point for the treatment of PD. Each surgical procedure has advantages and disadvantages. For this reason, analyzing the surgical outcomes of different surgical techniques is very important. The primary aim of of study was to present the outcomes of surgical procedures of PD at our clinic. The secondary aim of the study was to compare the satisfaction and efficacy of tunical lenghting techniques with tunical shortening procedures.

METHODS

The study was carried out with the permission of Tekirdağ Namık Kemal University Non-interventional Clinical Researches Ethics Committee (Date: 16.02.2022, Decision No: 2022-02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The patients who underwent surgical procedures for PD between January 2017 and May 2023 at a single center were retrospectively included in the study. The patients with penile curvature were evaluated with a full medical history and physical examination. Possible risk factors for erectile dysfunction including diabetes, hypertension, hyperlipidemia, frequency of exercise, and tobacco, and alcohol consumption were also assessed. The erectile function was evaluated via the short form of the International Index of Erectile Function Questionnaire-5 (IIEF-5).[6] Penile curvature was evaluated after the intracavernosal alprostadil injection or self-photos during the erection. Stretched penile length in detumescence phase was measured as the distance from the tip of the glans penis to the symphysis pubis and penile diameter was also measured at the circumcision line 1cm below the glans penis. Patients were excluded from the study if they were at the acute phase of PD, had the presence of penile trauma history, had previous penile curvature surgery or had congenital chordee.

Surgical procedures were only performed on patients who were at the chronic phase of PD (>1 year). The type of surgical procedure was selected according to the defined criteria mentioned in previous studies.[7] Patients with more than 60 degrees of penile curvature or complex penile deformity underwent plaque incision and grafting (double Y or H incision), whereas patients with less than 60 degrees of penile curvature underwent Yachia plication procedures. Two types of grafts including saphenous venous patch and bovine pericardium (Dutapech, Coloplast) were used for the filling defect in the corpus cavernosum. Phosphodiesterase type 5 inhibitors was applied to patients after post-operative 2 weeks of surgery and traction exercises were recommended to prevent contraction of graft material for tunical lengthening surgeries. Post-operative data including post-operative penile length, residual curvature, patient satisfaction, adverse events, and erectile function were obtained through face-to-face conservation or teleconference system at 1-12 months of the surgery.

Statistical Analysis

SPSS software version 25 (IBM, Armonk, NY, USA) was performed with all statistical tests. The sample mean and standard deviations were used to determine the average of collected data as quantitative variables met the normal distribution; otherwise, the sample median and min.-max. value was used for abnormal distribution. For nominal variables in the groups, a chi-square test was used. When the normality assumption was met for both groups, a Student's t-test was used to allow for group comparison. If the normality assumptions for groups were not met, the corresponding nonparametric Mann-Whitney U test was used.

RESULTS

A total of 36 patients were included in the study. The most common complaint of patients was inability to perform intercourse followed by penile pain during erection. The penile curvature was to the dorsal direction at 21 (58.3%) patients and to the lateral direction at 7 (19.4%) patients. The mean follow-up period for PD was 432.25±121.14 days and the mean duration of PD was 21.52±7.65 months. Comorbidities were also common, with 12 (33.3%) of patients having a history of diabetes, 7 (19.4%) having hypertension, and 5 (13.9%) having coronary artery disease (CAD). Clinico-demographic properties of patients were shown in **Table 1**.

There were no significant differences between preoperatively in penile length at tunical shortening and lengthening groups. (11,98±1,27cm , 12,21±1,44cm, respectively; p=0.621) Post operative penile length in PD who underwent penile plications was significantly shorter compared to tunical lengthening surgeries. (10.85±1.24,

13.04±1.62; respectively, p=0.001) It was observed that the average change in penis length was -1.13 cm in the tunic shortening methods, while it was +0.83 cm in tunical lengthening procedures. The differences in PD surgery-related penile length were shown in Table -2. Additionally, 12 (57.1%) of 21 patients in the tunical shortening group reported penile shortening, compared to 1 (6.7%) in the tunical lengthening group (p=0.001). Patient satisfaction was also higher in the tunical lengthening group, with 12 (80%) of 15 of patients reporting satisfaction with the surgical outcome, compared to 14 (66.7%) of 21of patients in the tunical shortening group (p=0.378).

Table 1. Clinico demographic properties of patients with Peyronie disease

	Patients with PD
Symptoms of patients at admission	
Difficulty during intercourse due to penile curvature	17 (47.2%)
Penile pain during erection	7 (19.4%)
Erectile dysfunction	3 (8.3%)
Penile lump	4 (11.1%)
Multiple complaints	5 (13.8%)
Side of penile curvature	
Dorsal	21 (58.3%)
Ventral	2 (5.6%)
Lateral	7 (19.4%)
Dorsolateral	6 (16.7%)
The presence of complex deformity	
Hour glass	2 (5,6%)
Pincer deformity	-
Co-morbidities	
DM	12 (33.3%)
HT	7 (19.4%)
CAD	5 (13.9%)
Age (year)	46.58±17.61
BMI (kg/m²)	21.14±11.47
The degree of penile curvature	49.21±21.76
Mean follow up period (day)	432.25±121.14
Duration of PD (month)	21.52±7.65
Stretched penile length (cm)	10.27±1.34
Penil diameter (cm)	9.19±0.83
Preoperavite IIEF scores	14.53±4.73
Mean operative time (min)	117.61±50.38
Patients satisfactions	
Yes	26 (72.2%)
No	10 (28.8%)

Table 2. The outcomes of stretched penile length between tunical lengthening and shortening procedures

	Penile plications	Plaque incision+ grafting procedures	p value
Penile length preoperative (cm)	11,98±1,27	12,21±1,44	0,621
Penile length postoperative (cm)	10,85±1,24	13,04±1,62	0.001
p value	0.781	0.478	

A total of 8 (38.1%) patients who underwent tunical shortening reported suture-related discomfort or pain (painful knots in the penis) whereas 1 (6.7%) patient in the tunical lengthening group reported this side effect (p=0.031). Pain during vaginal penetrations and incapability of intercourse were observed with higher rates in penile shortening group but there was no statistical difference between groups. (p=0.651, p=0.289 respectively) The average length of hospital stay was longer for patients who underwent tunical shortening surgery (1.27±0.27) than for those who underwent tunical lengthening surgery (1.79±0.39) (p=0.023) The comparisons of tunical lengthening technique and tunical shortening techniques at PD patients were shown at Table 3.

Table 3. Comparisons of tunical lengthening techniques and tunical shortening techniques in Peyronie's disease patients

	Penile plications (n:21)	Plaque incision+ grafting procedures (n:15)	p value
Age (year)	47.31±18.61	52.36±7.57	0.248
BMI	20.14±8.14	21.98±9.76	0.785
The degree of penile curvature	49.25±18.23	68.49±29.14	0.001
Mean hospital stay (day)	1.27±0.27	1.79±0.39	0.023
Mean operative time (min)	61.47±19.28	131.69±22.43	0.001
Residual curvature			0.371
< 30 degree	2	3	
30-60 degree	-	-	
Reduced penile sensation			0.151
Yes	1	3	
No	20	12	
Painful erection during intercourse			0.651
Yes	4	2	
No	17	13	
Incapability of Intercourse			0.289
Yes	4	1	
No	17	14	
Painful knots			0.031
Yes	8 (38.1%)	1 (6.7%)	
No	13 (61.9%)	14 (93.3%)	
Complaints about penile shortening			0.001
Yes	12 (57.1%)	1 (93.3%)	
No	9 (42.9%)	14 (6.7%)	
Patients satisfaction			0.378
Good	14 (66.7%)	12 (80.0%)	
Poor	7 (33.3%)	3 (20.0%)	
Penile hematoma			0.579
Yes	2	2	
No	19	13	
De nova ED			0.719
Yes	2	2	
No	19	13	

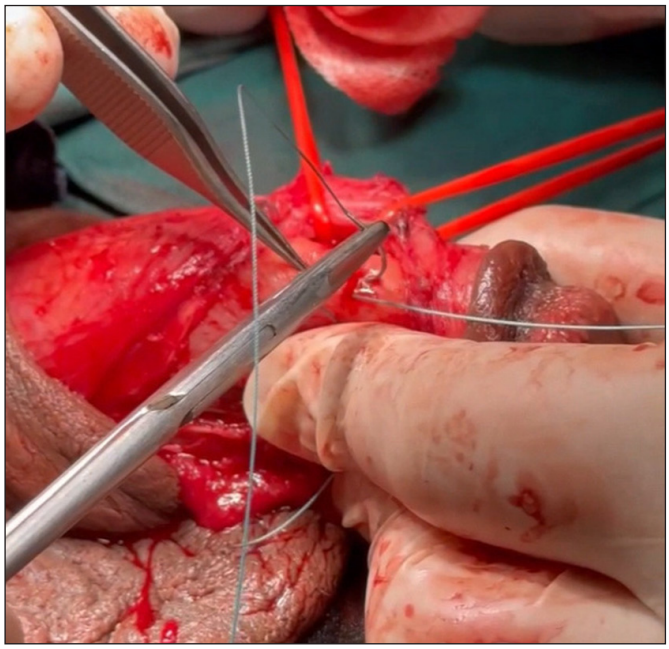

Figure 1. Penile plicatation tecniques for PD patients

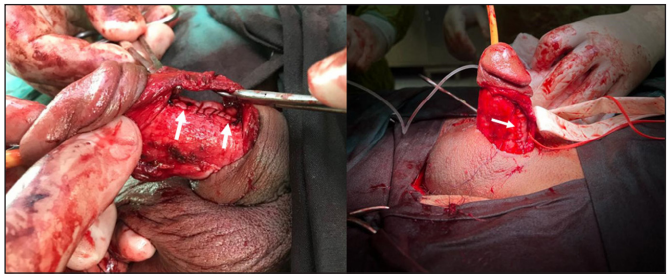

Figure 2. Plaque incision+ grafing technique

DISCUSSION

Many surgical treatment modalities with varying success rates were described for the treatment of PD.[8] As it was relatively easy to perform, tunica albuginea plication was commonly performed, in which the convex side of the penile curvature was shortened. This technique may lead to esthetically unsatisfying results. Furthermore, decreasing the penis length with this technique definitely caused patient disappointment. Peyronie's plaque incision and the grafting procedure was known to potentially result in penile elongation. However, it is important to note that this procedure is not without risks. There was a higher likelihood of decreasing penile sensation and the occurrence of postoperative erectile dysfunction at plaque incision technique.[7]

There is limited data in the literature evaluating the complaints of PD patients. We observed that the most common complaint reported by PD patients was difficulty in engaging in sexual intercourse due to penile curvature followed by penile pain during erection. However, a study conducted by Akkus et al.[9] stated that the most frequent complaint among PD patients was discomfort associated with penile plaques. It is worth noting that many articles focusing on Peyronie's disease often emphasize complications and patient satisfaction while neglecting to address the specific complaints reported by patients. The improvement in patients' complaints at admission is directly related to post-surgery satisfaction and success, so recording complaints during admission is critical. The regression of their problems is associated with the success of the procedure and patient satisfaction. For this reason, the complaints of Peyronie's patients should be documented, and questioned whether their complaints regressed after the intervention.

Plaque incision with graft procedure had a longer operation time compared to the tunical plicatation technique. This finding was consistent with studies that have utilized buccal mucosa as a graft material, which have reported similar operation durations ranging from 121 min to 145 min.[10-12] While surgical techniques were compared based on hospital stay, it was observed that tunical lenghting procedure had a longer hospital stay compared to the tunical shortening tecniques. In our study, saphenous vein and bovine pericardium were used as a graft material and hospital stay (1-4 days) was determined lower compared to the Ainayev et al.[10,11] studies in which buccal mucosal graft was used for PD. (9-10 days)

Our results revealed that the plication technique led to a reduction of approximately 1 cm in penile length, whereas the graft and incision technique resulted in an increase of 0.6 cm. These findings were consistent with previous studies. These studies reperted a mean of 1.5 to 3.2 cm penile lenghting during plaque incision and grafting techniques. It is worth noting that long-term follow-up studies have indicated a more modest increase of 0.5 cm.[13-15] There are also publications in the literature reporting that the length of the penis is shortened after plaque incision and grafting procedure.[16,17] Therefore, patients should carefully consider their options when choosing a surgical method. It is important for individuals to consult with their healthcare providers to discuss their specific condition, personal preferences, and expectations in order to make an informed decision regarding the preferred surgical approach.

Patients who underwent plaque incision with graft procedure complained of less penile shortening compared to those in the penile plication group. Additionally, the penile plication group reported higher suture-related discomfort on the suturing side. There was a statistical difference between the groups. Based on the evaluation of patient satisfaction, it is evident that the penile plication group had a lower satisfaction rate compared to the plaque incision with graft group. These findings were consistent with existing literature.[8] We believe that patient satisfaction is directly related to postoperative penile length. De nova ED and reduced

penile sensation was more common in plaque incision with grafting procedure compared to penile plications but there was no significant difference between groups.

Study Limitations

Our study had some limitations. Main limitation of study was the retrospective design of study. However, the data of the study was obtained during surgery in the operation theatre, which might decrease the bias that may possibly result from retrospective evaluation. The second limitation; there was no penile prostheses implantation treatment modalities in our study for PD. Further randomized studies are needed to confirm these findings and determine the long-term outcomes of these surgical techniques.

CONCLUSION

In Conclusion, tunical lengthening procedures are more effective and safer than tunical shortening procedures for the treatment of Peyronie's disease in terms of penile length and suture related discomfort. Clinicians should consider many factors including penile length, the degree of penil curvature and surgeon experience when selecting the appropriate surgical technique for Peyronie's disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tekirdağ Namık Kemal University Non-interventional Clinical Researches Ethics Committee (Date: 16.02.2022, Decision No: 2022-02).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The initial psychological impact of the COVID-19 pandemic on healthcare professionals in a children's hospital

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ABSTRACT

Aims: The pandemic has had devastating effects across the world particularly on healthcare professionals. We assessed anxiety and depression with somatization to discover the psychological effects of the pandemic.

Methods: 250 healthcare workers in a tertiary pediatric teaching hospital were asked to respond to the questionnaire between 1 and 30 June 2020 and the responses were assessed via three scales including the Patient Health Questionnaire 9 (PHQ-9), the General Anxiety Disorder 7 (GAD-7) and the Somatosensory Amplification Scale (SSAS).

Results: A total of 242 participants responded (response rate 97%); 29% of the participants reported moderate or severe anxiety while 49% reported moderate or more severe depression. There was no significant difference between the degrees of depression and moderate to severe anxiety through different professions (p= 0.480, p=0.384, respectively). Somatization was significantly lower in doctors and higher in female participants (p=0.001). Participants with chronic diseases and ones that had a dependent relative were at higher risk for anxiety and depression.

Conclusion: Most of the healthcare workers had depression and anxiety in the beginning of the pandemic, and it was independent of gender, profession, or workplace. Healthcare professionals with chronic diseases and dependent relatives are at risk for severe depression.

Keywords: COVID-19, pediatric, anxiety, depression, healthcare workers

INTRODUCTION

The viral infection COVID-19 quickly spread all over the world after first appearing in Wuhan, the largest metropolitan city in China's Hubei Province, in late 2019 and was declared a pandemic in March 2020.[1,2] The cooperation of all healthcare workers is essential to fight the pandemic; consequently, healthcare workers, particularly the ones caring for COVID-19 patients, carry the biggest burdens and risks. Increased workloads, physical fatigue, insufficient personal protective equipment (PPE), nosocomial contamination risk, ethically difficult decisions needed in patient care and restrictions on work hours and leave have been reported to increase the psychological burden on the physical and mental health of healthcare professionals.[3] Similarly, the fear of infecting their families and social isolation due to long shifts have contributed to healthcare workers' mental complaints.[3]

Several studies have reported that the mental complaints of people and particularly healthcare workers have been altered during the pandemic period.[3–5] In their meta-analysis, Papa et al.[3] reported that almost 23% of healthcare workers experienced anxiety, 22% suffered from depression and 34% complained of sleep disturbance during the COVID-19 pandemic. More mental complaints, particularly for healthcare workers treating COVID-19 patients, are expected. Previous studies have reported that women and nurses were more at risk for frequent psychological symptoms than other healthcare workers.[6,7] Furthermore, healthcare workers are anxious about

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becoming infected through COVID-19 patients while doing their work.[3] Regarding this concern, individuals report experiencing a variety of COVID-19-like symptoms that affect them both mentally and physically. Feeling COVID-19-like symptoms was claimed to be related to experiencing somatic situations more than normal and to catastrophically interpreting these symptoms.[8] It can be said that studies have found that the tendency to somatization may be important regarding the anxiety experienced in relation to COVID-19.

Somatization is the perception of bodily sensations that are normal or not clearly indicating a disease in an intense, harmful and disturbing manner.[9] For example, a tingling sensation in the throat or a runny nose may be perceived as a COVID-19 symptom. Conversely, in cases where the tendency to somatization is low, although an individual has symptoms compatible with COVID-19, the symptoms may not be strongly felt. Therefore, it is important to understand the mental complaints and their relationship with the somatization tendencies of healthcare workers during the pandemic. Differences between healthcare workers such as profession, gender or directly working in COVID-19 departments may differently affect existing mental symptoms.

In this study, we aimed to evaluate the psychological effects of the pandemic period on healthcare workers in a pediatric hospital and to reveal the risk groups in terms of depressive symptoms and anxiety.

METHODS

The study was carried out with the permission of Keçiören Training and Research Hospital Clinical Researches Ethics Committee (Date: 10.06.2020; Decision No: 2117). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We invited by email 250 healthcare professionals working in our tertiary pediatric teaching hospital to participate in the study. A total of 242 people completed the study questionnaire, for a response rate of 97%. The study data were collected from June 1 to June 30, 2020, using the snowball sampling method. Participants were included if they were over 18 years old, actively working in healthcare in the hospital whether working with COVID-19 or not and have consent to participate to the study. The participants without consent and the illiterate ones were excluded from the study. Online consent was obtained from all the participants.

Participants were first asked to provide their demographic information, such as age, gender, occupation, marital and educational status, having children, having a dependent relative at home, having a chronic disease and whether they worked in COVID-19-related services. The participants were questioned about COVID-19-related symptoms, such as fever, sore throat, cough, weakness, runny nose, nasal congestion, nausea, vomiting, diarrhea, or other symptoms. Respondents with these symptoms were asked if they thought their symptoms were related to COVID-19 and to rate their concerns between 1 and 10. Participants with symptoms were also asked how they responded when the symptoms occurred and whether they thought the symptoms could be psychological.

Participants were then asked to complete three different scales: the Patient Health Questionnaire 9 (PHQ-9) to evaluate their level of depression, the General Anxiety Disorder 7 (GAD-7) for anxiety assessment, and the Somatosensory Amplification Scale (SSAS) to assess their level of exaggeration of sensations. All these scales were in Turkish, and their Turkish translations have all been validated.[10–12]

PHQ-9 (Patient Health Questionnaire)

This is a nine-question depression module derived from the original PHQ scale. If a participant marks "more than half of the day" for five or more of the nine depressive symptom questions or indicates a depressive mood or anhedonia (inability to enjoy doing something), it is considered severe depression. Each item is scored on a four-point Likert scale: 0, never; 1, some days; 2, more than half of the days; and 3, almost every day. Total scores for the PHQ-9 scale can be between 0 and 27; answers to each question are scored from 0 to 3 points. A total score between 1 to 4 is the minimum result, between 5 to 9 indicates mild depression, between 10 to 14 reflects moderate depression, between 15 to 19 implies partially severe depression and between 20 to 27 is considered severe depression.

GAD-7 (General Anxiety Disorder)

This test developed according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders) can be easily applied to evaluate a generalized anxiety disorder. It consists of seven questions evaluating the respondent's experiences over the last two weeks and uses a four-point Likert scale (0= none, 1=many days, 2=more than half of the days and 3=almost every day) to score the answers. A total score of 0 to 4=mild, 5 to 9=moderate, 10 to 14=high and 15 to 21=severe anxiety. When a total score threshold of 10 was chosen, the test's sensitivity for the diagnosis of GAD was found to be 89% and its specificity 82%.[13]

N-SSAS (Somatosensory Amplification Scale)

N-SSAS is a self-assessment scale developed to measure the augmentation/exaggeration that people use while somatizing. This 10-item scale with proven validity and reliability was developed by Barsky et al.[14] Patients score each item between 1 and 5. A total amplification score is obtained by summing the scores from the items.

Statistical Analysis

A statistical analysis was performed with SPSS 20.0 for Windows (IBM SPSS Inc., NY USA). According to the analysis of 95% confidence (1-α), 95% test strength (1-β) and d=0.5 effect size, the number of samples to be taken was determined as minimum of 150 samples in power analysis. We used a Shapiro-Wilk test to assess the normal distribution of the data. Continuous variables were expressed as the mean and standard derivations, and categorical variables such as gender were summarized as frequencies and percentages. The depression and anxiety scores were categorized according to cut-off scores, and groups for sociodemographic and other categorical parameters associated with COVID-19 were compared with a chi-squared test. Any p-values less than 0.05 were considered significant.

RESULTS

General Characteristics of the Study Population

Of the 242 participants, 147 were women and 95 were men. The mean age of the participants was 31.6±8.1; 193 were younger than 40 years old, and 49 were 40 years or older. Regarding marital status, 126 were married, 109 were single and 7 were divorced. Almost half of the participants (105 of 242) had one or more children, and 62 had a dependent relative at home. While 34 participants suffered from a chronic disease, 208 stated that they were completely healthy. The education levels of the participants were as follows: 103 (42.6%) participants had a master's degree, 129 (53.3%) had a bachelor's degree and 10 (4.1%) were high school or below graduates. Of the 242 participants 108 were doctors, 82 were nurses and 52 were other medical staff. There were 117 participants working in a service related to COVID-19 and 122 working in non-COVID-19 services (Table 1).

During the study period, 76 (31.9%) participants experienced no symptoms, 60 (25.2%) had one symptom, and 102 (42.9%) had more than one symptom. The most common symptoms were fever and sore throat. The symptoms were considered psychological by 122 participants, while 49 believed them to be real. Of the participants, 119 (49%) were worried about having COVID-19; their mean anxiety score was 2.65±2.6. Of those who exhibited symptoms, 19 (8%) ignored the symptoms, 78 (32%) waited to see if their symptoms would continue, 8 (4%) called a doctor friend and 6 (2.4%) were admitted to a hospital.

Comparison of neuropsychological features

Approximately 29% of the participants had moderate or severe anxiety and 49% had moderate or more severe depression during the pandemic period. Moderate to severe anxiety was detected in 32% of the doctors, 31% of the nurses and 22% of the other medical staff (p=0.384). Approximately 16% of the doctors had no depression, 61% had mild to moderate depression and 23% had severe depression. On the other hand, 31% of the nurses and 20% of the other healthcare personnel had severe depression. There was no significant difference between the degrees of depression in different professions (p=0.480). While 31.5% of the women who participated in the survey had moderate to severe anxiety, only 25% of the men did (p=0.765). Severe depression rates were 28% and 18% in men and women, respectively. Of the severely depressed woman participants, 51% were nurses, 40% were doctors and 9% were assistant health personnel (Figure 1).

Table 1. Demographics trough different professions and working places

	Doctors (n=108)	Nurses (n=82)	Other Medical Staff (n=52)	p	COVID workers (n=117)	None COVID workers (n=122)	p	Total (n=242)
	n (%)	n (%)	n (%)		n (%)	n (%)		n (%)
Age								
<40 years	96(89)	69(84)	28(54)	<0.001	93(80)	99(81)	0,747	193 (80)
≥40 years	12(11)	13(16)	24(46)		24(20)	23(19)		49 (20)
Gender								
Female	64(59)	66(81)	17(33)	<0.001	69(59)	78(64)	0,431	95 (39)
Male	44(41)	16(19)	35(67)		48(41)	44(36)		147 (61)
Education								
Master	103(95)	0	0	<0.001	54(46)	49(40)	0,363	103 (43)
Bachelors	5(5)	82(100)	42(81)		60(51)	66(54)		129 (53)
High School or below	0	0	10(19)		3(3)	7(6)		10 (4)
Marital Status								
Single	52(48)	48(59)	9(17)	<0.001	52(44)	57(47)	0,871	109 (45)
Married	56(52)	31(38)	39(75)		62(53)	61(50)		126 (52)
Other	0	3(4)	4(8)		(3)	4(3)		7 (3)
Fertility								
One or more children	37(34)	29(35)	39(75)	<0.001	50(43)	52(43)	0,986	105 (43)
No children	71(66)	53(65)	13(25)		67(57)	70(57)		137 (57)

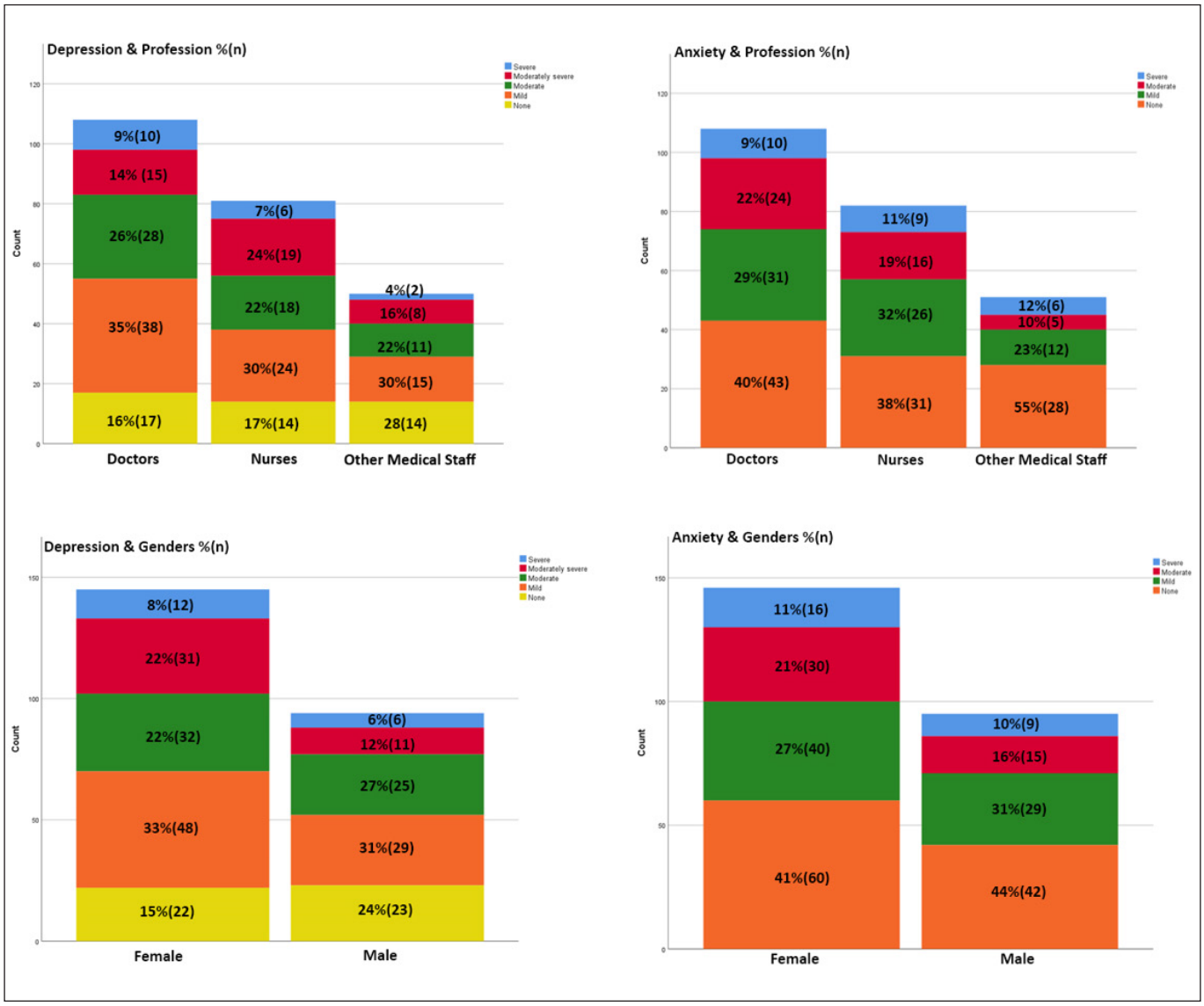

Figure 1. Proportions of the severity of depression and anxiety through the profession and gender of the participants

In total, 62 of the participants (26%) had a chronic illness, and 34 (14%) of them had a relative in need of home care. The depression, anxiety and somatization scores of the participants who had dependent relatives at home were significantly higher than those who did not (Table 2). In addition, while the depression levels of the participants with a chronic illness were significantly higher than those without a chronic illness, their anxiety and somatization evaluations were similar (Table 3).

Table 2. Comparison of PHQ-9, GAD-7, and N-SSAS Scores between participants with or without dependent relative

	Participants with dependent relative		Participants without dependent relative		Statistics	
	Mean	SD	Mean	SD	t	p
PHQ-9	11.98	6.50	9.41	5.81	-2.738	0.007
GAD-7	8.84	5.71	6.90	5.29	-2.345	0.021
N-SSAS	29.66	6.75	27.04	6.95	-2.591	0.011

PHQ-9: Patient Health Questionnaire-9, GAD-7: General Anxiety Disorder-7, N-SSAS: Somatosensory Amplification Scale

Table 3. Comparison of PHQ-9, GAD-7, and N-SSAS Scores between participants with or without chronic disease

	Participants with chronic disease		Participants without chronic disease		Statistics	
	Mean	SD	Mean	SD	t	p
PHQ-9	12.62	6.56	9.64	5.91	-2.672	0.008
GAD-7	8.91	5.61	7.15	5.40	-1.752	0.081
N-SSAS	20.09	6.56	27.48	7.03	-1.247	0.214

PHQ-9: Patient Health Questionnaire-9, GAD-7: General Anxiety Disorder-7, N-SSAS: Somatosensory Amplification Scale

Somatization was significantly lower in doctors than in other healthcare workers (nurses and staff) (p= 0.04). The participants with high somatization had significantly higher depression (p<0.001) and anxiety (p<0.001) rates. In addition, somatization was significantly higher in female participants (p=0.001).

DISCUSSION

The anxiety and depression levels of healthcare professionals of a tertiary pediatric hospital that provides diagnosis and treatment services to COVID-19 patients were assessed in this study. The most important findings in the study were that approximately half of the healthcare workers had depressive symptoms and that 30% had anxiety during this period. Moreover, depressive symptoms and anxiety did not differ by profession (doctors, nurses, or medical staff), age, gender, marital status, having children and education level. Anxiety and depressive symptoms were significantly higher in healthcare workers who had a chronic disease or a dependent relative at home. Another important finding of our study was that the medical staff and nurses had higher somatization compared to doctors.

Studies investigating the effects of the pandemic period on healthcare workers have shown that gender, occupation, workplace, and several social factors increase their stress, anxiety, and depressive symptoms. It has also been reported that the pandemic period is an independent risk factor for stress in healthcare workers.[4] Unsurprisingly, a significant portion of the healthcare professionals participating in our study reported depressive symptoms and anxiety. In particular, healthcare workers with chronic disorders and those with a dependent relative at home had significantly higher anxiety and depressive symptoms. COVID-19 is reported to be more severe in patients of advanced age and with comorbid diseases.[15] In addition, healthcare workers reported having serious concerns about infecting their families or colleagues during the pandemic.[16,17] This concern may explain the higher anxiety and depressive symptoms of the participants with chronic diseases or a dependent relative in this study.

It is known that women have more anxiety than men.[18] In addition, studies have shown that female healthcare workers are experiencing higher anxiety and depressive symptoms during the pandemic period.[3] In this study, although higher anxiety and depressive symptoms were found in females, no significant increase in these symptoms was found compared to their male colleagues. It has also been reported that, during the pandemic, anxiety and depressive symptoms were higher in doctors and those with a high level of education.[19] This has been attributed to their workload and their need for more frequent travel. However, we observed no significant relationship between education level and profession with anxiety and depressive symptoms in this study. Most healthcare workers have had to stay at home like any other citizen, as well as having had to be more engaged in other aspects of life, such as the health of family members or family income. In addition, in Turkey, nurses and medical staff have had to work under similar stresses and conditions during the pandemic period. It was an important finding of our study that this situation created a similar anxiety in all healthcare providers, regardless of their profession.

A recent study reported the anxiety and depressive symptoms of healthcare workers in a children's hospital in China, where the pandemic started. The authors reported that self-reported depression and anxiety were significantly higher in employees compared to the ordinary population.[20] They concluded that pediatricians working in departments related to COVID-19 should be given more psychological support during the pandemic period. Similarly, the anxiety and the depressive symptoms of the doctors, nurses and healthcare staff working in the tertiary children's hospital in our study were all markedly high. That there are similar results between the two countries may indicate that this situation affects all healthcare workers globally during the pandemic period.

Another important finding in our study was that the nurses and healthcare staff had more somatization than doctors. It is known that somatization decreases with the increase of cognitive skills,[21] which may explain the doctors' lower somatization. Contrary to our study, Lung et al.[22] reported more somatic symptoms in physicians than in other healthcare professionals. They attributed the difference to the different stress levels of jobs and gender, as well as to cultural and behavioral patterns. Due to differences in cultural and working conditions, physicians may have reported less somatic symptoms in our study, but it could also indicate that doctors may have been demonstrating less concern about symptoms related to COVID-19. Hence, this study suggests that doctors should be more careful concerning their personal symptoms.

Limitations

The major limitation of our study was that the participants did not record the number of hours they worked and that the participants were grouped according to their occupations. There are differences in the weekly working hours of different professions, which may affect the psychological status of the participants. In addition, other limitations of the study can be the relatively small sample size, the use of single item ratings, potential choice bias and the lack of knowledge about workload.

CONCLUSION

Depression and anxiety are common among healthcare providers working under severe conditions due to the COVID-19 pandemic, independent of their gender and profession. In particular, healthcare workers who have chronic illnesses or have dependent relatives at home are

at risk of severe depression and anxiety. These workers should thus be closely monitored as a high-risk group for depression and anxiety. Working conditions for treating COVID-19 patients should be regulated or appropriate psychological support should be provided for those who are at risk for severe depression and anxiety.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Keçiören Training and Research Hospital Clinical Researches Ethics Committee (Date: 10.06.2020; Decision No: 2117).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Lowering propionic acid levels by regulating gut microbiota with ursodeoxycholic acid appears to regress autism symptoms: an animal study

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ABSTRACT

Aims: Patients with autism have altered gut microbiata, including higher frequency of bacteroidetes and clostridiales that produce of propionic acid (PPA) –a compound that is established as an autism-inducing agent. We hypothesized that lowering the PPA levels by regulating gut microbiata with ursodeoxycholic acid (UDCA) can regress the autism symptoms. The aim of this study is to examine the potential ameliorating effects of UDCA on a PPA-induced rat model of autism.

Methods: Thirty male Wistar albino rats were divided into three groups: controls, PPA-induced (5 days of intraperitoneal 250 mg/kg/day dosage) autism model receiving oral saline, and PPA-induced autism model receiving oral UDCA (100 mg/kg/day). Oral treatments were applied for 15 days. At the end of the 15th day, all rats underwent behavioral tests and MR spectroscopy. At the end of the study, all animals were sacrificed and brain tissue / blood samples were collected for histopathological and biochemical analyses.

Results: Sociability test, open field test and passive avoidance learning tests were impaired, similar to the autism behavioral pattern, in PPA recipients; however, results were closer to normal patterns in the PPA+UDCA group. Biochemically, MDA, TNF-alpha, IL-2, IL-17, NF-kB, lactate, NGF and NRF2 levels in brain tissues showed significant differences between controls and the PPA+Saline group, and between the PPA+Saline group and the PPA+UDCA group ($p< 0.05$, for all). Histopathology showed that PPA injection caused increased glial activity, neural body degeneration, decreased neural count and dysmorphic changes in hippocampal and cerebellar tissues ($p<0.01$, for all). UDCA treatment significantly ameliorated these changes.

Conclusion: UDCA administration has ameliorating effects on PPA-induced autism-like behavioral, biochemical and histopathological changes in rats.

Keywords: Autism, ursodeoxycholic acid, propionic acid, gut microbiota

INTRODUCTION

Autism spectrum disorder is a complex neurobehavioral disorder, with a large phenotypical spectrum, usually including impaired social interaction and communication, restricted and/or repetitive behavioral characteristics, altered cognitive patterns (learning, memory etc.) and sensory perception.[1] The fact that its prevalence has been increasing significantly in recent years has drawn more attention to the potential causes of this disease. While some studies suggest that autism develops on a multigenetic basis[2] many environmental factors, including metabolism-related compounds have been associated with this disorder.[3] Although proteomic analyses are still at the center of determining potential pathways associated with various diseases, advances in recent decades have demonstrated that

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other metabolites including low molecular weight compounds, lipids, and other compounds associated with gut microbiota may contribute to the pathological basis of various diseases.[4-6] Similar relationships have also been demonstrated in autism,[7-10] indicating the need for further research assessing the roles of these compounds in autism.

One such compound is propionic acid (PPA), which is a short-chain fatty acid that enters human metabolism both as an intermediate product of fatty acid metabolism and as a metabolic end product of bacteria in the gut.[11] In gut microbiata, bacteroidetes and clostridiales are largely responsible for the production of PPA, and autistic patients have been shown to have an altered microflora with relatively greater levels of these bacteria.[12] PPA can pass the brain-blood barrier and was shown to alter the release of some neurotransmitters.[1,13] High PPA levels were also shown to induce behavioral, electrophysiological, neuropathological and biochemical effects similar to those observed in autism.[2,3] Autism-like experimental rat models have been established by administration of PPA in various ways, including subcutaneous (500 mg/kg), intragastric gavage (250 mg/kg), intraperitoneal (250 mg/kg), and intracerebroventricular (4 μL, 0.26 M) administration.[14-16]

Although most autistic patients receive pharmacological treatment, there is no accepted medical treatment procedure that can significantly reduce the core symptoms of autism. Based on the potential effects of the gut microbiata on this disease,[2] studies assessing microbiome-mediated therapies have gained importance. One of the most important metabolites of gut microbiota are bile acids. Novel studies provide evidence about the role of bile acids in regulating gut microbiata as well as gastrointestinal functions and intestinal permeability. Furthermore, the pathogenic and therapeutic roles of bile acids and related sterols have been shown in several metabolic,[17,18] and neurodegenerative diseases,[4,19] have been shown. Ursodeoxycholic acid (UDCA) is a secondary bile acid which has therapeutic value in gallstones and inflammatory diseases including primary biliary cholangitis.[20]

Considering studies suggesting that increased PPA production due to alterations in gut microbiata may lead to autism-like symptoms,[21] we hypothesized that lowering PPA levels by regulating gut microbiota could regress symptoms. Therefore, the aim of this study was to examine the potential ameliorating effects of UDCA administration in a PPA-induced rat model of autism.

METHODS

Animals

In this study, thirty 10-12 week old male Wistar albino rats weighing 150-200 g were used. The experiments performed in this study were carried out according to the regulations put forth in the 'Guide for the Care and Use of Laboratory Animals' by the National Institutes of Health (U.S.A). The Animal Ethics Committee's approval for the study procedure was obtained (İstanbul Science University, Date: 2022, Decision No: 31210835). The rats used in the experiment were obtained from Experimental Animal Laboratory of Science University. Rats were fed ad libitum and housed in pairs in steel cages kept in a temperature-controlled environment (22±2°C) with automated 12-hour light/dark cycles.

Experimental Procedures

Thirty male Wistar rats were included in the study. Twenty rats were administered PPA intraperitoneally, at 250 mg/kg/day dosages for 5 days to induce an autism model. PPA-administered rats were randomly divided into 2 groups. Study groups were designed as follows: Group 1: Normal control (orally fed control, n=10); Group 2 (PPA+Saline, n=10): received PPA and were administered saline via oral gavage (1 ml/kg/day%0.9 NaCl), Group 3 (PPA+UDCA, n=10): received PPA and were administered UDCA via oral gavage (100 mg/kg/day; Ursactive capsule 250 mg, Pharmactive). In the literature, UDCA was mostly administered at 100 mg/kg/day, so we used the same dose according to the literature (22, 23). All treatments (saline, UDCA) were administered for 15 days, after which behavioral tests were performed. All behavioral experiments were conducted between 10:00 AM and 3:00 PM. After behavioral tests, animals underwent MR spectroscopy under ketamine anesthesia (50 mg/kg; Ketasol, Richterpharma AG, Austria).

At the end of the study, all animals were sacrificed (cervical dislocation) under high-dose ketamine/xylazine anesthesia (100 mg/kg / 50 mg/kg) (Xylazine Rompun, Bayer, Germany). Targeted brain tissues for histopathology and tissue biochemical analyses were dissected, prepared and stored appropriately. Blood samples for biochemical analyses were collected via cardiac puncture and plasma was obtained via centrifugation.

Behavioral Tests

Three-chamber sociability and social novelty test: Sociability test was performed as previously described with minor modifications (3, 24). Briefly, a Plexiglas cage (40 × 90 × 40 cm) was divided into three equal regions (40 × 30 × 40 cm). On the first day, the rats were allowed to habituate in the test cage for 5 min (pre-test session). Twenty-four hours later, a stranger rat (Stranger 1) was

placed inside a small plastic cage with mesh-like holes in one side chamber and an empty cage in the third chamber. Then, the test rat was placed in the center chamber and the time spent in each region by the test rat was recorded for 10 min. Presence in a chamber was defined when the rats head and two front paws entered the chamber. Time spent with the Stranger rat was calculated and reported as a percentage of total time. All chamber floors were cleaned between each test (70% alcohol) to remove traces of olfactory stimuli (Figure 1).

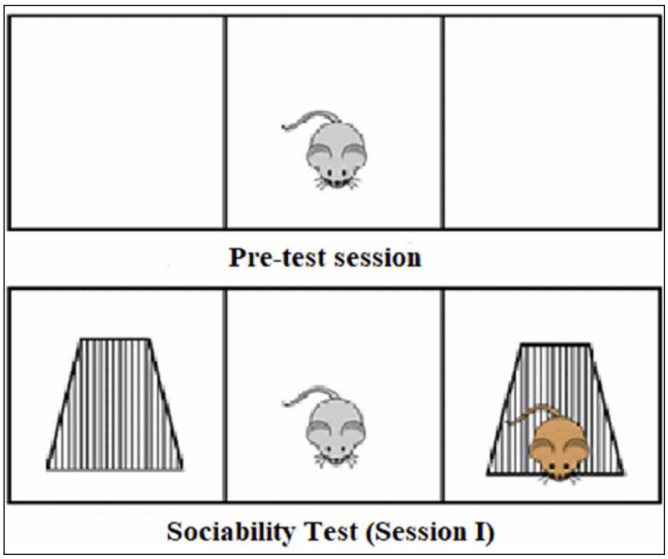

Figure 1. Three- chamber sociability and social novelty test

Open-field: The open-field (OF) paradigm assesses locomotion and exploration. Altered OF behavior is relatively simple to observe; however, concluding the reasons for the observed changes is a complex task. Generally, there are two factors that determine the behavior in this paradigm: The first is the natural exploratory drive of rodents to explore new environments (for food and shelter), while the second concerns precautionary avoidance of open and brightly lit spaces. The OF test is considered useful in determining stereotyped behavior, auto-grooming, and restriction of exploration in autism models.[24] The test was conducted in an open-aired box (50 × 50 × 40 cm). Rats were gently placed in the center of the box and allowed to explore the arena freely for 5 min. Then, each rat was observed for 5 min to evaluate its spontaneous activity level. The total number of ambulation events, defined as the number of floor divisions crossed with four paws, was recorded. The chamber was cleaned between each test (70% alcohol) to remove traces of olfactory stimuli.

Passive avoidance learning (PAL): The passive avoidance learning (PAL) test, as described previously,[26] is comprised of the assessment of fear-motivated avoidance. The healthy rat learns to refrain from stepping through a door leading into an apparently-safe dark chamber (preferred environment) due to prior experience of the fact that the door leads into a chamber in which an electrical shock is delivered. The PAL box was sized 20 × 20 × 20 cm and had dark and lighted chambers. After a 10-second habituation period in the lighted compartment, the guillotine door separating the light and dark chambers was opened. When a rat passed into the dark chamber, the door separating the light and dark compartments was closed. Then a 1.5-mA electric shock was delivered over 3 seconds, and the rat was subsequently removed from the dark chamber and returned to its cage (pre-test experience). Twenty-four hours later, the rats were placed into the lighted chamber of the PAL box again. The duration of time (or latency period) for the rat to travel from the lighted to the dark chamber was recorded, but a shock was not delivered. The latency period was recorded up to a maximum of 300s. The amount of time that the rat refrained from crossing into the dark chamber was recorded as PAL latency.

Magnetic Resonance Imaging Studies

Conventional MRI: All rats were examined using the 3.0-Tesla MRI/MRS scanner (Magnetom, Siemens Healthcare). Conventional MR sequences were as follows: sagittal fast spin-echo T1 weighted imaging for location; axial spin echo T1 weighted imaging [repetition time (TR)/echo time (TE) = 400/11 ms; field of view (FOV) = 60 mm; matrix = 256 × 256; number of excitation pulses = 2; bandwidth = 12.5 kHz; slice thickness = 1 mm; interslice gap = 0.2 mm; total number of scan slices = 16]; fast spin echo T2 weighted imaging (TR/TE = 4000/120 ms; other parameters were identical to the T1 weighted spin echo sequence); and fast fluid attenuation inversion recovery (TR/TE = 4000/120 ms; time of inversion = 2200 ms; matrix = 256 × 192; number of excitation pulses = 1; other parameters were identical to the T1 weighted spin-echo sequence).

MR spectroscopy: An automated multivoxel 2D chemical shift imaging sequence (TR = 1000 ms; TE = 35 ms; phase encoding x = 24; phase encoding y = 24; number of excitation pulses = 1) was used for 1H-MRS. FOV diameter was 60 mm, slice thickness was 4 mm and voxel size of the MRS was 1.87 × 1.87 × 4 mm^3. The volume of interest was chosen within the right striatum. The total duration for 2D 1H-MR spectrum acquisition was 580 seconds. Magnetom software (Siemans Healthcare) was used to process raw data stored in a workstation (Figure 2).

Hippocampus and Cerebellum Histopathology

The Cornu Ammonis (CA) 1 and CA 3 regions of hippocampus and cerebellum were chosen as the target areas to be examined for hippocampus damage. Briefly, following behavioral tests, animals were euthanized

and their brains removed and fixed for 3 days in 10% formaldehyde in 0.1 M phosphate-buffer saline (PBS). Then, they were moved into 30% sucrose and stored at 4°C until infiltration was complete. The brains were cut coronally on a sliding microtome (40 µm) and mounted on gelatinized glass slides. For glial fibrillary acidic protein (GFAP) immunohistochemistry, brain sections were incubated with H_2O_2 (10%) for 30 min to eliminate endogenous peroxidase activity and blocked with 10% normal goat serum (Invitrogen) for 1 h at room temperature. Subsequently, sections were incubated in primary antibodies against GFAP (Abcam, Inc., MA, US; 1/1000) for 24 h at 4°C. Antibody detection was performed with the Histostain-Plus Bulk kit (Invitrogen) against rabbit IgG, and 3,3' diaminobenzidine (DAB) was used to visualize the final product. All sections were washed in PBS and photographed with an Olympus C5050 digital camera mounted on an Olympus BX51 microscope. To calculate the GFAP immunostaining index, GFAP-positive cells were counted at 40X magnification in 3 to 4 randomized sections for each rat. All histopathological examinations were performed by the same investigator who was blinded to the study groups. This procedure was performed with an image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc. USA) in four sections per studied group. Cresyl violet staining to quantify the number of surviving neurons were performed in six sections with the same image analysis system.

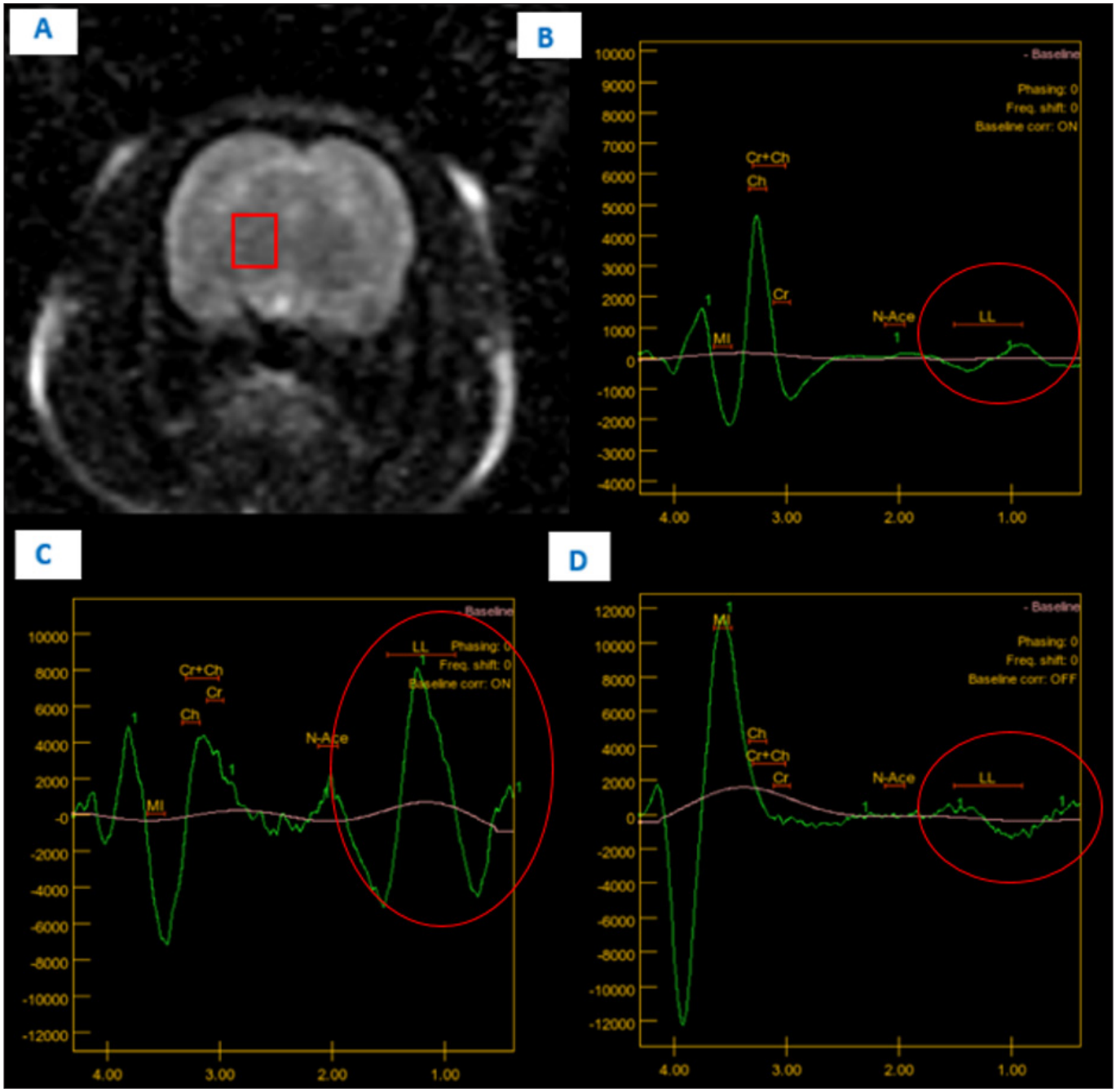

Figure 2: MR spectroscopy image (in the red circles, LL refers to lactate levels). A: MR spectroscopy chosen area (Red box), B: Normal Control Group male Rats, C: PPA and saline group male rats, D:PPA and UDCA group male rats.

Biochemical Analyses

After decapitation, brains were rapidly removed and stored at −20°C until biochemical analysis. For tissue analysis, whole cerebral tissues were homogenized with a glass homogenizer in 5X volumes of phosphate buffered saline (pH=7.4) and centrifuged at 5000×g for 15 min. The supernatant was then collected and total protein concentration in the brain homogenates was determined according to Bradford's method using bovine serum albumin as standard.[27]

The brain levels of TNF-alpha, Nerve Growth Factor (NGF), interleukin (IL)-17, IL-2, NF-kB, NRF2 and lactate in the supernatants were measured using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits. All samples from each animal were measured in duplicate according to manufacturer guidelines. A microplate reader was used for the measurement of absorbances (MultiscanGo, Thermo Fisher Scientific, NH, USA).

Measurement of brain and plasma lipid peroxidation (MDA): Lipid peroxidation was determined in the plasma and brain tissue samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS). Briefly, trichloroacetic acid and TBARS reagents were added to brain tissue samples, then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve (prepared using tetraethoxypropane) and were expressed as nmol/gr protein.

Statistical Analysis

Statistical evaluation was performed using SPSS version 15.0 for Windows (IBM, Armonk, NY, USA). The Shapiro-Wilk's W and Levene's tests were used to check normality of distribution and homogeneity of variance, respectively. Comparisons between groups were performed with the one-way ANOVA test. The results are presented as mean±standard error of the mean (SEM) values. p values of <0.05 were accepted to be statistically significant.

RESULTS

Behavioral tests revealed that, in the PPA+Saline group, time spent with stranger rat (%) and PAL latency were significantly shorter, and ambulation events were significantly fewer compared to the control and PPA+UDCA groups (p<0.05, for all) (Table 1).

Compared with the control and PPA+UDCA groups, the levels of brain MDA, plasma MDA, brain TNF-alpha, IL-2, IL17, NF-kB, and lactate were significantly higher, while NGF and NRF2 levels were significantly lower in the PPA+Saline group (p<0.05, for all) (Table 2).

Table 1. Results of behavioral tests

	Control (n=10)	PPA+Saline (n=10)	PPA+UDCA (n=10)
Open field test: number of ambulation	10.2±1.2	6.1±1.08*	8.3±1.3#
Passive avoidance learning (PAL) latency (Sec.)	268.8±20.1	109.1±41.5**	182.5±17.3#

Results were presented as mean±SEM. Statistical analyses were performed by one-way ANOVA. * p< 0.01, ** p<0.001 different from normal groups; # p<0.05, ## p<0.001 different from PPA and saline group.

Table 2. Biochemical parameters obtained from brain tissue and blood samples

Parameters	Control (n=10)	PPA+Saline (n=10)	PPA+UDCA (n=10)
Brain MDA level (nmol/gr protein)	48.8±2.3	195.3±12.9**	92.1±5.9#
Plasma MDA level (nmol/gr protein)	1.63±0.1	4.55±0.4*	3.76±0.2#
Brain TNF-alfa level (pg/mg protein)	14.2±4.4	117.5±9.3**	79.4±7.2#
Brain IL-2 level (pg/g protein)	2.1±0.2	274.1±10.1**	186.1±8.7#
Brain IL-17 level (pg/g protein)	240.9±19.6	598.1±14.5*	405.2±15.6#
Brain NF-KB level (pg/g protein)	19.2±2.1	201.7±14.3**	144.3±17.5#
NGF level (pg/mg protein)	88.1±5.9	39.5±2.3**	66.2±7.03#
NRF2 level (pg/mg protein)	98.2±10.6	45.8±8.4**	88.5±6.08##

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA. * p< 0.01, ** p<0.001 different from normal groups; # p<0.05, ## p<0.001 different from PPA and saline group.

Neuronal counts of CA1, CA3 and Purkinje cells were significantly lower in the PPA+Saline group compared to control and PPA+UDCA groups (p<0.05, for all). GFAP immunostaining index for CA1, CA3 and cerebellum, also MRI spectroscopy lactate values were all increased in the PPA+Saline group, compared to controls. On the other hand, the same indexes decreased for CA1, cerebellum and MRI lactate values (p<0.01, for all) in the PPA+UDCA group compared to PPA+Saline group; whereas CA3 values were similar (Table 3).

Table 3. Cell-count results of hippocampus and cerebellum and MR spectroscopy lactate value

Parameters	Control (n=10)	PPA+Saline (n=10)	PPA+UDCA (n=10)
Neronal Count CA1	87.8±4.4	54.6±2.1 **	66.7±3.3#
Neronal Count CA3	46.5±1.04	28.2±3.2 *	37.5±1.8 #
GFAP immunostaining index (CA1)	31.3±4.5	43.5±2.1 *	35.1±2.5 #
GFAP immunostaining index (CA3)	32.8± 2.1	43.7±2.9 *	38.5±3.3
Purkinje Count Cerebellum	20.8±1.4	10.7±2.8 *	16.5±0.9 #
GFAP immunostaining index (Cerebellum)	16.2±2.5	27.02±1.6*	19.7±0.8#

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA. * p< 0.01, ** p<0.001 different from normal groups; # p<0.05, ## p<0.001 different from PPA and saline group.

Figure 3 shows that PPA+Saline injection caused neural body degeneration and decreased neural count and dysmorphic changes in CA3 and CA1 cells; whereas UDCA treatment increased neural count and improved neural morphology.

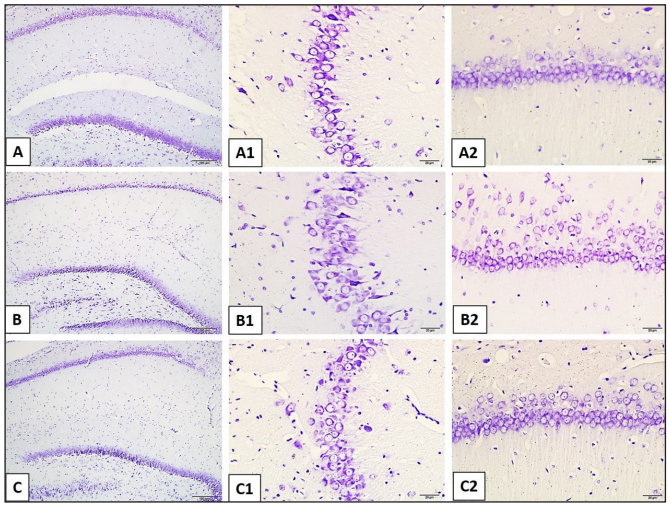

Figure 3: CA3 and CA1 regions of hippocampusCresyl violet stain4 and x 40 magnification. A-A1-A2: Normal Control Group Male Rats CA3 and CA1. Normal pyramidal neuron. B-B1-B2: PPA and saline group male rats have neural body degeneration & decreased neural count and dysmorphological changes CA3 and CA1. C-C1-C2: PPA and UDCA group male rats have increased neural count and improved neural morphology changes CA3 and CA1

Figure 4 shows that PPA+Saline injection caused increased glial activity in hippocampal CA3 and CA1 cells; whereas UDCA treatment reduced this effect of PPA.

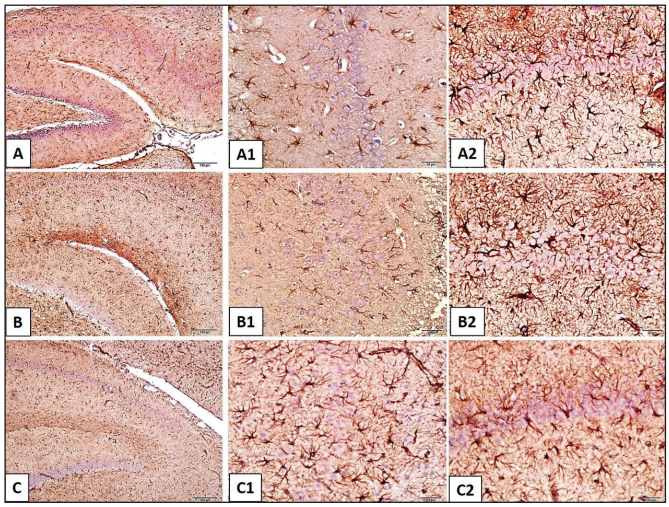

Figure 4: CA3 and CA1 of hippocampus x 40 magnification. Astrogliosis was characterized by GFAP immunostaining(Brown staining). A-A1-A2: Normal Control Group Male Rats CA3 and CA1, B-B1-B2: PPA and saline group male rats have increased glial activity CA3 and CA1.C-C1-C2: PPA and UDCA group male rats have have decreased glial activity CA3and CA1

Figure 5 shows that PPA+Saline injection decreased cell count and caused dysmorphic findings in Purkinje neurons. UDCA treatment increased cell count and improved neural morphological changes in Purkinje neurons.

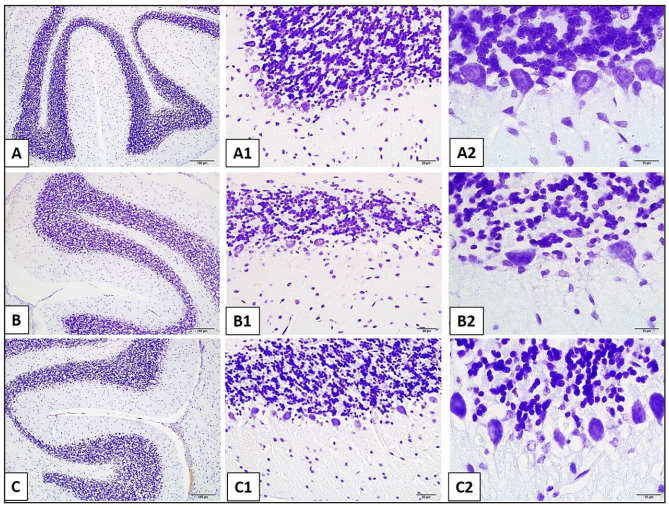

Figure 5: Cerebellum x 4, x 40, x 100 magnification. Cresyl violetstain.A-A2-A3, Normal Control Group Male Rats cerebellum, normal Purkinje Neuron, B-B1-B2: PPA and saline group male rats have decreased count and dysmorphological Purkinje Neuron. C-C1-C2: PPA and UDCA group male rats have increased count and improved neural morphological changes Purkinje Neuron.

Figure 6 shows that the PPA+Saline injected group had increased glial activity in the cerebellum and this activity was found to be decreased in PPA+UDCA recipients.

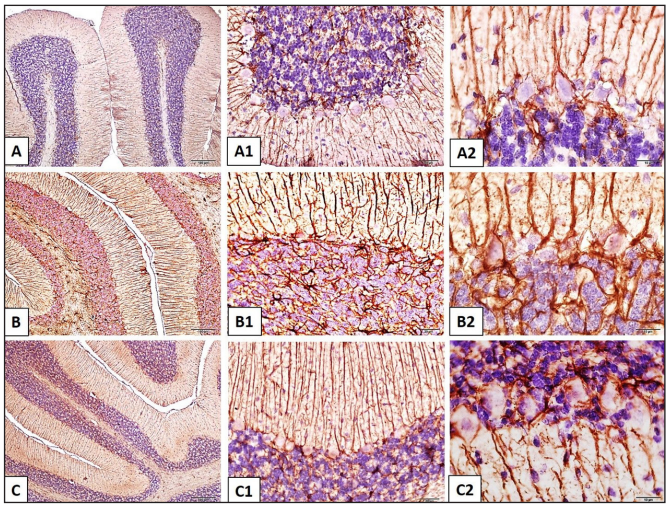

Figure 6: Cerebellum x 4, x 40, x 100 magnification. Astrogliosis was characterized by GFAP immunostaining (Brown staining). A-A1-A2, Normal Control Group Male Rats cerebellum, normal Purkinje Neuron, B-B1-B2: PPA and saline group male rats have increased glial activity cerebellum, C-C1-C2: PPA and UDCA group male rats have decreased glial activity cerebellum.

DISCUSSION

In this study, the results of UDCA treatment were evaluated in a rat model of PPA-induced autism, and it was determined that UDCA yielded ameliorating effects on PPA-induced effects such as disturbances in behavioral tests, biochemical alterations in the blood and brain tissues, and histopathological and MRI findings in brain tissues.

Intraperitoneal or intracerebroventricular injection of PPA (250-500 mg/kg doses) is a widely used

experimental model of autism in rats.[28] In the light of the data obtained in last decades, it has been found that the gut microbiota has significant effects via gut-brain axis on pathophysiology of several neurobehavioral disorders, including autism.[29] These findings are critical in the context of demonstrating the emerging roles of different metabolic compounds in various diseases. Some of these compounds can affect autism pathophysiology and studies support various hypotheses suggesting the functional contributions of lipids and sterol-related compounds in neurodegeneration and autism.[4,8,30] Considering that PPA is a fatty acid and is well-established as a compound that can induce autism-like findings in rats[14,15] the possible roles of altering gut microbiota and influencing the levels of related lipids may be crucial for not only determining treatment targets, but also identifying the pathophysiological contributions of these compounds in autism development.

It is known that most children with autism exhibit gastrointestinal symptoms, such as abdominal pain, vomiting, diarrhea, constipation, intestinal gas problems, and these symptoms are correlated with severity of behavioral or cognitive impairment.[31] When the feces content of children with autism were evaluated, it was seen that microbiota was altered and the levels of clostridiales and bacteroidetes were increased.[32] Some metabolites including PPA produced by these bacterial species can cross the blood-brain barrier and may have neurotoxic effects. Previous studies reported that PPA-treated rats display restricted locomotor activity and attention, impaired cognition, increased repetitive behaviors, and aggressive social behaviors,[15] as well as overexpression of pro-inflammatory cytokines,[33] and astrogliosis in brain tissues.[16] The results were similar in human studies. In autopsy and neuroimaging studies of patients, decrease in cerebellum Purkinje cells, glial activation and cytoplasmic volume change and neuronal cell loss are reported.[32] A novel study revealed that increased plasma levels of proinflammatory cytokines such as interleukins, TNF-alpha and TGF, as well as excessive cellular immune responses were evident in children with autism, and these inflammatory parameters were found to be associated with the severity of autism-related behavioral symptoms.[34] Crawley and colleagues report that the ideal animal model of autism should have at least three diagnostic symptoms unique to people with autism, including deficit in social interaction.[35] We observed that the autism animal model created in this study met this criterion.

The effects of current pharmacological treatment of autism is challenging. The treatment protocol is based on behavioral therapies and rehabilitation. Experimental treatment protocols, on the other hand, focus on different aspects including modulation of gut microbiota by using probiotics, prebiotics, fecal microbiota transplantation, antioxidants, and appropriate diet.[23] The common goal of these supportive treatments is to regulate the gut microbiota, to reduce the permeability of the intestinal barrier to toxins, and to reduce the oxidation and inflammation of brain tissue. Tomova et al.[36] investigated the effects of probiotic treatment on fecal microbiota and assessed plasma hormone and cytokine levels in children with autism. They found that probiotic supplementation altered the composition of gut microbiota and the level of plasma cytokines were decreased after treatment. Similarly, Varesio et al.[37] reported that ketogenic diet therapy had beneficial effects in improving behavioral symptoms in autism due to changes in gut microbiota. To the best of our knowledge, this is the first research assessing the therapeutic effects of UDCA in an experimental autism model. Our results are promising with the therapeutic effects of UDCA on all behavioral, biochemical, and histopathological changes induced by PPA, and support prior studies in terms of the importance of gut microbiota and its alteration which may influence the resultant levels of metabolic compounds. UDCA is a secondary bile acid that can modulate the composition of the gut microbiota via activation of the innate immune system.[38] Tang et al.[39] reported that a 6-month course of UDCA treatment ameliorated gut dysbiosis while not affecting microbial diversity in patients with primary biliary cholangitis.

Behavioral impairment is the most important diagnostic criteria of autism and is also observed in animal models of autism.[40] Correction of behavioral changes may be possible by eliminating the underlying neuroendocrine disorder(s). In this study, the molecular mechanism of the effect of UDCA on neurobehavioral characteristics has not been studied, but the most plausible mechanism is the influence on gut-brain axis through altered microbiota and microbiota-related metabolites. A recent study showed that mice devoid of gut microorganisms exhibited abnormal social behaviors, and restoration of the gut microbiota improved these disturbances.[41]

Increased TNF-alpha, IL-2, IL-17, NF-kB levels in brain tissue are signs of acute inflammation and activation of the innate immune response. Increased MDA and lactate levels and also decreased NGF and NRF2 levels are signs of acidosis and oxidative stress.[42] Today, there is strong evidence concerning the role of brain oxidative stress in the pathophysiology of autism.[43] Accordingly, we can theorize that UDCA treatment may ameliorate oxidative stress in the brain of autistic patients via regulation of gut microbiome. We preferred

the cerebellar and hippocampal regions of the brain to evaluate the histopathological changes, including cell loss, astrogliosis and neurodegeneration. These areas of brain are associated with motor and cognitive functions. Their damage can disturb the functions of connected areas, playing an important role on social behavior, motor, sensory, and memory functions.[44] Therefore, cell loss and neurodegeneration in these areas can explain behavioral changes. As expected, the observed improvement in behavioral disorders may be related with the indirect regenerative effects of UDCA treatment on neuronal tissues.

In some pathological processes, increased concentrations of specific metabolites, such as lactate, may be observed. Lactate is not found in normal brain tissue, and it most commonly arises/increases as a result of anaerobic glycolysis. In cerebral hypoxia, ischemia, seizures and some metabolic diseases, lactate increase can be detected in the brain by MR spectroscopy. Due to the interaction (spin-spin interaction/coupling) between the protons in the methyl and methine groups of lactate, it is observed as a doublet peak in MR spectroscopy and is distinguished from lipid/macromolecules by these two features.[45] Lactate level is thought to be an important biomarker in autism cases. In a study conducted on rats, it was found that while the brain lactate level was quite high in animals with pharmacologically-induced autism, lactate levels decreased in the autism group treated with finasteride.[46] In another study conducted in humans, it was found that lactate doublets increased by 13 times on MR spectroscopy in autistic patients when compared to a healthy control group.[47] In the present study, lactate elevation was also shown in MR spectroscopy in rats with PPA-induced autism, which supports the data in the literature. Consistent with the literature, MR spectroscopy also revealed a decrease in lactate level after UDCA administration in our study.

Limitations of the Study

The most important limitation of our study can be noted as the absence of investigating changes in the composition of gut microbiota with UDCA treatment. Therefore, with current data, the mechanism of UDCA-induced improvements in the autism model cannot be directly associated with PPA levels or changes in PPA levels due to the expected alteration of microbiota via UDCA. It is also possible that UDCA administration caused the observed effects through other mechanisms; however, since UDCA is a bile acid that was administered via oral gavage, overt systemic effects through other mechanisms are unlikely. Nonetheless, further studies are needed to explain the mechanism of action of UDCA on autism-related findings.

CONCLUSION

In Conclusion, we demonstrated that oral UDCA administration had ameliorating effects on PPA-induced autism-like behavioral, biochemical, and histopathological changes in rats. Our results suggest that UDCA administration may ultimately lead to neuroprotective and neuromodulator effects via regulating the gut microbiota. More animal studies examining the relationship between PPA-mediated autism and UDCA treatment could demonstrate the utility of this parameter in clinical practice in humans with autism in more detail.

ETHICAL DECLARATIONS

Ethics Committee Approval: İstanbul Science University Medical Ethics Committee approved all experiments and all procedures and processes in this study. Our study included animal subjects. We considered all ethical, scientific, and legal values. Animals were looked after properly and used in minimum numbers (İstanbul Science University, Date: 2022, Decision No: 31210835).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Relation of parathyroid hormone with malnutrition in peritoneal dialysis patients

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ABSTRACT

Aims: Protein-energy wasting syndrome is a risk factor specific to ESRD is protein-energy wasting (PEW) syndrome. Clinical and experimental studies have suggested that secondary hyperparathyroidism plays a vital role in increasing energy expenditure in patients with ESRD. The geriatric nutritional risk index (GNRI) is used to evaluate nutritional status in various pathological conditions. Considering the effect of parathormone on malnutrition and studies indicating that parathyroid hormone causes weight loss, we aimed to investigate the relationship between malnutrition and parathyroid hormone (PTH) in our patients using GNRI.

Methods: Forty-nine patients without known malignancy, liver disease, or chronic inflammatory disease who underwent peritoneal dialysis were included in the study. Patient data were recorded from these files. Height and weight were measured. GNRI was calculated by the formula 14.89 × serum albumin (g/dL) + [41.7 × bodyweight/ideal body weight

Results: Forty-nine patients (29 females, 59.2%) were included in the study. Three (6.1%), seven (14.3%), and seven (14.3%) patients had severe, moderate, and mild malnutrition, respectively. GNRI was positively correlated with albumin, hematocrit, and calcium levels (r=0.757, r=0.355, r=0.423; p<0.05, respectively). GNRI was negatively correlated with dialysis vintage (r=-0.303, p=0.038) and PTH (r=-0.287; p=0.046).

Conclusion: This study demonstrated a relationship between malnutrition and hyperparathyroidism. Increased PTH levels may cause phenotypic switching from white to brown fat via PTH receptors.

Keywords: Peritoneal dialysis, protein-energy malnutrition, secondary hyperparathyroidism

INTRODUCTION

The risk of mortality in people with chronic kidney disease, especially those receiving renal replacement therapy for end-stage renal disease (ESRD), is too high to be explained by traditional risk factors alone.[1] One risk factor for ESRD is protein-energy wasting (PEW) syndrome, which is characterized by malnutrition and changes in body composition.[2-4] PEW is common in patients with ESRD and is associated with a risk of hospitalization and death.[5-7] Therefore, new therapeutic approaches are needed to prevent and treat PEW. Previous clinical and experimental studies have suggested that secondary hyperparathyroidism plays a vital role in increasing energy expenditure in ESRD.[8-10]

The geriatric nutritional risk index (GNRI), calculated only by body weight, height, and serum albumin level, is used to evaluate the nutritional status in various pathological conditions.[11,12] It has been used in chronic hemodialysis,[12-21] and peritoneal dialysis (PD)[22-24] patients in the association between malnutrition and all-cause mortality and cardiovascular (CV) events.

Considering the effect of parathormone on malnutrition and studies indicating that parathyroid hormone causes weight loss, we aimed to investigate the relationship between malnutrition and PTH in our patients using the GNRI.

METHODS

The study was initiated with the approval by the Health Sciences University Haseki Training and Research Hospital Clinical Researches Ethics Committee (Date: 01.03.2023, Decision No: 217-2022). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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This was a retrospective cross-sectional study. A total of 49 patients who were followed up in the peritoneal dialysis unit of the Haseki Research And Training Hospital for four years without known malignancy, liver disease, chronic inflammatory disease, and who did not receive nutritional support were included in the study. An informed consent form was obtained from all patients. Demographic and laboratory data were recorded from their files. Height and weight were measured. GNRI was calculated according to the formula 14.89 × serum albumin (g/dl) + [41.7 × bodyweight/ideal body weight]. If body weight exceeded the ideal body weight, the body weight/ideal body weight was taken as 1. The GNRI was evaluated as <82 severe, 82-92 moderate, 92-98 mild malnutrition risk; and >98, normal.

SPSS for windows 20.0 package program was used for statistical analysis. The Shapiro–Wilk test was used to detect the normality of the parameters. For normally distributed parameters, variance analysis and Tukey's multiple comparison tests were used to compare more than two groups. To compare two groups in independent parameters, a t-test was used, and results are presented as mean± standard deviation. For the parameters not customarily distributed, more than two groups were compared using the Kruskal–Wallis test, and Dunn's multiple comparison tests were used. The Mann-Whitney U test compares two groups, and the results are presented as median (25%-75%) values. The chi-square test was used for the analysis of the cross tables. Spearman and Pearson's test was used for the analysis of correlation. The parameters that were found to be correlated were evaluated using linear regression analysis.

RESULTS

Forty-nine patients (29 females, 59.2%), with a mean age of 51±13 years, were included in the study. The etiology of ESRD was as follows: glomerulonephritis, 26.5% (13 patients);, diabetes in 22.4% (11 patients);, hypertension in 20.4% (10 patients);, pyelonephritis in 14.3% (4 patients); polycystic kidney disease, 10.2% (5 patients);, Alport in 2% (1 patient); and amyloidosis, 4.1% (2 patients). Thirty-one patients underwent CAPD, while 18 underwent APD (36.7%). The mean laboratory values of patients are shown in Table 1.

Mean GNRI was 102.16±14.53. According to the GNRI scores, three patients had severe (6.1%), seven (14.3%) had moderate, and seven (14.3%) had mild malnutrition. Thirty-two patients were not malnourished.

Table 1. Demographic values and laboratory measurements of the patients

Parameter	Value
Male/female	20/29
Age (years)	51±13
Dialysis vintage(years)	4±3
Primary disease	
Glomerulonephritis	13(26.5%)
Diabetes mellitus	11(22.4%)
Hypertension	10(20.4%)
Polycystic disease	5(10.2%)
Pyelonephritis	7(14.3%)
Amyloidosis	2(4.1%)
Alport	1(2%2)
Dialysis modality	
CAPD	31(63.3%)
APD	18(36.7%)
Urea (mg/dl)	98±25
Creatinine (mg/dl)	7.8±2.7
Albumin (gr/dl)	3.6±0.5
Ca (mg/dl)	8.9±0.8
Phosphorus (mg/dl)	5±1
PTH(µG/dl)	551±507
Hematocrit	31±6
Ferritin	318(16-2326)
Uric acid (mg/dl)	5.8±1
Residual renal volume (ml)	639(0-2800)
BMI (kg/m²)	25.88±5.28
GNRI	102.16±14.53

PTH: parathyroid hormone, BMI: Body Mass Index, GNRI: Geriatric nutritional risk index

Patients were divided into two groups based on the presence of malnutrition. PTH levels were increased in the malnutrition group. Seventeen patients had malnutrition, and 32 patients did not have malnutrition. The mean age was 53±12 years in the malnutrition group and 50±14 years in the non-malnutrition group. Albumin levels were significantly higher in patients without malnutrition (3.8±0.3 vs 3.1±0.3, p<0.05). Dialysis vintage, residual renal function, and urea and creatinine levels were not significantly different (Table 2). Creatinine was 7.8±2.9 vs. 7.6±2.2 in normal and malnutrition groups, respectively (p>0.05). The hematocrit level was significantly lower in the malnutrition group than in the non-malnutrition group (30±5 vs. 32±5; p<0.05). Uric acid, phosphorus, and ferritin levels did not differ between the groups (Table 2; p<0.05). PTH levels were found to be increased in the malnutrition group, but this was not statistically significant (659±594 vs. 494±453; p>0.05). Calcium levels were higher in patients with normal nutritional status (9.2±0.6 vs. 8.4± 1.0; p<0.05). Body mass index was lower in the malnutrition group (21.55±3.3 vs. 28.35±4.19; p<0.05).

Table 2. Comparison of groups according to presence of malnutrition

	Malnutrition (-)	Malnutrition (+)	p
Male/female	12/20	8/9	p>0.05
Age (years)	50±14	53±12	p>0.05
Dialysis vintage	4±3	5±3	p>0.05
Urea (mg/dl)	97±17	100±23	p>0.05
Creatinin (mg/dl)	7.9±2.9	7.6±2.2	p>0.05
Albumin (gr/dl)	3.8±0.3	3.1±0.3	p<0.05
Ca (mg/dl)	9.2±0.6	7.6±2.2	p<0.05
Phosphor (mg/dl)	5±1	5±1	p>0.05
PTH (µg/dl)	494 (48-1900)	659±594 (43-1900)	p>0.05
Hematocrite	32±5	30±5	p<0.05
Ferritin	281 (46-1038)	384 (16-2326)	p>0.05
Uric acid (mg/dl)	5.9±1.1	5.6±0.9	p>0.05
Residual renal volume (ml)	772 (0-2800)	289±427 (0-1200)	p>0.05
BMI(kg/m²)	28.35±4.39	21.55±3.3	p<0.05

PTH: parathyroid hormone, BMI: Body Mass Index, GNRI: Geriatric nutritional risk index

The GNRI was positively correlated with albumin, hematocrit, and calcium levels (r=0.757, r=0.355, and r=0.423, respectively; $p < 0.05$). GNRI was negatively correlated with dialysis vintage (r=-0.303, p=0.038) and PTH (r=-0.287; p=0.046) (Figure) (Table 3).

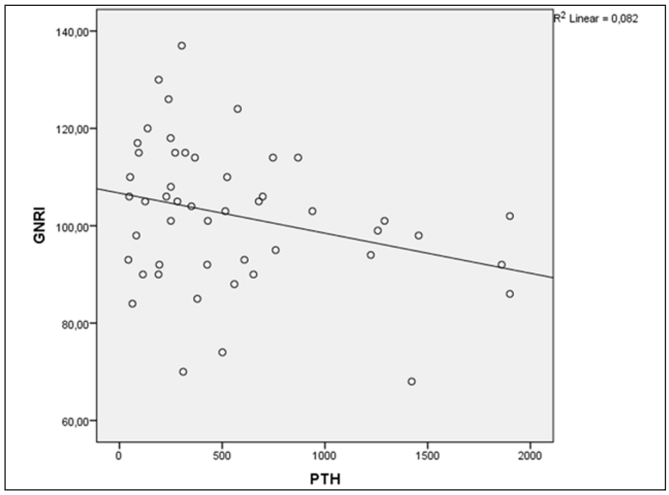

Figure. correlation of GNRI with PTH
PTH: parathyroid hormone, BMI: Body Mass Index, GNRI: Geriatric nutritional risk index

Table 3. Correlations of GNRI with studied parameters

	R	P
Dialysis vintage	-0.303	0.038
Hematocrit	-0.355	0.012
PTH	-0.287	0.047
Albumin	-0757	0.00
Ca	0.423	0.002
Residual renal volume	0.404	0.004

Regression analysis revealed that albumin, RRV, and PTH levels were significant independent determinants of GNRI (Table 4).

Table 4. Regression analysis of GNRI

	B	Beta	t	Sig.
(Constant)	39,874		2,596	,013
Albumin	19,660	,719	6,315	,0001
Dialysis vintage	-,276	-,054	-,557	,580
Residual renal volume (ml)	,005	,227	2,416	,020
Htc	,190	,069	,704	,485
Ca	-1,373	-,079	-,726	,472
PTH	-,006	-,194	-2,036	,048

PTH: parathyroid hormone, BMI: Body Mass Index, GNRI: Geriatric nutritional risk index

DISCUSSION

The main finding of this study was that PTH, albumin, and RRV were the major determinants of the GNRI.

Some studies have examined the GNRI in hemodialysis patients. One study reported that 31.6% of dialysis patients had malnutrition that could be detected using the GNRI.[12] Evidence suggests that the GNRI is a nutritional assessment tool that can be used for dialysis patients. It has been shown that GNRI can be used as a predictor of mortality in dialysis patients as well as its importance in the diagnosis of malnutrition.[12-24] Malnutrition is common in chronic kidney disease (CKD)[12,20] with approximately 18-75% of patients with CKD receiving maintenance dialysis, with evidence of malnutrition.[20] In our patient group, 34.7% of patients had malnutrition.

Serum calcium levels were significantly lower in malnourished patients. This decrease may be because we did not measure ionized calcium levels, and decreased albumin levels may have caused decreased total calcium levels. In addition, the vitamin D levels could not be measured. Decreased vitamin D levels can lead to reduced calcium levels.

Correlation analysis showed that the GNRI was negatively correlated with the RRV. This significance was obtained after the regression analysis. RRV may lead to patients eating independently. In addition, preservation of RRF was found to be associated with decreased inflammation, which is a component of the malnutrition syndrome.[25,26]

Chronic kidney disease and mineral and bone disorders increase mortality and morbidity.[27] The mechanism for this appears to be crosstalk between the bone and vascular wall. Malnutrition is associated with cardiovascular diseases. Although many factors can cause malnutrition in patients with CKD, inflammation is one of the most important causes.[28-30] Inflammation increases atherosclerosis.

The main finding of this study was the relationship between the GNRI and PTH levels. In the malnutrition group, the PTH levels tended to be higher, but this increase was not statistically significant. In the

correlation analysis, GNRI was negatively correlated with PTH levels. Decreased protein intake, as well as increased energy expenditure, is one of the contributing factors to PEW. Increased resting energy expenditure (REE) is caused by increased protein and fat catabolism. As a result, loss of adipose tissue and muscle tissue occurs. In a study of hemodialysis patients, Ikızler et al.[31] showed that the REE was higher in dialysis patients than in healthy controls. In addition, in this study, the REE increased even more during dialysis in the patient group.

An increase in REE occurs with a phenotypic transition from white to brown adipose tissue, which is called adipose tissue browning.[32] In a study by Cuppari et al.[9] the REE of patients was measured, and PTH was shown that PTH is an independent marker of REE.

After adjustment for lean body mass, REE was higher in patients with severe hyperparathyroidism than in patients with mild and moderate hyperparathyroidism and healthy individuals. In addition, the investigators measured the REE before and six months after parathyroidectomy (PTx) in patients with severe hyperparathyroidism. They found a 23% decrease in REE after surgery, parallel with a significant decrease in PTH levels. Therefore, these data suggest that severe SHPT may contribute to PEW by increasing the REE of ESRD patients and that PTx may reverse this condition.

Kir et al.[10] reported that PTH and PTH-related peptides (PTHrPs), which share the same receptor, act as mediators of fat tissue and muscle mass loss in mouse models of cancer and renal failure. In a previous study, Kir et al.[33] In cancer cachexia, PTHrP causes browning and wasting of adipose tissue by inducing the expression of UCP and other genes involved in thermogenesis and energy expenditure.

Tumor-bearing mice were then injected with antibodies that neutralized PTHrP. They observed that adipose tissue browning and the loss of muscle mass and strength were reversed. Since PTH and PTHrP share the same receptor, they conducted a study with 5/6 nephrectomized mice to understand the role of PTH in cachexia, which occurs in renal failure.[10] Fat browning and cachexia associated with secondary hyperparathyroidism developed in nephrectomized rats. Fat browning and muscle atrophy did not occur after nephrectomy in mice with PTH/PTHrP receptor deletion in the adipose cells. These data suggest that PTH and PTHrP may cause malnutrition via the PTH receptor. They also explained why PEW is common in secondary hyperparathyroidism and why it improves with hyperparathyroidism treatment.

This study had some limitations. First, the number of patients included in this study was low. Second, this was a cross-sectional study, so we could only discuss the status at that time. Third mortality and hospitalizations are not included in this population

CONCLUSION

As a result, PTH levels were negatively correlated with the GNRI values in our study. This may be caused by increased PTH, causing phenotypic switching from white adipose tissue to brown fat by PTH receptors. Therefore, the treatment of secondary hyperthyroidism may prevent and reverse malnutrition and wasting. Follow-up studies with larger cohorts are required to address this issue.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval by the Health Sciences University Haseki Training and Research Hospital Clinical Researches Ethics Committee (Date: 01.03.2023, Decision No: 217-2022).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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A scientometric analysis of the relationship between functional dyspepsia and anxiety

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ABSTRACT

Aims: Functional dyspepsia is a common disorder characterized by persistent or recurrent upper gastrointestinal tract symptoms without underlying disease or structural abnormalities that can have a significant impact on an individual's overall quality of life. Studies in the literature indicate that anxiety also plays an important role in the etiology of functional dyspepsia. Early diagnosis and treatment of the underlying anxiety disorder are important to relieve dyspeptic symptoms. In this study, the aim was to examine the studies related to functional dyspepsia and anxiety using the bibliometric method.

Methods: Clarivate Analytics' Web of Science database was used to search for articles and reviews on functional dyspepsia and anxiety between 1980 and 2022. VOSviewer, Tableau, and IBM SPSS Statistics were used for bibliometric network visualization and statistical analyses. A regression analysis using a nonlinear exponential model was used to forecast the number of publications in the next years. Keyword network visualization maps were used to identify recent trends and relationships.

Results: The Web of Science database included 560 articles and reviews about functional dyspepsia and anxiety between 1991 and 2023, with the top 5 research areas being Gastroenterology, Clinical Neurology, Neurosciences, Psychiatry, and General Internal Medicine. The collaborative clustering network map shows four distinct clusters based on total link strength scores, and the correlation between Gross Domestic Product (GDP) and the number of studies published suggests that countries with higher GDP tend to publish more studies. Since 2015, the amount of published materials on the topic has dramatically increased and is continuing to rise.

Conclusion: There has been an upward trend in publications about the relationship between functional dyspepsia and anxiety since the 2010s, with the top ten countries with the greatest number of publications being mostly wealthy nations. The most cited journal on the topic was the American Journal of Gastroenterology, with 1441 total citations. Our findings suggest that this is a rapidly evolving field with a broad range of research topics.

Keywords: Functional dyspepsia, anxiety, bibliometric analysis, dyspepsia

INTRODUCTION

Functional dyspepsia is a disorder marked by recurrent or persistent regurgitation, upper abdominal distress or discomfort, bloating, nausea, and early satiety in the absence of underlying disease or structural abnormalities. It is a functional gastrointestinal condition characterized by symptoms that can significantly decrease an individual's quality of life due to its impact on gastrointestinal motility and sensitivity. It is a highly prevalent disorder that accounts for 5% of visits to primary care clinicians.[1,2] Around 10–20% of the general population is thought to have functional dyspepsia.[3] The pathophysiological mechanism of functional dyspepsia is unclear. However, it is thought to be a mix of variables, including abnormal stomach emptying, altered gastrointestinal motility, visceral hypersensitivity, and psychological issues such as anxiety, stress, and depression. Functional dyspepsia, which manifests as gastric hypomotility and disorganized antral duodenal contractions, has been associated with gastrointestinal motor disorders.[4]

Anxiety is a mental health disorder characterized by feelings of worry, fear, or unease that can be mild or severe.[5] Anxiety disorders make up the majority of mental illnesses globally, with high comorbidity and morbidity.[6] The prevalence of anxiety disorders varies depending on the population studied and the diagnostic criteria used. Epidemiological studies show that anxiety disorders constitute the most frequent mental disorders in the community, with phobias being the most common.[7]

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There is a significant association between functional dyspepsia and anxiety.[8-11] Studies suggest that anxiety and depression play an important role in the etiopathogenesis of functional dyspepsia.[8] Patients with functional dyspepsia have a higher rate of anxiety disorder, depression, and somatoform disorder compared to the general population.[10] Anxiety is a significant predictor of healthcare utilization by patients with dyspepsia and irritable bowel syndrome.[12] Individuals with coexisting functional gastrointestinal disorders, particularly functional dyspepsia, exhibit a more intense symptom complex, lower health-related quality of life, higher somatization levels, and are more likely to suffer anxiety, depression, or sleeplessness than those without overlap.[11,13] Therefore, recognition of depression and anxiety is crucial to improve clinical outcomes in functional dyspepsia patients.[9]

A bibliometric analysis is a quantitative method used to evaluate and analyze scientific literature in a particular field.[14-16] It requires employing mathematical and statistical approaches to assess the external characteristics of literature in order to characterize, evaluate, and forecast research advancement in an area.[17] Bibliometric analysis is used to measure the productivity of researchers, groups, or institutions and to evaluate scientific production using various methods.[18] Bibliometric evaluation is an excellent method for assessing data characteristics and subject growth trends.[17] It is also used to explore research trends and characteristics of publications in a specific area. Following the studies on a particular topic and divulging their findings by examining these publications according to different features is the basis of bibliometric analysis. Bibliometric analysis is an advanced and efficient statistical technique that assesses secondary data acquired from digital databases from a quantifiable and objective standpoint and provides researchers with an overview of a particular research domain, such as gastroenterology, psychiatry and surgery.[19-21]

In this study, our purpose was to conduct a bibliometric analysis of the academic literature on functional dyspepsia and anxiety. Specifically, we sought to identify patterns in research, such as the number and types of studies conducted, frequently cited authors and publications, and common research themes and methodologies. By analyzing the bibliographic data, we aimed to gain insights into the current state of knowledge in the field, identify knowledge gaps, and suggest potential areas for future research. The findings from this study can be useful for researchers and clinicians to develop more effective diagnostic and treatment strategies for patients with functional dyspepsia and anxiety.

METHODS

Ethics committee approval is not required for this bibliometric study. This research was conducted in accordance with the World Medical Association Declaration of Helsinki's "Ethical Principles for Medical Research Involving Human Subjects."

Clarivate Analytics' Web of Science (WoS) database was used for the literature review. In WoS, the search terms "functional dyspepsia" and "anxiety*" were used. Only the "topic" section of the studies was used for the publication search. Using this search technique, all articles and reviews on functional dyspepsia and anxiety or articles containing terms derived from these were found and retrieved from the WoS database. The search dates were determined to be between 1980 and 2022 (access date: 24.04.2023). Researchers can use these reproducibility codes to access comparable documents (search results may differ based on access dates): (("functional dyspepsia" (Topic) OR "functional dyspe*"(Topic)) AND (("anxiety" (Topic) OR "anxi*" (Topic)) Timespan: 1980-2022 (Indexes Scanned: SSCI, SCI-E, ESCI, A&HCI, BKCI-SSH, BKCI-S, CPCI-SSH, CPCI-S)(Article Types Scanned: Article, Review Article)). For bibliometric network visualization, VOSviewer software was used (Leiden University's Center for Science and Technology Studies, Version 1.6.19).[22] The Tableau Software for Windows (Version 2019.4.1.; Tableau Software LLC, Seattle, WA) software was used to create a globe map. The IBM SPSS Statistics for Windows program was used for statistical analyses (Version 26; IBM Corp., Armonk, N.Y., USA). Data normal distribution was evaluated with the Shapiro-Wilks test. In line with the data distribution, Spearman's correlation coefficient was used to assess the relationships between the number of articles published by world nations and multiple economic development indicators of world countries to see whether there is a relationship between economic power and the number of scientific publications (Gross Domestic Product (GDP), and GDP per capita, World Bank, 2021 data).[23] To predict the number of publications in the next years, a nonlinear regression analysis (exponential model) was used. In the regression analysis, the R square (R2) value was utilized to measure the model's effectiveness. Results were considered statistically significant if the p-value was less than 0.05.

RESULTS

The Web of Science database included 560 articles and reviews regarding functional dyspepsia and anxiety published between 1980 and 2023. Of these publications, 494 were articles (88.21%), and 66 were reviews (11.79%). 98.04% (549) of these publications were in English, 1.25% (7) were in German, 0.36% (2) were in Turkish, 0.179% (1) were in Korean, and 0.179% (1) were in Polish. The average number of citations per article was

27.05, the total number of citations was 15147 (without self-citations: 13058), 72 publications were not cited, and the h-index of 560 articles was 60.

Active Research Areas

The top 10 research areas about functional dyspepsia and anxiety were Gastroenterology (319, 56.96%), Clinical Neurology (75, 13.39%), Neurosciences (66, 11.79%), Psychiatry (61, 10.89%), Medicine General Internal (60, 10.71%), Pharmacology (43, 7.68%), Psychology (42, 7.49%), Medicine Research Experimental (20, 3.57%), Pediatrics (14, 2.50%), Nutrition (10, 1.79%).

Development and Future Trends of Publication

Figure 1 depicts the yearly variation in the number of published papers. In addition, it presents the results of the non-linear exponential growth regression analysis conducted to predict the number of papers published in 2023 and beyond. The model demonstrated a statistically significant relationship with the data, with a degree of agreement of 90.4% (R2=0.904, p<0.001). The model predicts that a total of 61 (95% CI (Confidence Interval): 31–122) articles will be published in 2023, 68 (95% CI: 34–136) new articles in 2024, and 75 (95% CI: 37–151) new articles in 2025 (Figure 1). These predictions suggest a gradual increase in the number of published articles over the next three years. It is expected that more than 100 articles will be published each year starting in 2028 (projected 104, 95% CI: 49–189). However, it is important to note that unforeseen factors could impact these projections.

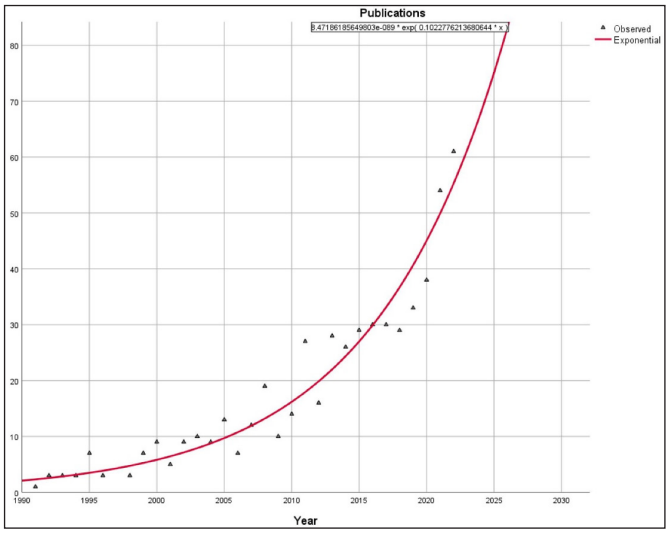

Figure 1 Distribution of functional dyspepsia and anxiety publications by year and projection of articles in the following years using the exponential growth model

Active Countries

A total of fifty-eight countries had publications about functional dyspepsia and anxiety. The countries that produced the most research publications on anxiety and functional dyspepsia were the United States of America (USA) (121, 21.61%), the People's Republic of China (PRC) (106, 18.93%), Australia (66, 11.79%), Japan (45, 8.04%), Sweden (43, 7.68%), Belgium (41, 7.32%), England (32, 5.71%), Germany (27, 4.82%), Italy (25, 4.46%), and South Korea (25, 4.46%) (Figure 2). The total link strength scores of 27 countries that published at least five articles on functional dyspepsia and anxiety and had international associations among

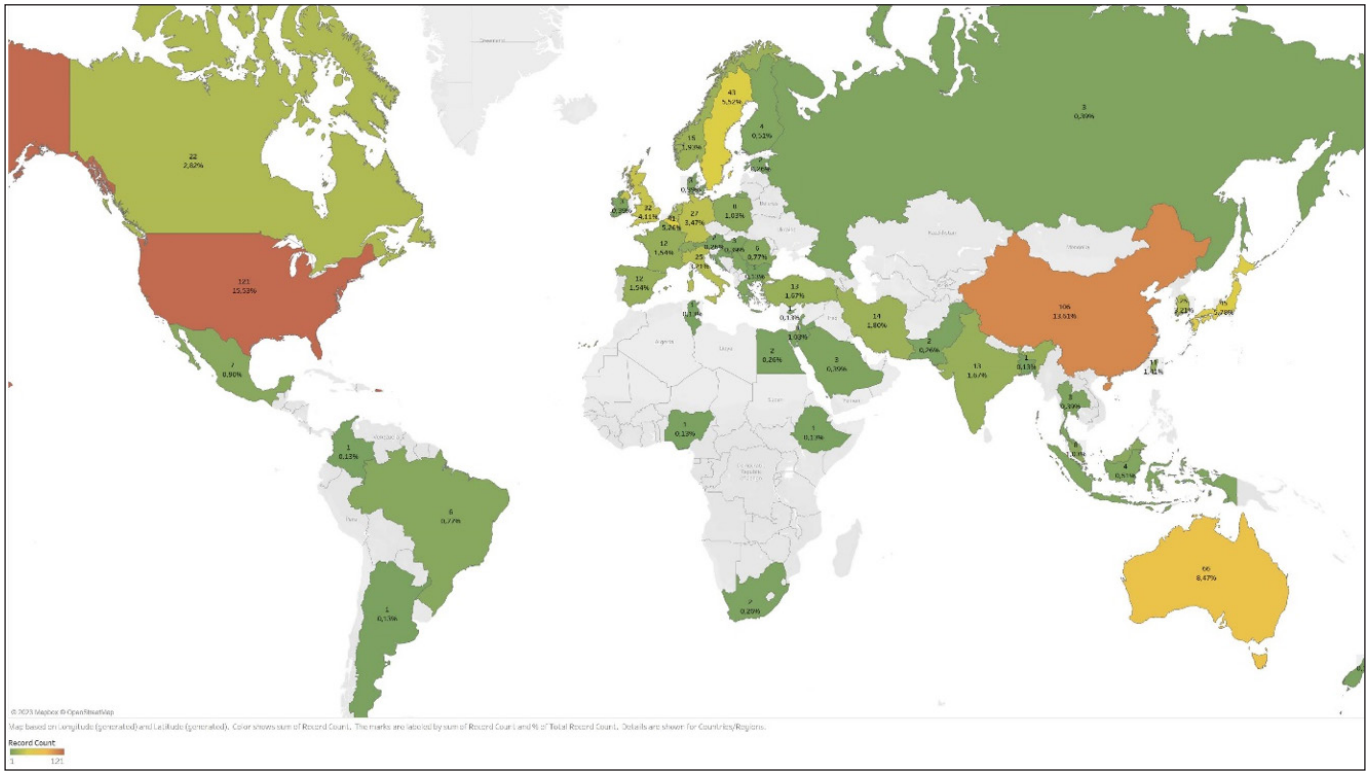

Figure 2 Global distribution of publications on functional dyspepsia and anxiety

their authors were calculated. Figure 3 displays the collaborative clustering network map using total link strength scores (Malaysia had no author connections to any other nation and was therefore omitted from the map). There were found to be four distinct clusters based on the data (Cluster 1: Belgium, France, Germany, the Netherlands, Norway, Poland, Romania, Spain, Sweden, Switzerland, Turkey; Cluster 2: Australia, Brazil, Canada, England, India, Iran, Israel, Mexico, PRC, USA; Cluster 3: Japan, Singapore, South Korea; Cluster 4: Italy, Taiwan). The internal collaboration density map is also illustrated in Figure 3.

Correlation Analysis of Publication Count and Gross Domestic Product

The GDP of a nation was significantly correlated with the number of studies that the country did on functional dyspepsia and anxiety ($r=0.758$, $p<0.001$). This was also true for the association between GDP per capita and the number of studies that the country published. ($r=0.444$, $p<0.001$). This suggests that countries with a higher GDP per capita tend to publish more studies, possibly due to having more resources and funding for research. However, correlation does not necessarily imply causation, and other factors may also be at play.

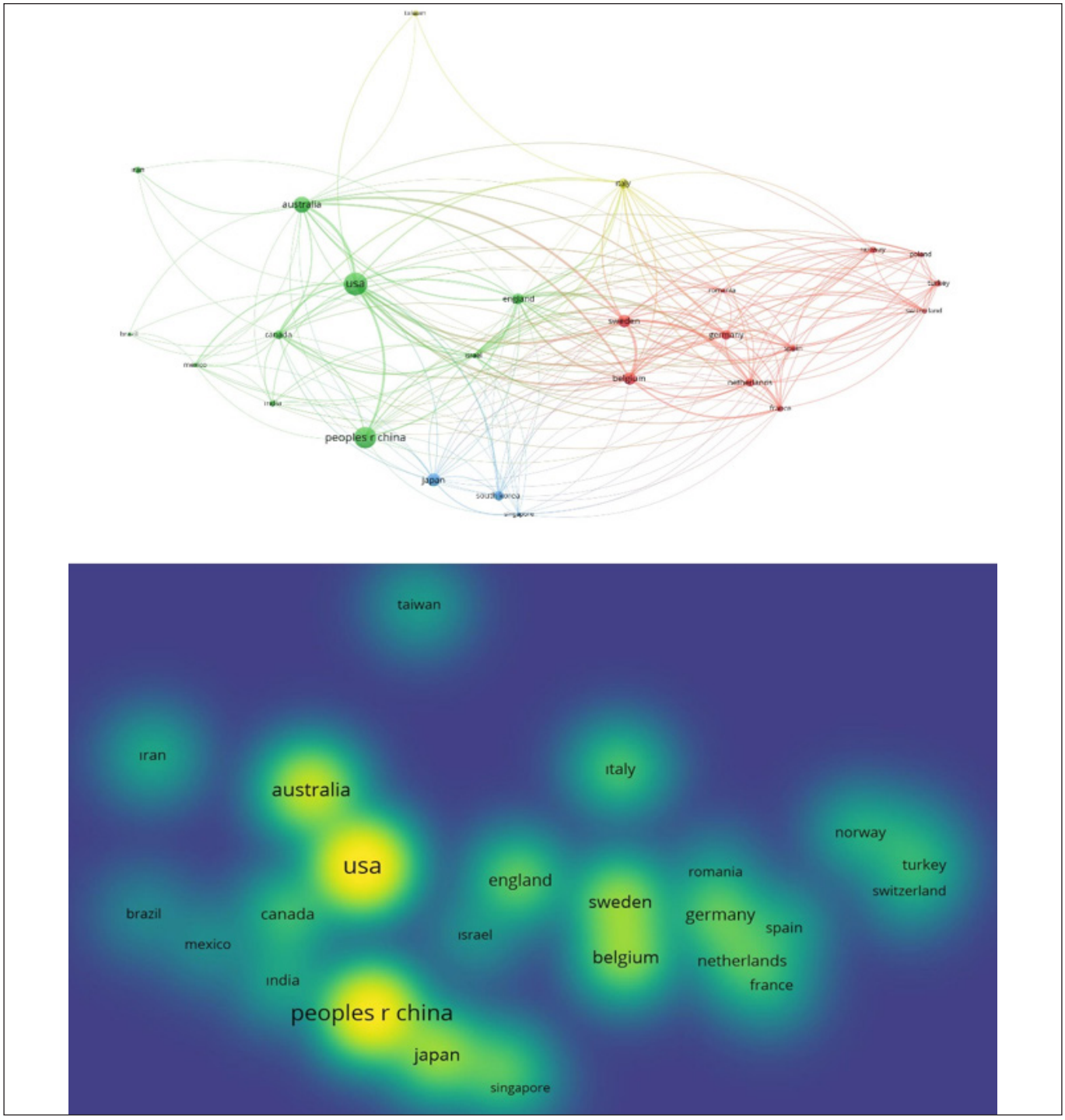

Figure 3. Network visualization, cluster and density map on worldwide cooperation on functional dyspepsia and anxiety

Active Authors

The top ten most active and productive writers with the most papers on functional dyspepsia and anxiety were Talley NJ (50, 8.93%), Tack J (25, 4.46%), Van Oudenhove L (22, 3.93%), Zeng F (19, 3.39%), Jones MP (17, 3.04%), Holtmann G (15, 2.68%), Liang FR (14, 2.50%), Miwa H (12, 2.14%), Qin W (12, 2.14%), and Walker MM (12, 2.14%). These influential authors have made significant contributions to the research on functional dyspepsia and anxiety, and their work has helped advance our understanding of the relationship between these conditions.

Active Institutions

From 1991 and 2023, the top ten institutions that produced the most articles on functional dyspepsia and anxiety were the Catholic University of Leuven (37, 6.61%), University of Newcastle (32, 5.71%), Mayo Clinic (29, 5.18%), University Hospital of Leuven (23, 4.11%), Chengdu University of Traditional Chinese Medicine (21, 3.75%), Karolinska Institutet (20, 3.57%), Macquarie University (20, 3.57%), University of Sydney (19, 3.39%), University of Bergen (14, 2.50%), Xidian University (14, 2.50%). It is interesting to note that the Catholic University of Leuven and the University Hospital of Leuven, both located in Belgium, are among the top four institutions producing articles on functional dyspepsia and anxiety. Additionally, it is worth mentioning that two Chinese universities, Chengdu University of Traditional Chinese Medicine and Xidian University, also made it to the top ten list.

Active Journals

Multiple years of research on functional dyspepsia and anxiety have resulted in the publication of 560 articles in 205 medical journals. This indicates the growing interest and importance of these subjects within the medical community. The top five active journals in this field are Neurogastroenterology and Motility (Publisher: Wiley) (51, 9.11%), Clinical Gastroenterology and Hepatology (23, 4.11%), Digestive Diseases and Sciences (20, 3.57%), Journal of Neurogastroenterology and Motility (Publisher: The Korean Society of Neurogastroenterology and Motility) (20, 3.57%), and Alimentary Pharmacology and Therapeutics (19, 3.39%). These journals have published more than 20% of the total articles on functional dyspepsia and anxiety. Table 1 lists the top 30 journals that published four or more articles, along with the total amount of citations received and the average amount of citations per article.

Table 1 The top 30 most active journals with articles on the relationship between functional dyspepsia and anxiety

Journals	RC	C	AC
Neurogastroenterology and Motility	51	805	110.27
Clinical Gastroenterology and Hepatology	23	933	111.79
Journal of Neurogastroenterology and Motility	20	541	64.32
Digestive Diseases and Sciences	20	357	24.77
Alimentary Pharmacology & Therapeutics	19	1026	90.82
Journal of Gastroenterology and Hepatology	19	336	43.27
World Journal of Gastroenterology	17	374	38.35
American Journal of Gastroenterology	15	1441	84.7
Scandinavian Journal of Gastroenterology	14	455	20.85
Psychosomatic Medicine	12	1082	54.93
PLoS One	11	247	27.67
Journal of Psychosomatic Research	11	530	26.37
Gastroenterology	10	890	72.35
Gut	10	950	86.22
Journal of Digestive Diseases	8	125	17.83
European Journal of Gastroenterology & Hepatology	8	225	14.96
Journal of Gastroenterology	7	346	36.88
BMC Gastroenterology	7	124	12.57
Medicine	6	25	5.15
Frontiers in Psychiatry	6	56	12.95
Psychotherapy and Psychosomatics	6	272	14.09
Digestion	5	186	17.6
Trials	5	29	4.27
Indian Journal of Gastroenterology	5	41	10.45
Gastroenterology Research and Practice	5	77	7.36
Journal of Clinical Gastroenterology	5	108	6.55
Clinical and Translational Gastroenterology	4	57	9.23
Journal of Pediatric Gastroenterology and Nutrition	4	241	15.35
Evidence-Based Complementary and Alternative Medicine	4	61	7.16
Frontiers in Neuroscience	4	7	3.5

(RC: Record Count, C: Number of Citations, AC: Average Citation per Manuscript

Citation Analysis

Table 2 shows the 20 papers with the most citations out of the 560 publications published between 1991 and 2022. In the last column of Table 2, the yearly average number of citations is shown.

Co-citation Analysis

There were 17,747 research papers in the references section of the 560 papers that were included in the analysis. The top ten publications with the most co-citations (more than 20) were Tack J (2006, Number of co-citations (NC):132), Zigmond AS (1983, NC:99), Aro P (2009, NC:77), Stanghellini V (2016, NC:63), Drossman DA (2006, NC:62), Koloski NA (2012, NC:56), Talley NJ (1999, NC:53), Van Oudenhove L (2008, NC:48), Tack J (2001, NC:47), Drossman DA (1993, NC:44).

Trending Topics

3433 distinct keywords were mentioned in the 560 papers on anxiety and functional dyspepsia. **Figure 4** depicts the cluster network visualization for 64 terms that occurred in at least five distinct publications. For those 64 terms, **Figure 4** also shows a network map for trend visualization and a network map for citation visualization.

Table 2 The top 20 most cited articles on functional dyspepsia and anxiety according to total citations

No	Article	Author Journal	PY	TC	AC
1	Medically unexplained physical symptoms, anxiety, and depression: A meta-analytic review	Henningsen, P et al. Psychosomatic Medicine	2003	578	27.52
2	The brain-gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study	Koloski, NA et al. Gut	2012	331	27.58
3	Epidemiology and health care seeking in the functional G1 disorders: A population-based study	Koloski, NA et al. American Journal of Gastroenterology	2002	242	11
4	Psychosocial factors are linked to functional gastrointestinal disorders: A population based nested case-control study	Locke, GR et al. American Journal of Gastroenterology	2004	213	10.65
5	Clinical Features of Idiopathic Gastroparesis Vary With Sex, Body Mass, Symptom Onset, Delay in Gastric Emptying, and Gastroparesis Severity	Parkman, HP et al. Gastroenterology	2011	204	15.69
6	Role of stress in functional gastrointestinal disorders - Evidence for stress-induced alterations in gastrointestinal motility and sensitivity	Monnikes, H et al. Digestive Diseases	2001	202	8.78
7	Somatic comorbidities of irritable bowel syndrome: A systematic analysis	Riedl, A et al. Journal of Psychosomatic Research	2008	182	11.38
8	Anxiety Is Associated With Uninvestigated and Functional Dyspepsia (Rome III Criteria) in a Swedish Population-Based Study	Aro, P et al. Gastroenterology	2009	177	11.8
9	Reliability and validity of the Gastrointestinal Symptom Rating Scale (GSRS) and Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire in dyspepsia: A six-country study	Kulich, KR et al. Health and Quality of Life Outcomes	2008	175	10.94
10	Evidence that independent gut-to-brain and brain-to-gut pathways operate in the irritable bowel syndrome and functional dyspepsia: a 1-year population-based prospective study	Koloski, NA et al. Alimentary Pharmacology & Therapeutics	2016	160	20
11	Chronic abdominal pain in children: A technical report of the American Academy of Pediatrics and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition	Di Lorenzo, C et al. Journal of Pediatric Gastroenterology and Nutrition	2005	157	8.26
12	Characteristics of Patients With Chronic Unexplained Nausea and Vomiting and Normal Gastric Emptying	Pasricha, PJ et al. Clinical Gastroenterology and Hepatology	2011	147	11.31
13	Predictors of health care seeking for irritable bowel syndrome and nonulcer dyspepsia: A critical review of the literature on symptom and psychosocial factors	Koloski, NA et al. American Journal of Gastroenterology	2001	147	6.39
14	Functional dyspepsia impairs quality of life in the adult population	Aro, P et al. Alimentary Pharmacology & Therapeutics	2011	139	10.69
15	Determinants of symptoms in functional dyspepsia: gastric sensorimotor function, psychosocial factors or somatisation?	Van Oudenhove, L et al. Gut	2008	136	8.5
16	Visceral hypersensitivity is associated with GI symptom severity in functional GI disorders: consistent findings from five different patient cohorts	Simren, M et al. Gut	2018	135	22.5
17	Multicenter, Randomized, Placebo-Controlled Trial of Amitriptyline in Children With Functional Gastrointestinal Disorders	Saps, M et al. Gastroenterology	2009	134	8.93
18	Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery	Marshall, JK et al. Gut	2010	133	9.5
19	Sleep disturbances in clinic patients with functional bowel disorders	Fass, R et al. American Journal of Gastroenterology	2000	126	5.25
20	Validity of a new quality of life scale for functional dyspepsia: a United States multicenter trial of the Nepean Dyspepsia Index	Talley, NJ et al. American Journal of Gastroenterology	1999	125	5

(PY: Publication year, TC: Total citation count, AC: Average citations per year)

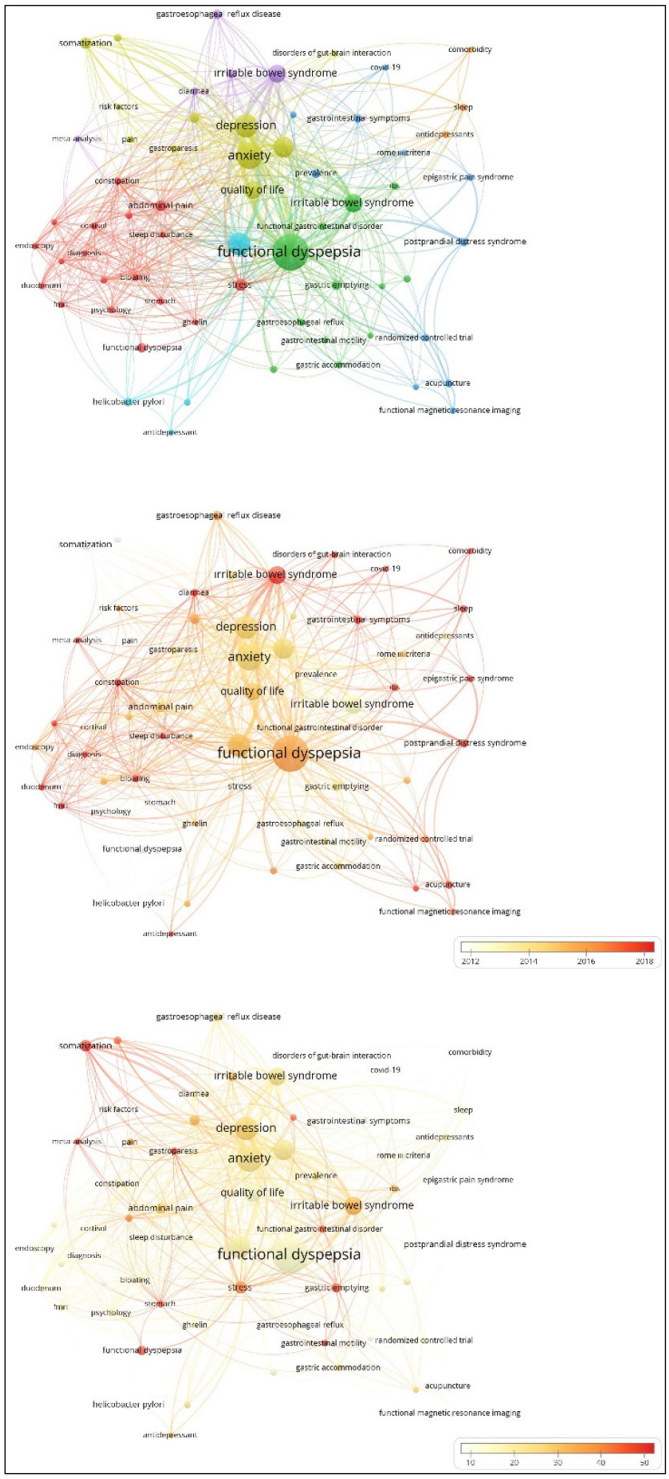

Figure 4 Keyword cluster analysis, keyword trend, and citation network visualization map of functional dyspepsia and anxiety

DISCUSSION

Functional dyspepsia is a major healthcare issue, particularly in modern times and in developed countries. It has a significant impact on the quality of life of patients.[24-26] Studies have reported that patients with functional dyspepsia have a reduced quality of life compared to healthy controls or the general population.[24] Dyspepsia is associated with increased rates of absence, decreased work efficiency, and increased use of medical and prescription drugs. Patients with functional dyspepsia also experience worse mental and physical quality of life.[27] The presence of other functional gastrointestinal disorders increases the burden of functional dyspepsia.[27]

Like functional dyspepsia, anxiety disorders also have significant economic costs and burdens, both in terms of direct medical treatment and indirect costs, such as lost productivity and reduced quality of life.[28-29] It is estimated that anxiety disorders impact 26.9 million people in the United States at some time in their life.[28] According to large population-based surveys, up to 33.7% of the general population of the world will experience an anxiety disorder at some point in their lifetime.[30] Over 6% of men and 13% of women have suffered from an anxiety disorder within the previous six months, according to a study conducted in the United States.[29]

Anxiety and functional dyspepsia are strongly connected.[8,9] Studies suggest that psychological factors, such as anxiety and depression, play an important role in the etiology of functional dyspepsia.[8] Cognitive-behavioral therapy (CBT) has been shown to be effective in reducing symptoms of functional dyspepsia in several studies. A randomized controlled trial found that CBT significantly reduced the severity of functional dyspepsia symptoms, including abdominal pain, bloating, and nausea, compared to a control group.[31,32] Another study conducted in China found that CBT improved the quality of life of functional dyspepsia patients and reduced anxiety and depression symptoms.[3] A systematic review and meta-analysis of randomized controlled trials also found that CBT was effective in reducing functional dyspepsia symptoms and improving quality of life.[34] Therefore, recognition and treatment of depression and anxiety are crucial to improving clinical outcomes in functional dyspepsia patients.[9]

There has been an upward trend in publications about the relationship between functional dyspepsia and anxiety since the 2010s. Even in the era of the COVID-19 pandemic, research on this topic continued without slowing. The evaluation of regression analysis suggests that the amount of research will keep increasing exponentially in the coming years. When analyzing the proportion of articles by country, the top ten countries with the greatest number of publications on the relationship between functional dyspepsia and anxiety were mostly wealthy nations. The correlation analysis reveals a significant relationship between article productivity and economic development variables, suggesting that a country's degree of economic development influences the number of articles published on functional dyspepsia. However, it is important to note that this does not necessarily mean that functional dyspepsia and anxiety are more prevalent

in wealthier nations, but rather that these countries may have more resources and funding available for research and publication. There was a significant correlation between GDP and GDP per capita and the number of publications regarding functional dyspepsia, although the association is significantly stronger with GDP than with GDP per capita, suggesting that national income is more important than individual income.

Based on international collaboration, the countries with the highest level of cooperation were the United States, Sweden, Belgium, England, and Australia. Clusters were generally formed based on the continent, with the exception of China (Cluster 1 was mostly comprised of European countries, Cluster 2 was mostly comprised of countries from America and the USA's close allies, and Cluster 3 was mostly comprised of Asian countries). Despite not working with any other nations, Malaysia also made a significant contribution to the research.

The journal with the most articles published to date on the topic was Neurogastroenterology and Motility (published by Wiley), followed by Clinical Gastroenterology and Hepatology, Journal of Neurogastroenterology and Motility (published by The Korean Society of Neurogastroenterology and Motility), Digestive Diseases and Sciences, Alimentary Pharmacology & Therapeutics, Journal of Gastroenterology and Hepatology, World Journal of Gastroenterology, and American Journal of Gastroenterology, respectively. When the journals were compared for the average number of citations per article per year, Clinical Gastroenterology and Hepatology had the lead with 111.79 average citations per article per year, followed closely by Neurogastroenterology and Motility with 110.27 average citations per article per year. The most cited journal on the topic of functional dyspepsia and anxiety was the American Journal of Gastroenterology, with 1441 total citations and an average number of citations per article per year of 84.7.

The most cited study was "Medically unexplained physical symptoms, anxiety, and depression: A meta-analytic review" published in Psychosomatic Medicine in 2003 by Henningsen P., and had 578 total citations and 27.52 citations per year.[35] The second most cited was "The brain-gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study" by Koloski NA., in 2012 in Gut, with 331 total citations and an average of 22.58 citations per year.[36] This was also the publication with the highest average citation count on this topic, owing its power to being a longitudinal study that followed a large population over 12 years, providing valuable insights into the bidirectional relationship between the brain and gut in functional gastrointestinal disorders. The third was "Epidemiology and health care seeking in the functional G1 disorders: A population-based study" also by Koloski NA., this time published in the American Journal of Gastroenterology in 2002 and having a total citation count of 242.[37]

The clustering analysis divided the keywords into seven different clusters. Functional dyspepsia, anxiety, depression, dyspepsia, functional gastrointestinal disorders, irritable bowel syndrome, quality of life, stress, somatization, and abdominal pain were the most frequently used keywords. After our search terms were removed from the list, the data showed that the terms frequently examined in recent years were gut-brain interaction, COVID-19, duodenum, proton pump inhibitors, and sleep disturbance. Gastric emptying, gastroparesis, somatization, gastrointestinal motility, and irritable bowel syndrome were the most frequently cited terms.

To our knowledge, there were no other bibliometric studies exploring the publications about the relationship between functional dyspepsia and anxiety. This suggests that our study fills a gap in the literature and provides valuable insights into the current state of research on functional dyspepsia and anxiety. This is the strongest aspect of this study. Additionally, another key strength of this article was that it covered the period from 1980 to 2023. Due to the inability to conduct citation and co-citation analyses in the PubMed and Scopus databases, they were excluded from the analysis. Because it incorporates citation analysis and indexes articles from higher-quality publications, WoS is preferred over competing databases. Usage of a reliable and comprehensive database such as WoS is another aspect of this study's strengths. Main limitation of this study was the reliance on a single database, even though it's a comprehensive one.

CONCLUSION

Functional dyspepsia is a common functional gastrointestinal disorder characterized by persistent or recurrent upper gastrointestinal tract symptoms. It is suggested that anxiety contributes to the pathogenesis and maintenance of functional dyspepsia symptoms, as it is a prevalent comorbidity in functional dyspepsia patients. The relationship between functional dyspepsia and anxiety is an important research topic in both internal medicine and psychiatry. Reducing the effects of functional dyspepsia on the general population requires early diagnosis of anxiety, rehabilitation, and, most importantly, knowledge. Our findings suggest that this is a rapidly evolving field with a broad range of research topics. We believe this article will lead to more research and publications on this critical topic..

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval is not required in this bibliometric study.

Informed Consent: Informed consent is not required.

Referee Evaluation Process: Externally peer reviewed.

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

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Original Article

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The impact of COVID-19 on patients with Parkinson disease

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ABSTRACT

Aims: Viral infections have been implicated in the development of Parkinson disease (PD). It has been observed that the presence of SARS-CoV-2 in dopaminergic cells can expedite the degeneration process and potentially exacerbate symptoms. The objective of this study was to assess the impact of the COVID-19 pandemic on individuals with PD.

Methods: A total of 60 patients were enrolled in the study. The severity of the disease was assessed using the using the Unified Parkinson's Disease Rating Scale (UPDRS), while the stage of the disease was determined using modified Hoehn &Yahr Rating Scale (m HYRS). Various measures were taken to evaluate the patients' well-being, including the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), Parkinson's Disease Quality of Life Questionnaire (PDQ-39), and Impact of Events Scale (IES-R) scores. The researchers also recorded the UPDRS scores, levodopa equivalent doses (LED), and BMI of the patients before and after the lockdown period. Subsequently, the collected data were compared to identify any significant changes.

Results: The difference in UPDRS, LED and BMI of the patients before and after the lockdown was statistically significant ($p<0.05$). Furthermore, there was a significant increase in UPDRS motor score, BAI, and PDQ-39 values among female patients compared to male patients ($p<0.05$). Comparing patients with and without COVID-19 infection, it was observed that patients who had contracted COVID-19 demonstrated a statistically significant increase in m HYRS and UPDRS motor scores, and PDQ-39 values after the lockdown ($p<0.05$). To assess the impact of various factors on the quality of life, multiple linear regression analysis was performed. The analysis revealed that COVID-19-positive patients and female patients experienced a more pronounced effect on their quality of life ($p<0.05$). Moreover, quality of life was found to be affected by disease stage, depression, anxiety, the IES-R scores increase, as well as by deteriorating sleep quality ($p<0.05$).

Conclusion: During the COVID-19 pandemic, PD patients experienced a worsening of clinical symptoms and increased treatment requirements. Furthermore, their quality of life was negatively affected, particularly among females and those who contracted COVID-19 infection. It is crucial to develop supportive treatment strategies targeting neuropsychological symptoms, as these could greatly contribute to the overall management and well-being of PD patients.

Keywords: Parkinson's disease, COVID-19, quality of life, sex

INTRODUCTION

Parkinson disease (PD) is a neurodegenerative condition characterized by both motor and non-motor features.[1] Initially, patients typically respond favorably to treatment, however, as the disease progresses, medication effectiveness may decrease, and side effects can emerge from prolonged use. These complications can significantly impact patients' ability to carry out their daily activities without assistance. Consequently, caregivers play a crucial role in monitoring patients' treatment progress and managing their daily living activities.[2]

Several notable observations have been made regarding the association between COVID-19 and PD. Studies have found higher levels of autoantibodies against coronaviruses in the cerebrospinal fluid of PD patients compared to healthy individuals, indicating a potential link between viral infection and the development of PD.[3]

The angiotensin-converting enzyme 2 (ACE2) protein is primarily responsible for the cellular entry of SARS-CoV-2, serving as a receptor for the virus.[4] ACE2 receptors are prominently expressed on dopaminergic neurons in the substantia nigra and striatum. It has been observed that viral infiltration of these cells accelerate the degeneration process, worsen symptoms, and increase the need for dopaminergic treatment, particularly in individuals with advanced age and longer disease duration.[3,5] Moreover, the SARS-CoV-2 virus is believed to induce protein misfolding and aggregation.

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Recent studies on PD have suggested a potential increase in alpha-synuclein accumulation in the context of COVID-19 infection, further implicating a connection between the two conditions.[4,6]

PD patients, like the general population, have been significantly impacted by disruptions in healthcare, quarantine measures, and the global stress caused by the COVID-19 pandemic.[7] While PD patients did not face a higher risk of infection compared to the general population, those who contracted SARS-CoV-2 experienced worsened symptoms, encountered challenges in receiving regular neurological assessments and medication adjustments due to limited access to outpatient clinics, and faced delays in planned surgical interventions.[8] Moreover, the pandemic has led to lifestyle changes among patients, including decreased physical activity, inability to exercise, and increased levels of psychological stress levels.[8] As a result of these clinical and lifestyle changes, the has been a noticeable increase in anxiety, depression, and stress, accompanied by a decline in oveall quality of life for PD patients.[2]

In the current research, we aim to evaluate the impact of the COVID-19 lockdown on patients with PD by assessing changes in their clinical findings, daily habits, quality of life, and the extent to which they were affected. Additionally, we seek to determine whether the implementation of the lockdown measures is associated with a deterioration of PD symptoms.

METHODS

This study was conducted with the approval of the Ethics Committee of the University of Health Sciences Fatih Sultan Mehmet Training and Research Hospital (Date: 11.03.2021, Decision No: 2021/29). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 60 patients in the neurology outpatient clinic at the University of Health Sciences, Fatih Sultan Mehmet Research and Training Hospital were included in the study. These patients were diagnosed with PD based on the Movement Disorder Association Parkinson's Disease Clinical Diagnostic Criteria. The study period encompassed December 2019 to December 2021.

To assess the impact of the COVID-19 lockdown, we divided the study period into two phases. The pre-lockdown period, referred to as "pre-COVID", included data collected until March 2020. The post-lockdown period, referred to as "post-COVID", included data collected from July 2021 onwards.

During these periods, we collected various data points including demographic information, daily levodopa equivalent doses (LED), scores from the Beck depression inventory (BDI), Beck anxiety inventory (BAI), Pittsburgh sleep quality index (PSQI), Epworth sleepiness inventory (ESI), Parkinson's disease quality of life questionnaire (PDQ-39) and impact of events scale (IES-R).

Disease severity was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS), while disease stage was determined using the modified Hoehn & Yahr Rating Scale (mHYRS). We recorded UPDRS scores, LED, and BMI of patients both before and after the lockdown, and compared the data obtained during these periods.

Statistical Analysis

For statistical analysis, we utilized IBM SPSS Statistics 25 software provided by IBM SPSS, Turkey. Descriptive methods such as mean, standard deviation, median, frequency, ratio, minimum, and maximum were employed to summarize the data. The Shapiro-Wilk Test was used to assess the distribution of the data.To compare qualitative data between the two groups we utilized the Mann-Whitney U Test. For comparisons between two time periods, the Wilcoxon test was employed. A chi-square analysis was performed to determine the association concerning qualitative data. Linear regression analysis was utilized to establish the parameters affecting the dependent variables. The significance level was set at $p<0.05$ to determine statistical significance.

RESULTS

The study included a total of 60 patients, of which 43.4% (n=26) were female and 56.7% (n=34) were male. The age range of the participants was between 32 and 83 years, with a mean age of 63.2±11.55 years. Detailed demographic and clinical information, such as disease duration, UPDRS and mHYRS scores, disease dominancy, educational status, habits, comorbid diseases, and lifestyle of the patients can be found in Table 1.

Table 1. Demographic and clinical data of patients with Parkinson's disease

		mean+SD	median (min-max)
Age		63.2±11.55	32-83 (67)
Sex	Female Male	26 (43.4%) 34 (56.7%)	
Disease Duration (year)		7.09±4.3	2-23 (6)
Disease Dominancy	Bradykinesia Tremor	44 (73.3%) 16 (26.7%)	
UPDRS		33.65±9.73	13-48 (35)
mHYRS		2.57±0.74	1-4 (2.5)
LED		527.33±290.4	110-1650 (450)
Person living together	With family Alone	55 (91.7%) 5 (8.3%)	
Comorbid diseases	Yes No	40 (66.7%) 20 (33.3%)	
Smoking	Yes No	9 (15%) 51 (85%)	
Education	Primary school High school University	40 (66.7%) 13 (21.7%) 7 (11.7%)	

UPDRS: Unified Parkinson's Disease Rating Scale, LED: levodopa equivalent dose, mHYRS: modified Hoehn-Yahr Rating Scale

When comparing the clinical findings of the patients before and after the COVID-19 pandemic lockdown, statistically significant differences were observed in UPDRS motor scores, daily levodopa intake, and BMI values after the lockdown (p=0.001, p=0.001, p=0.001, p=0.001; p<0.05). Detailed information regarding these findings can be found in Table 2.

Table 2. The impact of the COVID-19 pandemic on the clinical manifestations of patients with Parkinson's disease

	Before Lockdown mean+SD median (min-max)	After Lockdown mean+SD median (min-max)	p
UPDRS	33.65±9.73 13-48 (35)	49.53±14.24 20-78 (48)	0.001**
Levodopa equivalent dose	527.33±290.4 110-1650 (450)	695.92±299.55 150-1700 (650)	0.001**
Body mass index	27.6±4.65 20.76-43.28 (26.23)	28.62±5.34 20.76-48.93 (27.16)	0.001**

Wilcoxon test, *p<0.05, **p<0.01, UPDRS: Unified Parkinson's Disease Rating Scale

The study found a statistically significant rate of increase in UPDRS motor scores, BAI, and PSQI values among female patients compared to males (p=0.001, p=0.001, p=0.001, p=0.001; p<0.05). Moreover, there was a significant difference in the quality of life assessed by PDQ-39, and total score, with women exhibiting significantly higher scores than men (p=0.001; p<0.05). Specifically, women showed significantly higher scores in the "mobility" and "physical discomfort" domains of PDQ-39 compared to men (p=0.001, p=0.001; p<0.05). Detailed analyses of these findings can be found in Tables 3a and 3b.

We found statistically significant increases in mHYRS, post-lockdown UPDRS-motor scores and PDQ-39 values among patients with COVID-19 infection (p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001; p<0.05). A detailed analysis of these findings can be found in Table 4.

Table 3a. Comparison of clinical and neuropsychological findings of male and female patients

	Female (n=26) Mean±SD /Min-Max (Median)	Male (n=34) Mean±SD /Min-Max (Median)	p
UPDRS increase rate	17.54±6.64/8-31 (16)	14.62±7.06/4-34 (12.5)	0.047*
LED increase rate	167.88± 294.9/-700-1115 (175)	169.12± 168.56/-250-550 (150)	0.976
BMI increase rate	1.28±1.47/0-5.65 (0.82)	0.71±0.83/0-2.42 (0)	0.165
BDI	13.46±8.98/1-36 (14.5)	12.62±9.89/0-40 (10)	0.627
BAI	22.62±13.38/3-59 (21.5)	16.5±14.1 /1-56 (12)	0.028*
PSQI	13.38±3.9/4-21 (13)	10.29±4.14/2-17 (11)	0.007**
ESS	10.5±11.79/0-59 (7)	7.97±4/2-16 (8)	0.911
IES-R	35.08±12.27/12-58 (34.5)	32.94±15.1/4-58 (32.5)	0.591

Mann Whitney U Test , *p<0.05, **p<0.01, UPDRS: Unified Parkinson's Disease Rating Scale, LED: levodopa equivalent dose, BMI: body mass index, BDI: Beck Depression Inventory, BAI: Beck Anxiety Inventory, PSQI: Pittsburgh Sleep Quality Index, ESS: Epworth Sleepiness Scale, IES-R: Impact of Events Scale

Table 3b. Comparison of Quality of Life (PDQ-39) scales of male and female patients

	Female (n=26) Mean±SD /Min-Max (Median)	Male (n=34) Mean±SD /Min-Max (Median)	p
PDQ-39 total	64.77±29.56/4-124 (70)	43.59±28.37/6-124 (37.5)	0.004**
Mobility	21.69±11.02/0-44 (22.5)	11.82±11.51/0-44 (8.5)	0.001**
Activities of daily living	11±6.75/0-24 (11)	8.5±6.95/0-24 (6.5)	0.178
Emotional well-being	10.38±6.82/0-24 (9)	7.29±5.27/0-24 (6)	0.078
Stigma	3±3.25/0-11 (2)	2.32±3.01/0-12 (1)	0.293
Social support	2.96±2.92/0-12 (2.5)	2.5±2.3/0-8 (2)	0.649
Cognition	5.58±3.62/0-12 (5.5)	4.29±2.58/0-11 (4)	0.207
Communication	2.69±3.3/0-14 (2)	2.41±2.49/0-9 (2)	0.976
Bodily discomfort	7.19±3.63/0-12 (7)	4.56±3.58/0-12 (4)	0.007**

Mann Whitney U Test, *p<0.05, **p<0.01, PDQ-39: Parkinson's Disease Quality of Life Questionnaire

Table 4. Comparison of clinical data of patients with and without COVID-19

	COVID-19 (+) (n=17) Mean±SD/Min-Max (Median)	COVID-19 (-) (n=43) Mean±SD/Min-Max (Median)	p
Sex (f/m)	7/10	19/24	[a]0.533
Presence of comorbidities	13	27	[a]0.242
Smokers	3	6	[a]0.499
Dominancy (bradykinesia /tremor)	14/3	30/13	[a]0.256
Age	64.24±11.84/43-83 (65)	62.79±11.55/32-77 (67)	0.870
mHYRS	3±0.81/1.5-4 (3)	2.4±0.64/1-4 (2.5)	0.009**
Post-lockdown UPDRS	56.65±11.76/24-72 (58)	46.72±14.27/20-78 (46)	0.006**
Post-lockdown LED	803.24±350.57/150-1700 (800)	653.49±269.68/300-1300 (600)	0.075
Post-lockdown BMI	27.93±4.81/20.76-36.21 (26.81)	28.9±5.57/21.33-48.93 (27.82)	0.530
IES-R	34.35±14.51/8-58 (41)	33.67±13.78/4-58 (32)	0.812
PDQ-39	68.71±31.09/6-124 (66)	46.47±28.26/4-124 (42)	0.013*
Disease Duration	7.93±3.83/3-15 (7.5)	68±4.46/2-23 (6)	0.202

a Chi-square test, Mann Whitney U Test, *p<0.05, **p<0.01, UPDRS: Unified Parkinson's Disease Rating Scale, mHYRS: modified Hoehn-Yahr Rating Scale, LED: levodopa equivalent dose, BMI: body mass index, IES-R: Impact of Events Scale, PDQ-39: Parkinson's Disease Quality of Life Questionnaire

When comparing the habits of those who had COVID-19 during the pandemic with those who did not, no statistically significant changes were observed in terms of exercising, weight gain, worsening of clinical findings, disruption in treatment, reaching a doctor and using telemedicine (p=0.499, p=0.403, p=0.428, p=0.163, p=0.605, p=0.283; p>0.05). However, it was found that COVID-19-positive PD patients had significantly higher exposure to COVID-19-positive individuals compared to COVID-19-negative PD patients (p=0.001; p<0.01).

Upon examining **Table 5**, the multiple linear regression analysis conducted to assess the influence of independent variables on quality of life yielded statistically significant (F=13.064 p<0.05). A positive and highly statistically significant association was observed between independent variables and quality of life (R=0.866, p<0.05). The independent variables included in the model accounted for 75% of the total variance in quality of life (p<0.05). In the multivariate analysis of regression coefficients, it was found that COVID-19 status (β=-0.174, p<0.05) and gender (β=-0.197, p<0.05) had a negative impact on quality of life. Conversely, m HYRS (β=0.356, p<0.05), BDI (β=0.191, p<0.05), BAI (β=0.256, p<0.05), PSQI (β=0.189, p<0.05), and IES-R (β=0.281, p<0.05) had a positive and significant influence.

DISCUSSION

In this study, we investigated the clinical and neuropsychiatric effects of the COVID-19 lockdown on patients with PD. Our findings indicate that there was a deterioration in the clinical condition of individuals, as evidenced by worsening symptoms, weight gain, and increased daily LED doses during the post-lockdown period. Notably, clinical deterioration was more pronounced in female patients. Our observations revealed that the quality of life of the patients, particularly those who had COVID-19 infection and were female, experienced a more significant decline compared to male patients. Furthermore, we noted a significant increase in depression and anxiety levels among the patients following the lockdown. Additionally, their sleep quality deteriorated, and there was a progression in the disease stage. The overall impact of the COVID-19 pandemic situation further contributed to the deterioration of their quality of life.

The COVID-19 outbreak has had a significant unfavorable effect on individuals worldwide. To contain the spread of the virus, healthy individuals have been mandated to adhere to various social distancing rules, while those who have contact with the SARS-CoV-2 virus have been compelled to undergo isolation. Previous pandemics have demonstrated that a considerable number of people experienced ongoing psychological distress even after the pandemic had subsided. This highlights the crucial need for psychological support for susceptible individuals.[8-10] Moreover, the pandemic and associated lockdown measures may trigger or exacerbate neuropsychiatric symptoms such as anxiety, depression, and sleep disturbances among patients with PD. Given that physical activity has been shown to alleviate motor symptoms in PD, the sedentary nature of the lockdown measure is particularly unfortunate for this population and may exacerbate their motor symptoms.[8,9,11,12]

Likewise, in this study, we observed a parallel deterioration in motor symptoms, along with a notable increase in depression and anxiety levels, as well as a decline in sleep quality among our patients. Female patients have been found to be more pronounced to experience these deteriorations.

Cilia et al.[12] suggested that the exacerbation of motor symptoms could be attributed to either the degeneration of dopaminergic systems or changes in pharmacokinetics. They also found that patients required higher doses

Table 5. Results of multiple linear regression analysis for independent variables and Quality of Life (PDQ-39)

Independent variables	Univariable					Multivariable				
	B	S.Error	Standard (B)	t	p	B	S. Error	Standard (B)	t	p
COVID Status	-22.241	8.327	-0.331	-2.671	0.001**	-11.725	5.472	-0.174	-2.143	0.037*
Sex	-21.181	7.527	-0.347	-2.814	0.001**	-12.035	4.941	-0.197	-2.436	0.019*
Exercise Status	26.102	8.313	0.381	3.14	0.001**	2.068	6.09	0.03	0.34	0.736
Clinical worsening	-18.06	8.5	-0.269	-2.125	0.001**	-3.989	5.287	-0.059	-0.754	0.454
UPDRS	1.105	0.386	0.352	2.864	0.001**	-0.56	0.381	-0.178	-1.47	0.148
mHYRS	18.147	4.874	0.439	3.723	0.001**	14.707	4.913	0.356	2.993	0.004**
BDI	1.846	0.349	0.57	5.287	0.001**	0.617	0.294	0.191	2.096	0.041*
BAI	1.335	0.225	0.615	5.933	0.001**	0.555	0.204	0.256	2.722	0.009**
PSQI	3.531	0.812	0.496	4.352	0.001**	1.346	0.66	0.189	2.041	0.047*
ESS	1.655	0.429	0.452	3.854	0.001**	0.238	0.315	0.065	0.757	0.453
IES-R	0.891	0.264	0.405	3.371	0.001**	0.619	0.174	0.281	3.553	0.001*

UPDRS: Unified Parkinson's Disease Rating Scale, mHYRS: modified Hoehn-Yahr Rating Scale, BDI: Beck Depression Inventory, BAI:Beck Anxiety Inventory, PSQI: Pittsburgh Sleep Quality Index, ESS: Epworth Sleepiness Scale, IES-R: Impact of Events Scale

of dopaminergic therapy.[13] Similarly, Suzuki et al.[11] demonstrated a correlation between disease severity, disease duration, and the decline in the quality of life. The BMI revealed an increase, suggesting that a sedentary lifestyle has resulted in weight gain. However, it is important to note that this consequence may not be specific to PD.[14] Consistent with the aforementioned studies, we observed an increase in patients' UPDRS part III and PDQ-39 scores, m HYRS stages, and BMI among patients following the lockdown.

Although PD is more commonly diagnosed in men than in women, it is noteworthy that women tend to experience more accelerated disease progression and higher mortality rates.[15,16] The manifestation and diagnosis of PD predominantly rely on motor symptoms, which exhibit remarkable differences between men and women in terms of clinical progression and treatment outcomes.[17]

Regarding the impact on quality of life, studies have found that female gender has a detrimental effect on physical functioning and socio-emotional well-being. On the other hand, males have reported a more pronounced impact on the cognitive domain.[18] Consistent with these findings, our study demonstrated that female patients experienced a greater increase in UPDRS-motor scores, along with higher levels of depression, sleep disorders, and a decline in overall quality of life compared to males.

PD patients require regular outpatient visits for follow-up examinations and prescription management. However, due to the lockdown, these visits have been insufficient. Consequently, the patients had to change their daily routines and habits, leading to weight gain and worsening clinical symptoms. There were also disruptions in their treatment regimens, making it challenging for them to communicate effectively with their neurologists. In an attempt to overcome these obstacles, many patients resorted to utilizing telemedicine services.[12]

When examining the habits of PD patients with and without COVID-19 infection during the lockdown, we did not observe any remarkable difference. However, it is important to note that these changes in habits had an impact on their quality of life. The only notable difference between the patients was that PD patients who had been in contact with confirmed COVID-19 individuals were more susceptible to contracting the infection compared to COVID-19-negative PD patients. Our findings revealed a significant deterioration in the clinical stages of the disease, quality of life, and an increase in UPDRS scores following the lockdown among patients who had contracted COVID-19 compared to those who had not.

CONCLUSION

This paper sheds light on the undesirable outcomes of the COVID-19 outbreak on the quality of life of PD patients. The findings of this investigation have important implications for understanding and potentially managing the changes in clinical outcomes among these patients. We strongly believe that these results underscore the significance of multidisciplinary approaches for PD patients, enabling them to learn and employ self-management strategies effectively.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the University of Health Sciences, Fatih Sultan Mehmet Research and Training Hospital Ethics Committee (Date: 11.03.2021, Decision No: 2021/29).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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YouTube™ as an information source for speech and language disorders

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ABSTRACT

Aims: The purpose of this study is to evaluate the quality of the information provided by the top 100 videos when searching for "speech and language disorders" on YouTube™.

Methods: The results of the YouTube™ search were examined using the keyword “speech and language disorders”. The search was limited to the first 100 videos. Modified DISCERN, Global Quality Score (GQS), Video Information and Quality Index (VIQI), and the Journal of American Medical Association (JAMA) criteria were used for the evaluation of the videos. The Kruskal-Wallis H Test, Mann-Whitney U, and Spearman's RHO correlation were used for statistical analyses. The significance level was taken as $p<0.05$.

Results: A total of 83 videos were taken for evaluation and 38 (41%) of these videos were uploaded by Hospital/University staff, 23 (27.7%) of them were from Others, 15 (18.1%) of them were from Specialists and 11 (13.3%) of them were uploaded by laypersons. The average DISCERN, VIQI, and GQS scores were 3.1 and the JAMA score was 2.0.

Conclusion: We think that it is important for experts and academic institutions to upload high-quality, accurate, and precise videos that meet the expectations of society and include the experiences of the patients and their relatives.

Keywords: Language disorders, speech disorders, internet, information source

INTRODUCTION

When language and speech disorders are mentioned, various impairments for communication, language, and speech disorders of people come to mind. Among these, language-related ones are defined as developmental language and speech disorders, acquired language-speech disorders, and language disorders occurring because of any syndrome or disease. Speech disorders, on the other hand, are speech sound disorders associated with the acquisition and use of speech sounds of a certain language (articulation disorder-phonological disorder-childhood speech disorders). They include motor speech disorders (dysarthria-apraxia) related to the inability to use the motor muscle components of speech. In addition, stuttering and cluttering, which are related to the fluency of speech, are included in this classification, which also includes voice disorders.[1]

Recent studies show that 8 out of 10 users use the Internet to access health information online.[2,3] A recent study showed that more than 60% of adults use YouTube™ to search for health information online.[4] For those who have primary caregiver roles, there may be uncertainty about what the problem is and what can be done as an intervention.[5] For these reasons, parents prefer social media for answers about the health status of their children.[6] There has been a significant increase in the number of parents seeking online information and support regarding children's health, development, or disorder in the digital age we live in.[7]

It was reported in a study conducted by using the descriptive analysis of videos about speech disorders in recent years that the average frequency of watching these videos was 1 million 606 thousand.[8] In terms of the number of views, YouTube™ videos show that it is a frequently used area in terms of speech disorders, although it is not clear whether these are caregivers or not. The number of views of the videos and by whom they are uploaded is among the basic criteria for evaluating the quality.[9] Kollia et al.[10] conducted a study on autism spectrum disorder and reported that the most viewed videos were uploaded and provided by non-professionals (e.g., personal videos and television program videos). However, experts have doubts about the reliability of such videos in terms of answering people's questions.

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According to a study that evaluated internet searches of individuals with stuttering in 2022, it was found that people who did not start treatment were more likely to search for online resources associated with stuttering than those who received treatment.[11] This finding shows that people who are relatively less knowledgeable about the subject prefer videos as an information source. In this context, it is very important to determine the quality of these videos.

The quality of health-related videos uploaded to YouTube™ needs to be critically evaluated because of their wide use in professional education and because patients frequently turn to it for information. The purpose of the study was to evaluate the quality of videos uploaded to YouTube™ about language and speech disorders.

METHODS

Ethics committee approval was not required for the study because there was no human or animal involvement and the YouTube™ videos reviewed were public.

A video search was conducted on the online video-sharing website YouTube™ (www.youtube.com) on April 12, 2023. Browser history was deleted, cookies were cleared, and a new YouTube account was opened in the Google Chrome Browser to minimize user-targeted search results. The search was made by using the keywords "speech disorders", "language disorders", and "speech and language disorders". A total of 79% of YouTube™ users look at other pages when they cannot find what they are looking for on the first page, and more than 90% of search results are only on the first 3 pages.[12] Considering these, the first 100 search results were included in the present study. The access links of the detected videos were exported to an Excel file because the search results may change on different days. Videos that were not in English, silent videos with low video quality, closed likes and comments, and inconsistent titles and content were excluded from the study. The first 83 videos were included in the study after the videos were removed in line with the exclusion criteria.

The evaluation of the videos was performed by two Speech and Language Therapists who had master's and Ph.D. degrees specialized in speech and language therapy to avoid any possible bias. Quantitative data of videos were evaluated according to the duration of the video, the number of views, number of likes and dislikes, the number of comments, and elapsed time since uploading, and the videos were categorized as hospital/university, specialist, layperson, and others according to their upload sources.

Modified Discern, Global Quality Score (GQS), Video Information and Quality Index (VIQI), and Journal of American Medical Association (JAMA) were used to evaluate the quality of the videos.

The modified DISCERN score was used to specifically evaluate the clarity, reliability, bias, reference suffix, and areas of uncertainty for information in YouTube™ videos (Table 1). Each of the items was scored as 1 for Yes and 0 for No.[13]

Table 1. Modified DISCERN criteria

1. Are the aims clear and achieved?
2. Are reliable sources of information used? (i.e., publication cited, speaker is board-certified vascular surgeon)
3. Is the information presented balanced and unbiased?
4. Are additional sources of information listed for patient reference?
5. Are areas of uncertainty mentioned?

The Global Quality Score (GQS) takes into account the flow of information in online videos along with ease of use and video quality (Table 2). The evaluation is made with a 5-point Likert scale, with 1 point indicating the lowest quality and 5 indicating excellent quality.[14]

Table 2. The Global Quality Score (GQS) criteria

1. Poor quality, very unlikely to be of any use to patients
2. Poor quality but some information present, of very limited use to patients
3. Suboptimal flow, some information is covered but important topics missing, somewhat useful to patients
4. Good quality and flow, most important topics covered, useful to patients
5. Excellent quality and flow, highly useful to patients

The information accuracy, information flow, quality, and precision of the videos were evaluated with the Video Information and Quality Index (VIQI) Scale. When the videos were evaluated with VIQI, a 5-point Likert-style scale was used, with 1 point indicating the lowest quality and 5 indicating excellent quality.

The Journal of American Medical Association (JAMA) system evaluates the reliability of health-related online resources based on 4 criteria (authorship, citation, explanation, and timeliness). JAMA Criteria are given in Table 3. Each criterion is graded between 0 and 1, and a maximum of 4 points that can be obtained means the highest quality.[15]

Table 3: Journal of the American Medical Association (JAMA) criteria

Authorship: Authors and contributors, their affiliations, and relevant credentials should be provided
Attribution: References and sources for all content should be listed clearly, and all relevant copyright information should be noted
Disclosure: Website "ownership" should be prominently and fully disclosed, as should any sponsorship, advertising, underwriting, commercial funding arrangements or support, or potential conflicts of interest
Currency: Dates, when content was posted and updated, should be indicated

Statistical Method

Statistical analyses were performed using the IBM SPSS 21.0 software version 21. The variables were investigated using Kolmogorov-Smirnov/Shapiro-Wilk's test to determine whether or not they are normally distributed. As all the parameters were not normally distributed the Kruskal-Wallis test was performed to compare those variables among the video source. The Mann-Whitney U test was performed to calculate the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. While investigating the associations between the variables, the Spearman test was used to calculate the correlation coefficient and significance.%5 Type-I error was accepted statistical significance.

RESULTS

A total of 83 consecutive videos were analyzed within the scope of the study. Thirty-four (41%) of these videos were uploaded by Hospital/University staff, 23 (27.7%) of them were from Others, 15 (18.1%) of them were from Specialist and 11 (13.3%) of them were uploaded by Layperson (Figure 1).

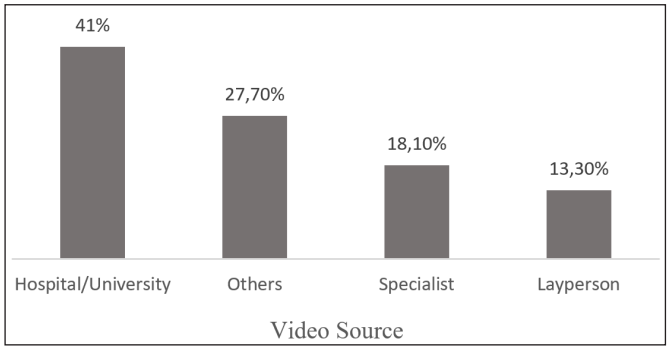

Figure 1.

When the contents of the 83 videos evaluated were examined, it was found that the videos were on fluency disorders (n=20), speech sound disorders (n=19), developmental language disorders (n=35), neurogenic-acquired language disorders (n=15), motor speech disorders (n= 7), voice disorders (n=4), language disorders secondary to autism spectrum disorder (n=12), delayed speech (n=9), and language disorders (n=12).

When all videos are evaluated together, the mean number of days since the upload of videos was 1635.6±1034.9 days, the mean total video duration was 587.6±717.3 seconds, the mean total view count was 24152.5±58899.7, the mean Likes number was 234.4±393.5, the mean Dislikes number was 7.6±22.5, the mean total number of comments was 15.5±34.9, the mean total DISCERN score was 3.1±1.2, the mean total VIQI score was 3.1±1.0, the mean GQS score was 3.1±1.0 and the mean total JAMA score was 2.0±0.9. The features of the videos were shown in Table 4.

Table 4. The features of the videos

Variable	Mean±SD
Number of days since upload (day)	1635.6±1034.9
Total video duration (seconds)	587±717.3
Total view count	24152.5±58899.7
Likes	234.4±393.5
Dislikes	7.6±22.5
Number of comments	15.5±34.9
DISCERN score	3.1±1.2
VIQI score	3.1±1.0
GQS score	3.1±1.0
JAMA score	2.0±0.9

When the videos were compared to their source, the mean total video duration was found to be statistically significantly shorter in the Hospital/University group compared to the Specialist and Layperson groups (491.5±810.2 vs 595.4±451.8 and 491.5±810.2 vs 703.0±480.0; p=0.007, p=0.005, respectively).

No statistically significant difference was detected between the groups in other features of the videos. Table 5 summarizes the comparison of the features of videos according to their source.

Table 5. The comparison of features of the videos according to their sources

Mean	Hospital/University (n=34)	Others (n=23)	Specialist (n=15)	Layperson (n=11)	P Value
Days since upload (day)	1856.4±1070.5	1634.1±1185.5	1404.6±805.8	1271.0±779.8	P=0.285
Total video duration (seconds)	491.5±810.2	669.5±818.9	595.4±451.8	703.0±480.0	P=0.007
Total view count	37622.3±87902.4	17814.9±23289.9	9855.2±15824.8	15266.1±17377.5	P=0.283
Likes	248.9±484.8	242.9±327.8	197.8±377.6	222.3±234.1	P=0.674
Dislikes	12.0±33.8	5.2±9.6	3.5±4.9	4.7±4.3	P=0.691
Comments	11.4±33.9	16.0±26.8	11.1±16.8	33.6±61.4	P=0.171
DISCERN score	3.1±1.2	2.9±1.2	3.4±1.1	3.5±1.1	P=0.452
VIQI score	2.9±1.1	3.0±0.9	3.7±0.9	3.3±0.7	P=0.124
GQS score	2.9±1.1	3.0±0.9	3.7±0.9	3.3±0.7	P=0.141
JAMA score	2.1±1.0	1.7±0.8	2.2±0.9	2.0±0.1	P=0.343

*Spearman correlation analysis

*Kruskal Wallis test, after Bonferroni correction, only Hospital/University-Specialist and Hospital/University-Layperson were found statistically significant with p values listed above. P values for pairwise comparison are as follows; Hospital/University-Others p=0.120, Others-Layperson p=0.274, Others vs Specialist p=0.344, Specialist vs Layperson p=0.281.

Correlation analysis revealed a positive and statistically significant correlation between the mean total video duration and VIQI and GQS scores (r=0.252, p=0.022; r=0.236, p=0.032, respectively). No statistically significant correlation was found with other video parameters. Table 6 shows the Spearman correlation analysis and coefficient values.

Table 6. The correlation coefficients between the video features and DISCERN, JAMA, VIQI and JAMA scores

Mean	DISCERN	VIQI	GQS	JAMA
Number of days since upload (day)	r=-0.098 P=0.378	r=-0.145 P=0.190	r=-0.131 P=0.238	r=-0.121 P=0.275
Total video duration (seconds)	r=0.053 P=0.631	r=0.252* P=0.022*	r=0.236* P=0.032*	r=0.080 P=0.470
Total view count	r=0.185 P=0.093	r=0.098 P=0.378	r=0.114 P=0.305	r=-0.005 P=0.965
Likes	r=0.156 P=0.159	r=0.123 P=0.267	r=0.130 P=0.240	r=-0.032 P=0.775
Dislikes	r=0.170 P=0.124	r=0.099 P=0.372	r=0.106 P=0.338	r=-0.033 P=0.766
Number of comments	r=0.054 P=0.626	r=0.033 P=0.766	r=0.037 P=0.737	r=-0.101 P=0.363
Video source	r=0.069 P=0.535	r=0.209 P=0.058	r=0.197 P=0.074	r=-0.037 P=0.742

*Spearman correlation analysis

DISCUSSION

Especially with the pandemic process, platforms such as YouTube™ have become a source of medical information that could reach large audiences free of charge.[16] It is very important to evaluate the credibility of sources because these platforms have high-quality videos as well as low-quality ones that might cause misleading information.

It was found in the present study that most of the videos (41%) uploaded about language and speech disorders were uploaded by professionals who were working in hospitals and universities and (18.1%) specialists. It was also found that less part of the videos was uploaded by non-professional (13.3%) laypersons and (27.7%) others. However, when the number of views of the videos was examined, no significant differences were detected between the groups. When the literature was reviewed, there are also studies reporting that the majority of those who watched health-related videos on the internet were not interested in the source of the videos.[17] The present study also supported this and showed that users did not consider uploader differences when choosing videos to watch.

Literature showed that more parents and other family members sought information online about the relevant speech and language disorder.[18] In the present study, it was seen that the videos uploaded to YouTube™, in line with the literature, mostly focused on language and speech disorders in childhood.

When the literature was reviewed, it was seen that the prevalence of delayed speech was 2.53%,[19] the prevalence of articulation disorder was 2.1%, stuttering was 2.1%, and voice disorder was 1.2%,[20] In the present study, in which the videos published on YouTube were evaluated, it was found that the number of videos about fluency disorders (n=20) and speech sound disorders (n=19), which had a high prevalence, was high, and there was little content about delayed speech even though the prevalence was high (n=9).

The scales recommended to be used for the evaluation of written scientific material such as JAMA and DISCERN were used in previous studies conducted on videos on the Internet, and it is recommended to develop appropriate methodology and scales for the evaluation of visual publications such as videos.[18] For this reason, the researchers used the Video Power Index (VPI) value, the Global Quality Scale (GQS), and the modified DISCERN scale in the present study, where the quality of videos on language and speech disorders on the digital content platform YouTube™ was evaluated.

Total Modified DISCERN scores were obtained as a mean of 3.1±1.2, which result shows that the quality of the videos is at moderate levels. It was seen that 17% (n=14) of the videos were of high quality. Total Global Quality Scale (GQS) mean was 3.1±1.0, the total Video Power Index (VPI) mean was 3.1±1.0, and the total JAMA score mean was 2.0±0.9. Similar to the modified DISCERN scores, the GQS, VPI, and JAMA scores indicated moderate levels of quality of the videos. When the evaluation was made according to the loaders, the differences between the groups were not significant. There is no consensus in the literature on which of these scales is more precise. However, in some previous studies[21,22] a more objective evaluation was aimed by using the two scales together. Obtaining similar results from the scales that were used in our study shows that the reliability of the assessment is high.

There are few studies evaluating Language and Speech Disorders videos uploaded to YouTube™. Akram et al.[18] examined the understandability and actionability of uploaded videos about language and speech disorders. As a result of their study, they reported that YouTube™ videos have low scores of understandability and action-ability. Similarly, in the present study, the quality of the uploaded videos was found to be at moderate levels.

In their study in which videos on YouTube™ about children with speech and/or language disorders were evaluated according to the video uploader of understandability and actionability, Bellon-Harn et al.[23] eported that videos uploaded by professionals were found to be superior to other uploading sources in terms of understandability, but no difference was detected in terms of action-ability among video sources. Similarly, according to the correlation analysis in our study, no significant difference was detected between the video quality scales according to the video uploader.

There were two main limitations in the study. This cross-sectional study includes Youtube™ searches on the specified date, and the data obtained is based on a specific search result. Another limitation was that language and speech disorders have a very wide spectrum. The evaluation of each sub-title in future studies will provide valuable contributions to the literature.

CONCLUSION

YouTube™ is increasingly accessed by patients and their families for information on the diagnosis, understanding, and treatment of language and speech disorders. However, the present study shows that the quality of the videos on these topics is not sufficient. We think that it is important to upload quality, accurate and precise videos that meet the expectations of society, especially the experiences of patients and their relatives, by experts and academic institutions.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval was not obtained as there was no human or animal participation in the study, and the videos were public. The study, which follows the World Medical Association Declaration of Helsinki, did not utilize any patient data or materials. All videos used in the study can be found on a public social media site (YouTube™).

Informed Consent: There was no human or animal participation in the study and the videos reviewed on YouTube™ were open to everyone. Therefore, it was not necessary to obtain informed consent.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of shaping performance and surface changes of two different minimally invasive shaping file systems used in resin blocks

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ABSTRACT

Aims: This study aimed to compare the shaping ability of T-endo MIS and TruNatomy (TRN) file systems in J-shaped resin root canals and to investigate the deformation of the files after two acrylic block preparation and two sterilization cycles.

Methods: Forty acrylic blocks were numbered, then four file groups (n=10) were established: T-endo MIS glider (15.04) + T-endo MIS Finisher (25.04) (first and second usage) and TRN Glider (17.02) + TRN Prime (26.04) (first and second usage). Preoperative and postoperative images after the root canal preparation of simulated acrylic blocks were taken for each sample, and superimposed. The amount of resin removed from both the inner and outer walls of the canal to the level of 3, 5, and 7 mm from the apical point was measured, and the amount of transportation was calculated. Additionally, the deformation of the files was examined under a scanning electron microscope before and after usage and sterilization process.

Results: Tip deformation of TRN files increased with the number of uses (p=0.007). With the second use, the surface deformation of the MIS files increased (p=0.015). There was no difference in cutting-edge deformation in either file type according to the number of uses (p>0.05). There was no difference in transportation values between the MIS and TRN file systems at any level (p>0.05). The file systems did not show a significant difference in the amount of transportation between first and second use (p>0.05).

Conclusion: When TRN (26.04) and MIS (25.04) NiTi rotary files were used twice on acrylic blocks, the amount of deformation did not differ. Both file types were similar in terms of transportation values.

Keywords: Acrylic block, canal transportation, file deformation, minimally invasive shaping

INTRODUCTION

Root canal instruments are commonly reused in clinical conditions to achieve economic benefits. However, the repeated usage of these instruments, coupled with increased mechanical stresses encountered within the root canals, as well as exposure to irrigation solutions, sterilization, and disinfection procedures, can lead to file deformation.[1] An increase in the number of autoclave sterilizations increases the surface roughness of the file and thus increasing the likelihood of file separation.[2] The increased working time of the instrument inside the root canal due to multiple uses may increase the risk of instrument fracture.[3] The precursors of the start of this process are the tip, cutting edge, and surface deformations that occur in the file.[4] The presence of a broken file in the root canal can impede access to the apical portion of the canal, posing challenges for effective treatment.[5]

Minimally invasive endodontics (MIE) aims to preserve as much tooth structure as possible and apply MIE principles to all root canal treatments. These principles encompass various objectives, ranging from the preparation of a smaller access cavity to the use of instruments with smaller tapers and tip diameters, and the utilization of instruments with different geometric designs and metallurgical properties.[6-8] Endodontic instruments conforming to this concept are designed to exhibit less taper, greater flexibility, and improved cyclic fatigue resistance than conventional instruments.[9]

Earlier research indicated that conducting minimally invasive root canal preparation with NiTi files having a smaller taper might result in the preservation of root dentin tissue to a greater extent than larger-tapered NiTi

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files.[10] Therefore, the introducing new rotary files to the dental market, which adheres to the principles of MIE and possesses the capability to shape root canals, has increased interest in conservative preparation.[11] One of these files is TruNatomy (TRN; Dentsply Sirona, Maillefer, Ballaigues, Switzerland), produced from a thin NiTi wire with a diameter of 0.8 mm and a unique off-centered square cross-sectional design. TRN is a multiple file system consisting of an orifice modifier (20.08), a glider (17.02), Small (20.04), Prime (26.04,) and Medium (36.03) shaping files.[11] Studies conducted on TRN have demonstrated its ability to preserve root canal dentin and respect the natural anatomy of the canal during instrumentation.[12] Another file introduced for conservative shaping is T-endo MIS multiple file system (Dentac, İstanbul, Türkiye), a rectangular-sectioned file with a high martensitic crystal content, providing it with the feature of memory control.[13] This system consists of four files and they are as follows: Orificer (25.09), Glider (15.04), Shaper (20.05), and Finisher (25.04) files. The taper of the main shaping file of both TRN and MIS is 4%.[13,14] Both file system movement kinematics are rotation.[11,13]

During the shaping of curved root canals, it is necessary to preserve the original canal curvature and to prevent the tendency to flatten in the apical region, which may disrupt the canal integrity in the inner part of the root curvature.[15] Evaluation of the performance of endodontic files is often associated with their ability to shape curved root canals and preserve the original anatomy.[16,17] However, our knowledge about the tendency to preserve or disrupt this anatomy as a result of multiple uses of files is limited. Contrary to a study stating that the repeated use of files increases the transportation rate,[3] there is also a study in which no significant difference in transportation was observed between the first and sixth use of files.[18]

To our knowledge, no study has yet compared the T-endo MIS file system with TRN regarding deformation and transportation. Therefore, this study aimed to examine the deformation, before use in J-shaped resin canals, after first and second usage, and post-sterilization, of T-endo MIS and TRN, which are newly developed heat-treated NiTi rotary file systems. Moreover, evaluation of transportation occurring at 3, 5, and 7 mm in acrylic blocks after the first and second usage was intended. The null hypotheses of this study can be listed as follows:

$H0_1$: There is no difference within and between the file systems in terms of the amount of transportation that occurs in the acrylic blocks after the first and second use.

$H0_2$: There is no difference within and between the file systems in terms of the deformation that occurs in the files after the first and second use.

METHODS

A selection of 40 J-shaped resin canals (Dentsply Maillefer, OK, USA) with a taper of 2% and a single-direction curvature of 40 degrees, measuring 17 mm in length, was chosen. The resin block images were captured using a Canon EOS 60D digital camera equipped with a Sigma 105 mm 1:2.8 DG macro-lens (Sigma Corp., Fukushima, Japan) under standardized conditions before canal preparation. The images were saved as JPEG files for further analysis and documentation. Then, all blocks were concealed with black adhesive tape, masking the root canals, and randomly divided into four groups. The working length was determined using a #10K type file. A single endodontist performed all procedures. Irrigation was conducted using a 30-G IrriFlex needle (Produits Dentaires SA, Switzerland) with 20 mL of distilled water. The same endo motor (VDW Gold, VDW, Munich, Germany) was used for all instruments.

Group 1 (n=10): For the resin canal preparation, the TRN Glider (17.02) and Prime (26.04) files were used for the first time at 500 rpm and 1.5 Newton-centimeter (N-cm) torque. Then the files were then sterilized for the first time.

Group 2 (n=10): For the resin canal preparation, the TRN Glider (17.02) and Prime (26.04) files were employed for the second time at the same rpm and torque values. Then the files were then sterilized for the second time.

Group 3 (n=10): For the resin canal preparation, the MIS Glider (15.04) and Finisher (25.04) files were used for the first time at 300 rpm and 2.5 N/cm torque. Then the files were then sterilized for the first time.

Group 4 (n=10): For the resin canal preparation, the MIS Glider (15.04) and Finisher (25.04) files were employed for the second time at the same rpm and torque values. Then the files were then sterilized for the second time.

Since both file systems underwent sterilization during packaging, no additional sterilization was performed before their use. Following the first and second use, all files were cleaned using a sponge and immersed in an ultrasonic bath (Ege Eagle Ultrasonic, Bornova, İzmir, Türkiye) for 15 minutes. After drying, each file was individually sealed in a sterilization package and autoclaved (Getinge Quadro Avanti, Getinge, Sweden) at 134°C under 30 psi for 20 minutes.

Following preparation, the black tape on the blocks were removed. Images of the prepared acrylic block were taken and recorded after the first and second usage of the file.

Scanning Electron Microscopy (SEM) Analysis and Assessment of Deformation

All deformation evaluations were performed on the main shaping files. The first SEM examination was conducted before using the files, right after they were removed from their packaging. After the first and second usage, once sterilized, the files were placed into the SEM apparatus without any contact with the surfaces to be examined. Examination of file deformation was carried out using SEM (FEI, Quanta 250 FEG, Eindhoven, Netherlands) before use, after the first and second use, and after the sterilization process. To ensure consistent examination of the file surfaces, a holder was used to position the files in a standardized manner for each examination. High-resolution SEM microphotographs were captured for each instrument and its 5 mm tip, magnified at 1500× and 5000×. In this study, the physical and visual changes in MIS and TRN files caused by mechanical stress and sterilization processes during canal preparation were evaluated according to the following parameters (Figure 1):[4]

Tip deformation: Flattening and distortion at the tip of the files,

Cutting edge deformation: Notching, waviness, and/or deterioration of the original helical structure on the cutting edges of the files,

Surface deformation: Micro-level surface separations.

The images were then evaluated based on criteria, by two endodontists who were not involved in the preparation process. Deformation parameters were scored as either present or absent.

Image Analysis and Assessment of Transportation

The Adobe Photoshop CS6 Extended program (Adobe Systems Inc., San Jose, CA, USA) was utilized to overlay the pre- and post-preparation images of the blocks (Figure 2). Three measurement zones were identified at the apical foramen levels of 3, 5, and 7 mm, and the images were analyzed using AutoCAD 2021 (Autodesk Inc., San Jose, CA, USA). After the superposition, the amount of resin removed from each canal's inner and outer walls at the 3, 5, and 7 mm levels was measured (Figure 3). Positive values indicate that transportation occurs at the inner surface of the canal curvature, and negative values indicate that transportation occurs mainly at the outer surface of the canal curvature.18 A difference of 0 indicated no transportation on the inner and outer wall.

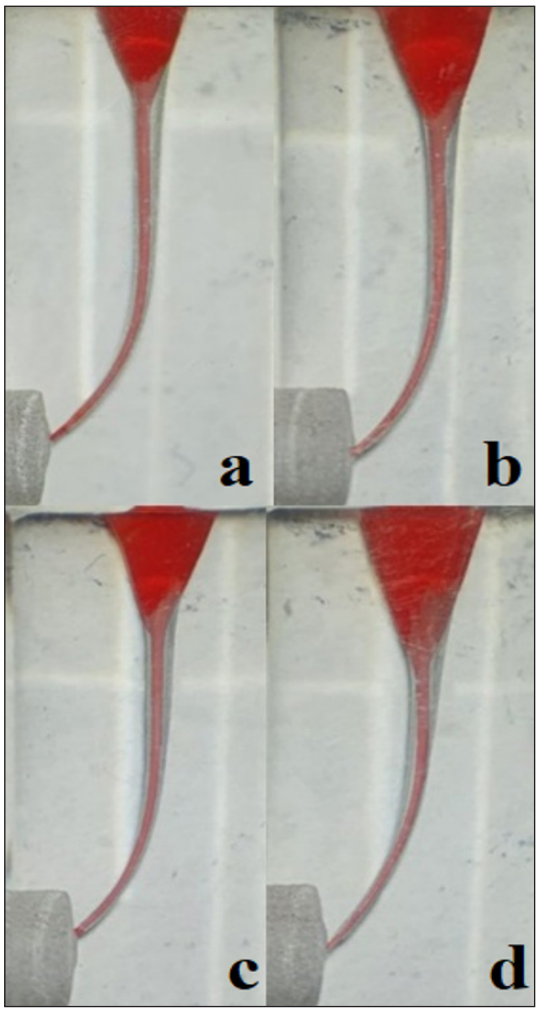

Figure 2. Superimposition of pre- and post-preparation block images a, MIS – first usage; b, MIS – second usage; c, TRN – first usage; d, TRN – second usage.

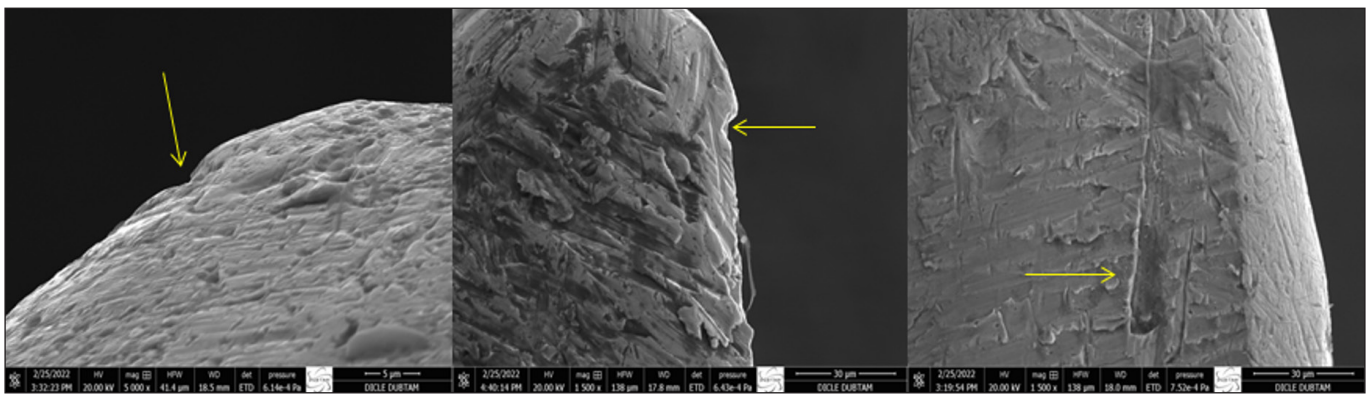

Figure 1. Tip, cutting edge, and surface deformation in file systems

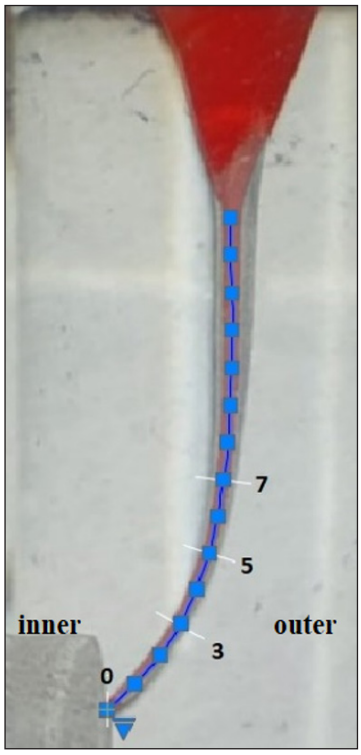

Figure 3. Identifying measurement points in the superimposed image

Statistical Analysis of Deformation

The relationship between categorical variables according to groups was examined using Fisher's exact test. Cochran's Q test was used to compare the deformation states within each file. Kappa tests were used to investigate the agreement between observers. Analysis results were presented as frequencies (Tables 1, 2, and 3). The significance level was taken as $p<0.05$.

Statistical Analysis of Transportation

The mean, standard deviation (SD), median, minimum (min), and maximum (max) values were calculated for the two file systems (Table 4). Conformity to normal distribution was evaluated using the Shapiro–Wilk test. Data were analyzed by one-way ANOVA, independent samples T, Kruskal–Wallis H, and Mann–Whitney U tests using SPSS 21.0 Software (IBM Corp, Armonk, NY, USA). The alpha-type error was set at 0.05.

Table 1. Comparison of tip deformation as a result of use within and between files

	Before usage	First usage	Second usage
TRN	0^{b}/10	0^{b}/10	5^{a}/10
MIS	0/10	0/10	2/10

Cohran's Q, Fisher's Exact test, a, b: The same letter indicates no difference between uses for each file.

Table 2. Comparison of cutting edge deformation as a result of use within and between files

	Before usage	First usage	Second usage
TRN	0/10	0/10	1/10
MIS	0/10	1/10	3/10

Cohran's Q, Fisher's Exact test

Table 3. Comparison of surface deformation as a result of use within and between files

	Before usage	First usage	Second usage
TRN	0/10	0/10	1/10
MIS	0^{a}/10	1^{ab}/10	5^{b}/10

Cohran's Q, Fisher's Exact test, a, b: The same letter indicates no difference between uses for each file.

RESULTS

Deformation

An excellent level of agreement between observers was obtained in all deformation assessments ($p<0.05$).

Tip deformation

In the TRN file, tip deformation increased significantly after the second use ($p<0.05$), and no difference was observed for the MIS file between the first and second uses ($p>0.05$) (Table 1).

Cutting-edge deformation

The file systems did not show a significant difference in cutting-edge deformation relative to each other and according to the number of uses ($p>0.05$) (Table 2).

Surface deformation

While the surface deformation of the MIS file increased significantly compared to before use ($p<0.05$), there was no significant difference in the TRN file ($p>0.05$) (Table 3).

Table 4. Evaluation of the transportation created by the files according to the number of uses and the level

Level (mm)	Transportation (mm)			
	Usage 1		Usage 2	
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)
MIS				
3	0.049±0.036	0.042 (0.014-0.139)^{xy}	0.160±0.284	0.029 (0.017-0.703)
5	0.024±0.015	0.025 (0.006-0.055)^{x}	0.111±0.251	0.031 (0.011-0.823)
7	0.101±0.053	0.121 (0.010-0.171)^{y}	0.102±0.053	0.105 (0.018-0.201)
TRN				
3	0.027±0.012^{a}	0.028 (0.002-0.049)	0.027±0.02^{a}	0.021 (0.001-0.066)
5	0.013±0.010^{b}	0.010 (0.001-0.032)	0.026±0.02^{a}	0.020 (0.003-0.069)
7	0.101±0.048^{c}	0.109 (0.033-0.162)	0.096±0.036^{b}	0.105 (0.044-0.154)

One way ANOVA, Independent Samples T, Kruskal Wallis H and Mann Whitney U test, a,b; x,y: Expresses differences within the same column.

Transportation

There was no difference in transportation values between the MIS and TRN file systems at any level (p>0.05). The file systems did not show a significant difference in the amount of transportation between the first and second use (p>0.05). For the MIS file, there was significantly more transportation at 7 mm than at the 5 mm level (p<0.05). For the TRN file, there was more transportation at 7 mm than at 3 and 5 mm levels (p<0.05).

DISCUSSION

Thanks to NiTi file technology, which continues to improve and progress from past to present, manufacturers aim to increase the fracture resistance and clinical performance of root canal instruments. Current NiTi files with increased flexibility and cyclic fatigue resistance compared to traditional ones make root canal preparation even easier.[19,20] With this technology (i.e., m-wire, blue-wire, gold-wire, t-wire, cm-wire, r-phase), clinicians use the files more than once because they find them more reliable against fracture and also to reduce the cost of treatment. However, it has been shown that cutting efficiency and flexibility decrease due to repeated use and sterilization cycles.[21] All these changes may cause an increase in the amount of transportation in the root canals.[22]

The fact that the TRN file system creates less transportation in the canal compared to many other files shows that this file system is suitable for use within the framework of MIE principles.[23,24] There is no study in the literature that measures the amount of transportation caused by this file when used multiple times. In addition, very few studies compare the TRN file with other files with similar tip diameters and tapers.[25]

In this study, the transportation that occurs at three levels in acrylic simulated canals as a result of the repeated usage and sterilization of two different minimally invasive file systems (TRN and MIS) was investigated in two dimensions. In addition, the deformation of the files as a result of use was evaluated by using SEM. According to the results of this study, the files did not show a significant difference in transportation between first and second use, within themselves and relative to each other. Therefore, the first H0 hypothesis was accepted.

In a study comparing the shaping ability of TRN (26.04), OneCurve (25.06), and Jizai (25.04) file systems, the Jizai file, which has the same tip diameter and taper with the MIS file we used in our study was not different from TRN in terms of transportation.[25] Kumar et al.[26] showed that TRN (26.04), Protaper Gold (25.08), and HyFlex EDM (25.08) files cause similar apical transportation in extracted teeth. In other words, although the apical diameter of the TRN file is larger than that of these files, there was no significant difference between them. The MIS Finisher (25.04) file we used in our study showed similar results to those for TRN regarding apical transportation, although it has a smaller apical diameter. The manufacturer states that TRN files' design and heat treatment give them more flexibility when shaping of root canal walls, resulting in efficient shaping.[14] Our result supports the manufacturer's claim.

It has been shown that multiple use of conventional NiTi files increases transportation in the apical region of resin canals.[3] On the other hand, increasing the use of another conventional NiTi file did not make a significant difference in transportation in resin blocks.[18] According to the results of our study, the first and second uses do not create a significant difference in the amount of transportation at the three levels in either file system. However, when both previous literature and our study are included, the microhardness and degree of curvature of the resin blocks, the number of uses, and the variety of files used may have affected all these results.

Transportation greater than 0.30 mm can have an adverse effect on apical sealing after obturations.[27] In our study, no transportation exceeding 0.16 mm was observed in either group at any level. This may be due to the flexibility of the TRN and MIS files and their smaller taper than most root canal files.

When the files were compared after the first and second use, there was no significant difference in deformation. In contrast, files from both systems showed increased deformation according to the number of uses. While tip deformation in the TRN file increased after the second use compared to the first, damage to the MIS file after the second use was insignificant compared to the first use. Therefore, the second H0 hypothesis was partially rejected. According to the manufacturers' information, MIS and TRN have a non-cutting tip design.[13,14] The apical 3 mm part of the MIS file is square, and the coronal part has a rectangular cross-section design.[13] The difference in cross-sectional design between the two file systems may have affected the rate of tip deformation.

When examined in terms of the cutting edge, there is no significant difference between the two file systems in terms of either the number of uses or the level of cross-section. The fact that there is no difference between the systems in terms of transportation confirms that the cutting efficiency of these files does not change with increasing use. In terms of surface deformation, while the MIS file was significantly deformed, the amount of deformation was insignificant in the TRN file. This may be due to TRN's NiTi alloy and off-centered parallelogram cross-section design. Clinically, the larger taper of a file causes more contact with the dentin surface and this

might increase the risk of the deformation and fracture of the file.[28,29] The TRN file has a variable regressive taper, while the MIS file has a constant taper. In this case, the surface deformation of the MIS file may have increased since it has more contact with the resin block.

Studies have been conducted examining the shaping ability, cutting efficacy, or deformation of files from 1 use to 10 uses.[4,21,30,31] In this study, we used the files twice and included them in the sterilization cycle twice. In addition to studies showing that sterilization causes file deformation and fatigue,[32] some studies claim the opposite.[33] In our study, we evaluated the deformation of the files after the shaping and sterilization process. Therefore, the effect of sterilization alone on surface deformation could not be tested. We repeated the same sterilization procedure after two uses to provide standardization. Uslu et al.[34] showed that exposure to 5.25% sodium hypochlorite (NaOCl) solution for 5 minutes and 17% EDTA for 10 minutes affects the surface roughness of the file. In our study, distilled water was used to irrigate acrylic blocks. Thus, NaOCl and EDTA's effect on the files' possible deformation was eliminated.

While human teeth with various anatomical variations can be used to examine the shaping ability of files, it is possible to perform this examination in standardized resin blocks. Although 3D evaluation cannot be done using this method, examining shaping ability in resin blocks has been widely accepted in the endodontic literature.[3,16,18,23] Although we preferred resin blocks to provide standardization and strengthen the methodology in our study, the fact that the hardness of these blocks is different to that of human teeth[35] is one of the limitations of our study. Another limitation of this methodology is that the deformation of the files was evaluated for two cycles of use and sterilization. Increasing the number of uses may affect the amount of transportation and deformation.

CONCLUSION

Within the limits of our study, when TRN (26.04) and MIS (25.04) NiTi rotary files were used twice on J-shaped resin blocks, the deformation they showed against physical stress and sterilization processes was not different from each other. In addition, neither has an advantage regarding transportation. Both file systems can be alternatives for minimally invasive shaping.

ETHICAL DECLARATIONS

Ethics Committee Approval: The authors declare that no experiments were performed on humans or animals for this study. Therefore, it does not require an ethical committee decision.

Informed Consent: The authors declare that no experiments were performed on humans for this study. Therefore, it does not require an informed consent.

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Original Article

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The impact of gastroenterology fellowship involvement on the ERCP outcomes

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ABSTRACT

Aims: This study aimed whether the companionship of the gastroenterology fellowships to operators performing high-volume ERCP, would adversely affect the safety and success of the procedure.

Methods: This retrospective observational study included 964 patients with naïve papilla who underwent ERCP between February 2019 and May 2022. Procedures with fellowship involvement were compared with procedures performed only by the expert operator in terms of cannulation success, procedure time, cannulation time, unintended PD cannulation, difficult cannulation, cannulation techniques, and post-procedure adverse events.

Results: The two groups were similar in terms of procedure difficulty, successful cannulation in the first session, overall successful canulation, unintended PD cannulation, difficult cannulation rates, and cannulation techniques. The median procedure time was significantly higher in the fellowship involvement group compared to the other group (p=0.008). There was no difference between the two groups in terms of adverse events after the procedure (p>0.05). Procedure difficulty were found an independent risk factor of adverse events in the multivariate analysis (Odds ratio: 2.3; 95% Cl 1.4-3.6; p<0.001).

Conclusion: Our study showed that the involvement of gastroenterology fellowships in the ERCP procedure prolonged the procedure and cannulation time but did not decrease the technical success and did not increase adverse events.

Keywords: ERCP training, gastroenterology fellowship, post-ERCP complications, endoscopic retrograde cholangiopancreatography

INTRODUCTION

As a result of the recent developments in Endoscopic Retrograde Cholangiopancreatography (ERCP) and other advanced endoscopic procedures and the widespread use of these procedures, the training and competence of operators have become one of the current issues in recent years.[1] Qualification and its measurement are of great importance in ERCP, considering the central position of ERCP in these procedures. Many guidelines on measuring competence in ERCP have been published.[2,3] Studies mostly focus on the relationship between the volume and duration of trainees and proficiency of trainees.

The number of studies evaluating the effect of trainee involvement in ERCP training on ERCP outcomes is relatively few.[4] In a recent study, investigating the effect of trainee involvement on ERCP outcomes, it has been found that trainee involvement does not increase the risk of ERCP complications and has no effect on the technical success of the procedure.[4] In the study, trainee endoscopists have been defined as persons with negligible ERCP experience.

In the literature, there are also studies on the success and proficiency of fellowship operators in gastroenterology education in ERCP procedures.5 In previous publications, Accreditation Council for Graduate Medical Education (ACGME) has stated that 3-year gastroenterohepatology training may not be sufficient in terms of competence in ERCP. For this reason, postgraduate ERCP training programs have become increasingly widespread in recent years.[6]

In the literature, previous studies on ERCP training have not specified the status of participants in the procedures as pre- or post-graduation during their training.[7] In this study,

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we aimed to shed light on whether the companionship of the gastroenterology fellowships to operators performing high-volume ERCP, would adversely affect the safety and success of the procedure.

METHODS

Ethical Statement

The study was carried out with the permission of Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 22/12/2021, Decision No: E2-21-1170), and the study was conducted in accordance with the Declaration of Helsinki guidelines. Signed informed consent was obtained from each participant prior to the study.

Study Design

This study was designed as a retrospective cohort study in a single tertiary center gastroenterology clinic. ERCP procedures of patients with naive papilla between February 2019 and May 2022 were reviewed for the study. Among those, procedures with grade 1 and grade 2 difficulty were included in the study. Patients who were hospitalized in other centers and whose clinical follow-up records were missing were also excluded from the study. Grade 3 procedures were also excluded since only experienced operators were involved and total procedure number was low. Demographic data, procedure indications, laboratory, and imaging data, ERCP findings, procedure-related complications, and all clinical follow-up data of all patients were obtained from electronic database and patient files.

Endoscopic Procedures

ERCPs were performed with a lateral scope (TJF 190; Olympus Optical, Japan) by an experienced operator (yearly ERCPs 800<) or by fellows (4 in total) under the supervision of the same experienced operator. The experienced operator in the procedures attended by the Fellowships intervened in the procedure verbally or practically at any stage of the process when deemed necessary. In ERCP procedures, selective biliary cannulation was first attempted classically, primarily with the guidewire method. If there was a failure in selective biliary cannulation, other methods such as a double guidewire, pre-cut techniques, and transpancreatic biliary sphincterotomy (TPBS) were performed. The routine Cannulation times and total procedure times of the procedures were also recorded.

Training Program

In Turkey, gastroenterology training is performed as a 4-year internal medicine residency followed by 3 years of upper specialization. Fellowships in our clinic are trained in upper gastrointestinal endoscopy and colonoscopy procedures in the first 2 years of gastroenterology residency. Fellowships who performed more than 2000 upper gastrointestinal endoscopies and more than 1000 colonoscopies in total participated in these procedures for ERCP training in the last year. Due to variations in the rotation schedules, some procedures were performed with fellowship participation while others were performed by an expert operator without a fellowship.

Definitions

Procedures resulted with deep selective biliary cannulation were defined as successful cannulation. The difficulty level of ERCP procedures was graded according to the Schutz scale.8 ERCP-related adverse events of all patients after the procedure were defined according to the criteria set by an international consensus.[9] In addition, cardiopulmonary complications which developed in some patients during the ERCP procedure, were defined as adverse events. The time between the visualization of the major papilla and the selective biliary cannulation was defined as cannulation time, while the time from the beginning to the end of the procedure was defined as total procedure time. Difficult cannulation was defined according to the criteria set by a recently published guideline. According to these criteria, biliary cannulation could not be achieved within 5 minutes and/or 2 or more unwanted pancreatic cannulations were considered as difficult cannulation.[10]

Study Outcomes

The primary outcome of the study was to determine the effect of fellowship participation on ERCP success and post-procedural adverse events. Outcomes of the ERCP procedures between fellowship involvement and expert operator were compared in terms of cannulation success, procedure time, cannulation time, unintended pancreatic duct (PD) cannulation, difficult cannulation, and cannulation techniques. In addition, the data of both groups were compared in terms of adverse events such as post-procedural complications, need for intensive care unit (ICU), and mortality.

Statistical Analysis

Data analyses were conducted using Statistical Package for the Social Sciences (SPSS 22.0 for Windows, Chicago, IL, USA) software. The variables were investigated using visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov tests) to determine whether they were normally distributed. In reporting descriptive statistics, data were expressed as mean ± standard deviation (SD) for normally distributed numerous variables, median (minimum-maximum) for non-continuous numerous variables and ordinal variables, and as frequencies and percentages (%) for nominal variables. The $\chi 2$ tests, Fisher's exact or Likelihood ratio tests were used to compare nominal variables or categorical variables between the groups. In addition, the independent samples T-test was used to compare continuous numerous variables and the Mann-Whitney U test was used to compare the

noncontinuous numerous variable or ordinal variables between the groups. A p-value of < 0.05 was considered statistically significant. Variables thought to affect post-ERCP side effects were first analyzed by univariate logistic regression. Then, the significant variables were included in the multiple logistic regression model and the odds ratio (OR) of the variables was calculated.

RESULTS

A total of 1003 patients with naive papilla underwent ERCP between February 2019 and May 2022. Five patients who met the Schultz 3 criteria and 34 patients whose follow-up was conducted at another center were excluded from the study. The remaining 964 patients were included in the study.

Table 1 shows the comparison of demographic characteristics and laboratory findings between the groups. There was no significant difference between the two groups in terms of age, gender, history of cholecystectomy, comorbidities and laboratory findings (p>0.05). There was a significant difference in the indication for the procedure between the two groups (p=0.006).

The two groups were similar in terms of procedure difficulty8, successful cannulation in the first session, overall successful canulation, unintended PD cannulation, difficult cannulation rates, and cannulation techniques (p=0.879, p=0.783, p=0.338, p=0.239, p=0.644, p=0.345, respectively). The cannulation time was significantly different between the two groups (p<0.020). The median procedure time was significantly higher in the fellowship involvement group compared to the other group (p=0.008). Sphincterotomy and stone removal rates were significantly higher in the procedures without fellowship involvement group compared to the other group (p=0.027, p=0.001, respectively) (Table 2).

There was no difference between the two groups in terms of adverse events after the procedure (p>0.05) (Table 3).

Table 3. Comparison of post-ERCP side effects of procedures with and without fellowship participation

	Procedures of only expert operators, n (%)	Procedures with fellows involvement, n (%)	p
PEP, n (%)	48 (12.1)	80 (14.1)	0.377*
Bleeding, n (%)	10 (2.5)	25 (4.4)	0.125*
Perforation, n (%)	3 (0.8)	8 (1.4)	0.540*
Cholangitis, n (%)	2 (0.5)	4 (0.4)	0.699*
Cardiopulmonary complications	5 (1.3)	8 (1.4)	0.847*
Need for ICU, n (%)	7 (1.8)	18 (3.2)	0.178*
Mortality, n(%)	4 (1.0)	8 (1.4)	0.771**
Any adverse events, n (%)	65 (16.4)	117 (20.6)	0.102*

PEP: Post-ERCP Pancreatitis, ICU: intensive care unit, *: χ2 tests **: Fisher exact test

Univariate analyses revealed that age (p=0.022), procedure difficulty (p<0.001), total procedure time (p<0.001), cannulation time (p<0.001), and difficult cannulation (p<0.001) were possible risk factors of adverse events. When these five possible risk factors were entered into multivariate analysis, only procedure difficulty was found an independent risk factors of adverse events (Odds ratio: 2.3; 95% Cl 1.4-3.6; p<0.001) (Table 4).

Table 1. Baseline characteristics of the patients

	Procedures of only expert operators N=396	Procedures with fellowship involvement N=568	
Age mean (SD)	59.27 (17.92)	60.09 (18.19)	0.489*
Female sex n(%)	230 (58.1)	311 (54.8)	0.306**
History of cholecystectomy n(%)	74 (18.7)	83 (14.6)	0.092**
Comorbidities			
HT	161 (40.7)	254 (44.7)	0.210**
DM	74 (18.7)	113 (19.9)	0.641**
Cardiovascular diseases	74 (18.7)	111 (19.5)	0.740**
Chronic obstructive pulmonary diseases	23 (5.8)	30 (5.3)	0.724**
Chronic kidney diseases	8 (2)	23 (4)	0.079**
Malignancy	14 (3.6)	32 (5.6)	0.288**
Indications for the procedures, n (%)			0.006**
CBD Stones	330 (83.3)	444 (78.2)	
Malignant stricture of the bile duct	28 (7.1)	56 (9.9)	
Bile leak or trauma	21 (5.3)	18 (3.2)	
Other	17 (4.3)	50 (8.8)	
Laboratory data			
Tbil	2.80 (0.3/24.0)	2.90 (0.2/25.1)	0.678***
Dbil	1.7 (0.1/16.0)	1.80 (0.1/17.7)	0.589***
AST	154.0 (12/1843)	147.5 (9.0/2072)	0.989***
ALT	203 (7/1496)	216.5 (4/1139)	0.572***
GGT	381 (10/2415)	353.5 (5/3090)	0.680***
CRP	12 (0.10/358.0)	13.6 (0.1/243.0)	0.973***

HT: hypertension, DM: diabetes mellitus, CBD: common bile duct, Tbil: total bilirubin, GGT: gamma-glutamyl transferase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, *: independent sample t-test, **: χ2 test, ***: Mann-Whitney U test, bold values show statistical significance p<0.05.

Table 2. Comparison of ERCP findings of procedures with and without fellowship participation

	Procedures of expert operators, n (%)	Procedures with fellowship involvement (%)	p
Procedure difficulty level n (%)			0.879*
Grade 1	354 (89.4)	506 (89.1)	
Grade 2	42 (10.6)	62 (10.9)	
Successful cannulation in the first session n (%)	378 (95.5)	540 (95.1)	0.783*
Overall success cannulation n (%)	392 (99.0)	558 (98.21)	0.338*
Cannulation time, n (%)			0.020*
<5 min	293 (74.6)	405 (72.1)	
5-10 min	54 (13.7)	58 (10.3)	
10<min	46 (11.7)	99 (17.6)	
Total procedure time (min) Median (min-max)	25 (13/72)	27 (11/76)	0.008**
Unintended PD cannulation			0.239***
None	301 (76)	411 (72.4)	
1 time	35 (8.8)	47 (8.3)	
2≤ times	60 (15.2)	110 (19.3)	
Difficult cannulation, n (%)	111 (28.0)	167 (29.4)	0.644*
Cannulation technique			0.345***
Wire-guided cannulation	304 (76.8)	433 (76.2)	
Double guidewire technique	77 (19.4)	97 (17.1)	
Pre-cut technique	9 (2.3)	21 (3.7)	
TPBS	2 (0.5)	7 (1.2)	
Failed cannulation	4 (1.0)	10 (1.8)	
Sphincterotomy	392 (99.0)	550 (96.8)	0.027*
Stone removal (Balloon or basket)	291 (73.5)	357 (62.9)	0.001*
Plastic stent placement	250 (63.1)	333 (58.7)	0.169*
Covered metal stent placement	4 (1)	10 (1.8)	0.338*
EBD	21 (5.3)	33 (5.8)	0.736*
Brush sitology	39 (9.8)	74 (13.0)	0.338*

PD: pancreatic duct, TPBS: trans-pancreatic biliary sphincterotomy, EBD: endoscopic balloon dilatation, *: $\chi 2$ tests, **: Mann-Whitney U test, ***: Likelihood test, bold values show statistical significance.

Table 4. Evaluation of the causes of post-ERCP side effects by multivariate analysis

Variables	No-Adverse Events	Adverse Events	Univariate p value	Multivariate p value	OR (95%CI)
Gender			0.072	-	-
Male	354 (83.7)	69 (16.3)			
Female	428 (79.1)	113 (20.9)			
Age	59.1 ± 19.1	62.5 ± 17.6	0.022	0.052	
Tbil	2.9 (0.2 - 25.1)	2.65 (0.20 - 24.0)	0.921	-	-
Procedure difficulty level					
Grade 1	714 (83.0)	146 (17.0)	<0.001	<0.001	2.3 (1.4 - 3.6)
Grade 2	68 (65.4)	36 (34.6)			
Total procedure duration	26 (11 - 76)	29 (12 - 68)	<0.001	0.585	-
Cannulation time					
<5 min	601 (86.1)	97 (13.9)	<0.001	0.936	-
5-10 min	76 (67.9)	36 (32.1)			
10<min	98 (67.6)	47 (32.4)			
Difficult cannulation					
No	593 (86.4)	93 (13.6)	<0.001	0.078	-
Yes	189 (68.0)	89 (32.0)			
Fellowship involvement					
No	331 (83.6)	65 (16.4)	0.103	-	-
Yes	451 (79.4)	117 (20.6)			

Tbil: total bilirubin, Continuous variables with normal distribution were expressed as mean±standard deviation, while others were expressed as median (min - max). Categorical variables were shown as n (%)

DISCUSSION

The most important results of the study were that the involvement of fellowship in ERCP does not decrease cannulation success and does not increase post-procedural adverse events. The fact that the involvement of fellowship in the ERCP procedure significantly increases the total procedure and cannulation time is another important result of study.

The fact that ERCP has been included in the therapeutic treatment of many pancreaticobiliary diseases with the developments in recent years and has become increasingly widespread has revealed the importance of ERCP training. On this subject several guidelines have been published in many regions and each guideline has put forward different competency criteria.[11,12] Those guidelines sought to answer the question of the minimum number of procedures an independent ERCP operator should perform during the ERCP training.

While discussing this very important issue, another question has been raised, regarding how fellowship involvement in ERCP training will affect the results of ERCP. In this concept, current studies in the literature revealed conflicting results. In a recent study of 1843 ERCP procedures, fellowship involvement and control group were compared in terms of adverse events. Contrary to expectations, moderate and severe adverse events were found to be significantly higher in the control group. We believe that this study is very valuable with its multicentred and prospective nature. However, the fact that serious and moderate adverse events were higher in the control group is the most important controversial finding of the study. The reason for these controversial results may be the fact that the data were collected from different databases due to the multicentred nature of the study, and some centers in the study performed ERCP in low volumes.[4] However, in another study by Voiosu,[13] the principal investigator of the above article, no differences were found in terms of adverse events between procedures with and without fellowship involvement. The fact that this study was single centered supports our hypothesis about the previous study. In our study, no significant difference was found between both groups in terms of complications. Notably, PEP was fount to be the most common adverse event with a rate of 13.3% in total. Although this is slightly higher than the sample studies mentioned above, it seems to be an acceptable rate considering that the incidence of PEP in meta-analyses in the literature ranges between 8.4 and 14.7%.[14] In addition, almost all patients who developed PEP had mild pancreatitis. Severe PEP developed in only 3 patients in the group with fellowship involvement followed by 2 patients in the other group. Mortality secondary to post-procedural cardiopulmonary complications were observed in 2 patients. In 1 patient from both groups, mortality developed due to prolonged ICU hospitalization (i.e., infection) after the surgical intervention for ERCP related perforation.

The success of the procedure has also been among the main topics investigated in studies with fellowship involvement. While some studies have compared only cannulation success,[13,15] some studies have compared the technical success of the procedure as a total evaluation of manoeuvres such as biliary cannulation, stone removal, and stent placement.[4,16] Frost et al.[15] found no difference in successful biliary cannulation rates between the groups with and without trainee involvement in a study of 219 procedures. In addition, both studies by Voisu et al.[4,13] reached similar findings and demonstrated that trainee involvement did not affect cannulation and technical success. In a Chinese prospective study, no significant difference was found between the trainee involvement group and the control group in terms of technical success.[16] Similar results were also observed in our study and no significant difference was found between the two groups in terms of successful biliary cannulation. Notably, successful biliary cannulation rates in both groups were above 90%, which is the quality criterion of the European Society for Gastrointestinal Endoscopy (ESGE).[17] It was observed that there was a difference between the two groups in terms of stone removal and sphincterotomy rates among the interventions performed during the procedure. We think that the higher rate of stone removal in the expert operator group may be due to the higher number of patients who underwent ERCP with the indication of Common Bile Duct stones in this group.

Whether the fellowship involvement may lead to an increase in the number of difficult cannulations is undoubtedly another important concern for expert operators. Fellowship initiation of the procedure and unsuccessful cannulation attempts may result with an increase in difficult cannulation situations which may cause some operators to have negative thoughts about ERCP training. Voiosu et al.[13] reported that trainee involvement prolonged cannulation time and increased the use of the pre-cut technique. Although difficult cannulation was not defined in the study, it is suggestive in this respect that pre-cut techniques, which are among the methods used in the case of difficult cannulation, were found to be higher in the trainee group. In this study, the duration of cannulation was longer in the fellowship involvement group, which is consistent with this study. However, no difference was found between both groups in terms of difficult cannulation and cannulation techniques.

The most important limitation of this study is that the study was single-centered and retrospective. The low number of fellowships and expert operators is another limitation of the study. Finally, the fact that there may be inter-fellowship variability accompanying the procedures, which may affect the results, can also be counted among the limitations of the study. However, we think that the high number of subjects recruited for the study is sufficient.

CONCLUSION

This study demonstrated that the involvement of gastroenterology fellowships in the ERCP procedure prolonged the procedure time yet did not decrease the technical success. Notably, another important result of this study is that fellowship involvement is not an independent risk factor for ERCP related adverse events. ERCP training of fellowships under the supervision of experienced operators may ensure that independent ERCP operators and centres may become more common in the future, but this statement requires further studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 22/12/2021, Decision No: E2-21-1170).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Is restrained eating behaviour associated with pre-pregnancy weight and weight-gain in gestational diabetes?

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ABSTRACT

Aims: The aim of this study was to investigate the association between eating behaviors and pre-pregnancy body mass index (BMI) and body weight gain in individuals with gestational diabetes mellitus.

Methods: This cross-sectional study was conducted in 34 pregnant women diagnosed with gestational diabetes mellitus and 50 nondiabetic women above the 28th week of pregnancy. The Dutch Eating Behavior Questionnaire was used to assess eating habits. A quantitative food frequency questionnaire was used to assess food consumption.

Results: Individuals with gestational diabetes had significantly higher levels of restrained eating behavior scores (27.11±5.98 vs 20.32±6.84) compared to nondiabetic women ($p<0.05$). Pre-pregnancy body mass index value was found to be related to restrained eating behavior score in individuals with gestational diabetes ($p<0.05$), and restrained eating score was found to be higher in individuals with gestational diabetes who had body weight gain above the recommendations than in individuals with body weight gain in accordance with the recommendations ($p<0.05$). The energy intake (1965±433.02 kcal/day vs. 1731.04±462.64 kcal/day), carbohydrate (201.19±59.35 g/day vs. 170.56±56.49 g/day) consumption of pregnant women diagnosed with gestational diabetes were higher than those nondiabetic women, whereas vitamin B6 (1.12±0.31 mg/day vs. 1.33±0.46 mg/day), and calcium consumptions (682.88±265.12 mg/day vs. 963.03±391.63 mg/day) were lower ($p<0.05$).

Conclusion: Women with gestational diabetes showed a higher restrained eating behavior score, which may be attributed to pre-pregnancy body mass index and body weight gain above the recommendations. Furthermore, it has been emphasized that this may be associated with an inadequate intake of certain nutrients.

Keywords: Eating behaviour, gestational diabetes, restrained eating

INTRODUCTION

The term gestational diabetes mellitus (GDM) refers to a glucose tolerance disorder that occurs during pregnancy for the first time and factors such as age, genetics, black race, obesity, environment, and lifestyle have been reported to play a role in its etiology.[1] It was reported that the prevalence of GDM in the United States increased from 4.6% to 8.2% from 2006 to 2016,[2] and in a meta-analysis covering the period 2004-2016 in Turkey that the prevalence ranged between 1.9-27.9%, with an average rate of 7.7%.[3]

A number of complications (macrosomia, birth injuries, cesarean section, hydramnios, preeclampsia, metabolic disorders in the newborn) have been shown to be associated with GDM during pregnancy and postnatally for both the mother and the newborn.[4] It was reported that approximately 20-50% of individuals with GDM were diagnosed with diabetes mellitus (DM) within 5-10 years after birth and the risk of type 2 diabetes increased 7.4-fold.[5] It has been emphasized that screening and identification of these individuals are extremely important for improving short- and long-term maternal and fetal outcomes.[6] Medical nutrition therapy is defined as a preliminary therapy for controlling blood sugar levels and preventing ketosis.[7] In general terms, the nutritional requirements of individuals with GDM do not differ from pregnant women without GDM, but it has been suggested that is particularly important to focus specifically on nutritional therapy to ensure and maintain maternal euglycemia, prevent variations in blood glucose levels, and protect maternal and newborn health by ensuring appropriate maternal body weight gain.[8]

It has been pointed out in recent years that individuals with GDM develop unhealthy eating habits as a result of the emotional changes caused by pregnancy, the rules imposed on a comfortable diet, and the stress and anxiety

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generated by having to adhere to treatment regimens.[9] In the literature, there are a limited number of studies evaluating eating behaviors in individuals with GDM.[9,10]

In this study, it was aimed to investigate the relationship between eating behaviors and pre-pregnancy body mass index (BMI), body weight gain, and food consumption in individuals with GDM. We tested the hypothesis that eating behavior problems are more common in individuals with GDM than nondiabetic pregnant women and that this is associated with body weight gain and food consumption.

METHODS

This cross-sectional study was conducted between March 2022 and November 2022. Ethics committee approval to conduct the present study was obtained from the Clinical Research Ethics Committee (Date: 02.02.2022, Decision No: 2022/20). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was conducted on individuals who were >18 years of age, signed an informed consent form, did not have any risky pregnancy status other than GDM, and were >28th week of pregnancy. Pregnant women who were <18 years of age, multiparous, had abnormal laboratory findings, intrauterine growth retardation, and risky pregnancies other than gestational diabetes were excluded from the study.

Sample calculation was calculated via the student-t test with a power of 0.80 at a significance level of 0.05, according to **Table 1** in the study of Lan et al.[11] and it was determined that a minimum of 30 pregnant individuals for each group ((a) with a diagnosis of gestational diabetes pregnant women (b) nondiabetic pregnant women) with a gestational week >28, were required. The study was conducted on a total of 84 pregnant individuals, including 34 subjects who met the inclusion criteria and were diagnosed with GDM and 50 subjects without any risky pregnancy and nondiabetic.

Table 1. Data on the sociodemographic and obstetric characteristics of individuals

Variable	Total (n=84)	With GDM (n=34)	Without GDM (n=50)	p value
Age (years)	28.49±5.06	29.09±4.75	28.40±5.01	0.398
Marriage age (years)	21.81±5.08	21.79±6.71	21.82±3.70	0.980
Number of pregnancy	2.43±1.10	2.41±1.13	2.44±1.09	0.909
Gestatioanl age (week)	30.62±4.79	28.18±4.09	29.34±6.35	0.392
Pre-pregnancy BMI (kg/m²)	24.94±4.23	25.42±4.73	24.61±3.88	0.411
BMI (kg/m²)	28.84±4.46	28.60±4.80	29.04±4.26	0.666

General information, eating behaviors, and food consumption of pregnant women were obtained by the researchers through a questionnaire form by face-to-face interview method. The body weight and height of the subjects were recorded during outpatient clinic visits by the obstetric nurse using a calibrated scale (SECA 711) and a height meter (SECA 220). Pre-pregnancy measurements were obtained from the hospital's electronic registry system. BMI value was calculated for each individual and categorized based on the guidelines of WHO.[12] In addition, body weight gain during pregnancy was evaluated according to the Institute of Medicine recommendations.[13]

Eating behaviors were analyzed by administering the Dutch Eating Behavior Questionnaire (DEBQ), a Turkish validity and reliability study of which was conducted by Bozan et al. (2009). The questionnaire includes 33 items and consists of 3 subgroups assessing emotional eating behaviors (e.g., do you eat sweets when you are unhappy?), external eating behaviors (e.g., if something smells good, do you eat more than you normally eat) and restrained eating behaviors (e.g., do you eat less than you want to eat to avoid getting fat). The items in the questionnaire are rated on a 5-point Likert scale and high scores indicate the presence of eating problems. For the assessment of food consumption, a quantitative Food Frequency Questionnaire (FFQ) was administered by an expert dietitian and analyzed via Nutritional Information System Package Software (Bebispro for Windows, Stuttgart, Germany; Turkish version, 2010) to determine daily energy, micro, and macronutrient consumption.

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 22.0 for Mac (SPSS Inc., Chicago, IL), and the data distribution was evaluated using the Kolmogorov-Smirnov test (p:0.200>0.05). As confirmed by the kurtosis value (skewness: 0.381, kurtosis: -0.192), the data was found to be normally distributed. Descriptive statistics are presented as the mean and standard deviation. Independent Samples T-test was used to analyze the difference between individuals with GDM and pregnant women without GDM in terms of sociodemographic, obstetric data, eating behavior scores, and food consumption. Multiple regression analysis was used to assess the relationship between pre-pregnancy BMI and eating behaviors (emotional, external, and restraint). The difference between body weight gain in pregnant women with and without GDM was analyzed by chi-square test. Besides, the difference between eating behavior (emotional, extrinsic, and restraint) scores according to body weight gain was determined by a one-way ANOVA test, and the Tukey test was used to find out in which groups there was a difference. The level of significance was set at p<0.05.

RESULTS

The mean age, gestational age, marriage age, number of pregnancies, gestational week, pre-pregnancy, and current BMI (kg/m²) values of the individuals were 28.49±5.06 years, 21.81±5.08 years, 2.43±1.10, 30.62±4.79 weeks, 24.94±4.23 kg/m² and 28.84±4.46 kg/m², respectively. No significant difference was found between age, marriage age, number of pregnancies, gestational week, pre-pregnancy, and current BMI (kg/m²) values in pregnant individuals with and without GDM, and it was shown that they were similar (Table 1).

Among the individuals, 6.0% were underweight, 54.8% were normal, 26.2% were overweight, and 13.1% were obese before pregnancy. Before pregnancy, 8.8% of women with GDM were underweight, 44.1% were normal, 29.4% were overweight, and 17.6% were obese, while 4.0% of without GDM were underweight, 62.0% were normal, 24.0% were overweight, and 10.0% were obese.

The mean restrained, externality, and emotional eating behavior scores of the individuals were 23.07±7.29, 35.61±7.88, and 22.30±8.49, respectively. Individuals with GDM had higher restrained eating behavior scores (27.11±5.98 vs. 20.32±6.84) compared to without GDM (p<0.05), whereas there was no difference in externality and emotional eating score (Table 2).

Table 2. Mean values of individuals' restrictive, extrinsic and emotional eating scores

Variable	Total (n=84)	With GDM (n=34)	Without GDM (n=50)	p value
Restrained eating score	23.07±7.29	27.11±5.98	20.32±6.84	0.000*
Externality eating score	35.61±7.88	35.17±7.62	35.92±8.11	0.670
Emotional eating score	22.30±8.49	23.94±8.36	21.52±8.19	0.332

* p<0.05

It was shown that pre-pregnancy BMI was decisive on restrained eating behavior score (p:0.006) in individuals with GDM and emotional eating score (p:0.042) in without GDM (p<0.05). Restrained eating score was associated with pre-pregnancy BMI in individuals with GDM and emotional eating in individuals without GDM. (Table 3).

A total of 39.3% of the individuals had a body weight gain above the recommendations, 31.0% had a body weight gain below the recommendations and 29.8% had a body weight gain in accordance with the recommendations. It was found that 70.7% of individuals with GDM had a body weight gain above the recommendations, 8.8% had a body weight gain below the recommendations and 20.5% had a body weight gain in accordance with the recommendations, while 18.0% of the without GDM individuals had a body weight gain above the recommendations, 46.0% below the recommendations, and 36.0% in accordance with the recommendations. It was determined that individuals with GDM gained body weight above the recommended weight at a higher frequency (70.7% vs 18.0%) than without GDM (p<0.05).

Restrained, externality, and emotional eating scores according to body weight gain during pregnancy in GDM and without GDM individuals, are presented in Table 4. The restrained eating score was higher in individuals with GDM with body weight gain above the recommended body weight gain than in individuals with body weight gain in accordance with the recommendations (p<0.05). No difference was found between the groups in terms of externality and emotional eating scores (Table 4).

The energy and nutrient intakes of pregnant women with GDM and without GDM pregnants are shown in Table 5. The energy intake (1965±433.02 kcal/day vs. 1731.04±462.64 kcal/day), carbohydrate (CHO)

Table 3. Multiple linear regression analysis of the effect of pre-pregnancy BKI on restrained, externality, emotional eating score

Variable	Birth status	Beta	t	p	95% confidence interval	
Restrained eating score	GDM Without GDM	0.480 0.055	2.999 0.371	0.006* 0.712	0.023 -0.026	0.121 0.038
Externality eating score	GDM Without GDM	-0.216 -0.305	-1.229 -1.730	0.229 0.090	-0.067 -0.059	0.017 0.004
Emotional eating score	GDM Without GDM	0.077 0.342	0.487 2.088	0.630 0.042*	-0.026 0.001	0.043 0.060

*p<0.05

Table 4. Restrained, externality and emotional eating scores in individuals with GDM and without GDM according to body weight gain status during pregnancy

Variable		Under recommended (n=24)	Recommended (n=27)	Above recommended (n=33)	p
Restrained eating score	GDM Without GDM	27.66±3.78 21.08±7.21	21.85±2.54[a] 20.38±6.59	28.58±6.14[b] 18.22±6.70	0.027* 0.576
Externality eating score	GDM Without GDM	29.00±6.55 37.65±8.58	36.00±7.52 33.22±7.81	35.70±7.70 36.88±6.71	0.349 0.208
Emotional eating score	GDM Without GDM	19.00±5.56 19.30±7.88	28.28±11.37 20.00±7.07	24.70±7.47 24.66±10.47	0.273 0.240

a,b: It shows that the difference between the groups is significant.

(201.19±59.35 g/day vs. 170.56±56.49 g/day), B1 (0.85±0.33 mg/day vs. 0.70±0.17 mg/day) and B2 (1.47±0.55 mg/day vs. 1.24±0.41 mg/day) vitamin consumption of individuals with GDM were higher than without GDM, whereas vitamin B6 (1.12±0.31 mg/day vs. 1.33±0.46 mg/day), and calcium consumptions (682.88±265.12 mg/day vs. 963.03±391.63 mg/day) were lower (p<0.05). The consumption of other nutrients was similar between the groups (Table 5).

Table 5. Daily energy and nutrients intake of individuals

Variable	With GDM (n=34)	Without GDM (n=50)	p
Energy (kcal/day)	1965±433.02	1731.04±462.64	0.022*
CHO (g/day)	201.19±59.35	170.56±56.49	0.019*
CHO (%)	43.25±9.97	38.91±9.28	0.045*
Protein (g/day)	67.±53±20.32	71.51±15.49	0.313
Protein (%)	14.76±2.51	15.80±3.20	0.099
Fat (g/day)	93.95±24.71	86.12±27.10	0.183
Fat (%)	45.08±7.56	43.05±6.58	0.208
Fibre (g/day)	19.53±8.58	19.10±5.78	0.803
Vitamin A (mcg/day)	1294.95±877.14	1223.91±976.19	0.729
Vitamin E (mg/day)	25.75±13.15	25.97±7.73	0.923
Vitamin B1 (mg/day)	0.85±0.33	0.70±0.17	0.021*
Vitamin B2 (mg/day)	1.47±0.55	1.24±0.41	0.042*
Vitamin B6 (mg/day)	1.12±0.31	1.33±0.46	0.023*
Vitamin B12 (µg/day)	1.24±0.39	1.71±0.70	0.001*
Folate (mcg/day)	277.56±100.88	279.34±83.38	0.932
Calcium (mg/day)	682.88±265.12	963.03±391.63	0.021*
Iron (mg/day)	11.26±4.46	9.93±2.50	0.084
Zinc (mg/day)	9.92±3.52	9.26±2.69	0.339
Vitamin C (mg/day)	117.40±80.30	120.60±73.24	0.851

*p<0.05

DISCUSSION

During pregnancy, eating behaviors may be affected by hormonal and emotional changes.[14] In particular, women diagnosed with GDM may develop anxiety about consuming foods without restriction. As a result of stopping eating certain foods that they previously enjoyed and changing their eating habits, they become insufficient in their food consumption and make wrong food choices.[15]

In our study, the Turkish version of DEBQ was used and restraint, external, and emotional eating behaviors were measured. There was no difference in external and emotional eating behavior scores between individuals with and without GDM, but restrained eating behavior showed a difference between the groups. The restrained eating behavior score was found to be higher than in without GDM individuals, and it was shown that restrained eating behavior was associated with pre-pregnancy BMI and resulted in excessive body weight gain during pregnancy.

Restrained eating behavior refers to the tendency of chronically limiting food intake to lose weight or prevent weight gain.[16] Researchers have demonstrated that this type of moderate eating, though adaptive in today's obesogenic environment, is paradoxically associated with extreme dietary restriction, increased impulsivity, and increased reactivity to food.[17] In the literature, there is no study evaluating restrained eating behavior in individuals with GDM, and the number of studies evaluating restrained eating behavior in individuals without GDM is limited.[18-21]

The findings of a prospective study in which eating behaviors and body weight gain were assessed in 463 healthy pregnant women in Italy indicated that excessive body weight gain was a sign of unhealthy eating habits and a tendency to diet.[18] In the Irish study, restrained eating behaviors before pregnancy and body weight gains at the 15th week of pregnancy were prospectively analyzed in 799 pregnant individuals, and restrained eating behavior was found to be associated with higher weight gain.[19] In a study in which restrained eating behavior and body weight gain were evaluated with a three-factor eating behavior test in individuals who had quit smoking before pregnancy and in early pregnancy, it was shown that the restraint tendency was associated with increased body weight gain.[20] Eating behaviors were analyzed via the Revised Restraint Scale in 62 Caucasian pregnant individuals who were about to give birth to their first or second baby, and body weight gain above or below the recommended level during pregnancy was found in those who showed restrained eating behavior.[21] In a sample of 204 infant-mother, maternal restrained eating behaviors and pre-pregnancy BMI were assessed using maternal self-report measures and was determined that maternal restrained eating behavior waslinked with an increased risk of overweight in early infancy.[22]

It is well-known that appropriate body weight gain during pregnancy is crucial for maternal and newborn health.[23] In the present study, body weight gain above recommendations was found to be higher in individuals with GDM than without GDM, and it was demonstrated that restrained eating behavior scores were higher in individuals with GDM who had body weight gain above recommendations during pregnancy. In our study, restrained eating behavior was also associated with increased body weight gain in individuals with GDM.

In a prospective study in which 156 pregnant women were followed up and their body weight gains were evaluated, 56% of the individuals were above the recommendations, 27% were in accordance with the recommendations and 16% were below the recommendations,[24] while in another study in Greece in which 977 pregnant individuals were

evaluated, it was found that 45% of the individuals gained body weight above the recommendations, 32% gained body weight in accordance with the recommendations and 23% gained body weight below the recommendations.[25] In the Polish study, in which 42 pregnant individuals with GDM and 28 pregnant individuals without a diagnosis of GDM who were in the 24-28th gestational week of pregnancy were evaluated, no difference was found between the groups in terms of body weight gain.[26]

Moreover, in this study, pre-pregnancy BMI was shown to be a determinant of restrained eating behavior scores in individuals with GDM. Likewise, in the Pregnancy, Infection, and Nutrition study, on 2006 pregnant women with a gestational week <20, it was found that anxiety and restrained eating behaviors were more common in individuals with a high pre-pregnancy BMI.[27] In a study evaluating 795 pregnant individuals in the 2003-2012 National Health and Nutrition Examination Survey, it was shown that diet quality decreased as pre-pregnancy BMI increased.[28] In a Chinese study in which 106 individuals with GDM were evaluated, high pre-pregnancy BMI was associated with excess body weight gain during pregnancy.[29]

We found no relationship between eating behaviors and nutrient consumption of individuals in our study; however, energy, CHO, vitamin B1, and B2 intakes were higher among individuals with GDM, and vitamin B6 and Ca intakes were lower among without GDM. The higher energy intake among individuals with GDM may associated with higher over-recommended body weight gain than among individuals without GDM. In particular, CHO consumption was found to be high, whereas consumption of animal-derived nutrients, B6 and Ca, which are important during pregnancy, was low.

In a Vietnamese study in which the food consumption of 60 individuals with GDM was evaluated with 24-hour food consumption records, the energy consumption of individuals was found to be 1841.7±92.2 kcal/day, dietary CHO was 243.4±16.5 g/day, the dietary protein was 84.3±3.9 g/day and Ca consumption was 700 mg/day.[30] In our findings, meanwhile, energy consumption (1965.0±433.02 kcal/day) was higher and CHO consumption (241.19±59.35 g/day) were similar, whereas dietary protein (67.53±20.32 g/day) and Ca (682.88±265.12 mg/day) consumptions were lower.

A moderate-intensity lifestyle intervention that optimizes consumption of whole grains, vegetables and fruits; reducing the intake of excessively processed food and simple sugars; and the Mediterranean diet intervention are noted to be the most successful options in the dietary treatment of GDM.[31]

CONCLUSION

In our study restrained eating behavior score was found to be higher in individuals with GDM than in nondiabetic pregnant women, and it was revealed that pre-pregnancy BMI was determinant and resulted in body weight gain above the recommendations. Furthermore, it was found that body weight gain was higher than the recommendations in individuals with GDM, more energy and CHO consumption was noted, and vitamin B6 and Ca consumption, both of which are essential during pregnancy, was less.

GDM is a critical condition for which optimal nutrition should be ensured. With planned pregnancies, identifying risky groups early, controlling body weight by assessing eating behaviors, raising awareness of individuals about healthy eating behaviors and the amount of nutrients they need, as well as ensuring their compliance and follow-up will contribute significantly to maternal and newborn health.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Etlik Zübeyde Hanim Health Practice Clinical Research Ethics Committee (Date: 02.02.2022, Decision No: 2022/20).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Review

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A comprehensive survey: prevention of female infertility by nutrition

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ABSTRACT

There is an important relationship between nutrition and infertility in women. Most diseases that cause infertility in women can be prevented and treated with proper nutrition. The present study aims to determine the role of nutrition in women's fertility. Samples of this study are all articles published in magazines, books, and theses from the Web Of Sciences, PubMed, Medline, Elsevier, and Google Scholar search engines with the keywords of "female infertility," "nutrition," "diet," "fertility outcomes," "folate," "iron," "omega-3 fatty acids", and "Vitamin." In the beginning, 1052 articles were found, and after reviewing the STROBE checklist, 38 articles were selected. After reviewing the inclusion and exclusion criteria and evaluation, 23 articles were included in the study. Various studies showed that proper nutrition improves women's ovulation parameters and pregnancy outcomes. The reviewed studies suggested a balance between protein and carbohydrates, consuming foods with a low glycemic index, high fiber diet, eating five meals a day, daily consumption of essential fatty acids such as omega 3 and 6, use of organic materials, low-fat proteins, complex carbohydrates, folic acid, antioxidants, vitamins, and foods that cause regular ovulation such as cod liver oil, licorice plant, five-finger plant, thistle, and cinnamon. The results of this comprehensive study indicate that proper nutrition through adequate consumption of micronutrients can positively impact women's fertility. These findings highlight the potential contribution of diet interventions to clinical practice for infertility prevention and management.

Keywords: Infertility, nutrition, women, survey

INTRODUCTION

Infertility is an increasing problem. According to the World Health Organization (WHO) statistics, 15% of couples suffer from this problem, and 30% of these infertility problems are caused by women.[1] Infertility is defined as the inability to conceive a child after one year of sexual intercourse without the use of contraceptive methods. Infertility may have symptoms in women, such as irregular periods and hormonal changes.[2] Several factors affect women's fertility, including diet and consumption of micronutrients. Micronutrients include vitamins and minerals required in small amounts as dietary components.[3] Most diseases that cause infertility in women can be prevented and even treated with proper nutrition. In order to have proper nutrition for infertility, it is necessary to have a proper diet and also to know the effects of food in preventing infertility. Although these micronutrients do not contain energy, they are necessary for the catabolic and anabolic processes of the body. Therefore, investigating the relationship between nutrition and infertility is important for preventing and treating this condition.

The causes of infertility include various diseases such as polycystic ovary syndrome (PCOS), thyroid disorders, and endometriosis. Also, lifestyle, exercising, smoking, alcohol consumption, and stress are factors affecting infertility.[4,5] Age is also a significant factor in female infertility, and with age, the quality and number of eggs decrease.[6] Considering these factors can be effective in choosing treatment strategies and preventing infertility.

Nutrients play a significant role in preventing pregnancy, and a balanced diet with a variety of proteins, healthy fats, vegetables, fruits, and whole grains can improve the nutrients necessary for the optimal functioning of the reproductive system. Proper nutrition through adequate intake of micronutrients is important for optimal reproductive health.[4-6] Studies have shown that certain nutrients such as iron, omega-3 fatty acids, and folate positively affect pregnancy outcomes.[7,8] Also, not being overweight through proper nutrition and regular exercise can improve pregnancy outcomes and reduce risks during pregnancy.[9,10] Therefore, with proper nutrition,

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women can play an important role in pregnancy health and preventing diseases leading to infertility.

Considering that much research has been conducted on treating infertility, the factors affecting it, and the effect of nutrition on the progress of fertility in women, the present study aims to determine the role of nutrition in women's fertility. This article aims to provide a comprehensive survey of articles on preventing infertility in women through proper nutrition. By examining the relationship between nutrition and infertility, this article tries to help raise awareness about nutrition and a healthy diet in preventing and treating infertility. We hope this information can be useful to women seeking to conceive and infertility professionals.

METHODS

This survey study was conducted to determine the role of micronutrients in women's fertility. Samples of this study are all articles published in magazines, books, and theses. Articles were extracted from the Web Of Sciences, PubMed, Medline, Elsevier, and Google Scholar search engines. To find all the desired articles, the keywords "female infertility," "nutrition," "diet," "fertility outcomes," "folate," "iron," "omega-3 fatty acids", and "Vitamin" were used.

These keywords were searched in the mentioned databases published from 2012 to 2022. Database search was done with high sensitivity by a researcher familiar with a database search. In the beginning, the titles of the articles that were searched by the research team with the mentioned keywords were 1052 titles. To avoid bias, the search was conducted by two experts independently, then similar and repeated searches were excluded from the study. A checklist was designed based on the objectives, and by studying other available sources, the abstracts of the collected articles were studied, and the articles that did not have the required information according to the checklist were excluded from the study. A list of all available articles reviewed in the mentioned databases was prepared based on the search strategy and keywords. The searched articles' titles were reviewed, and those with duplicate titles were removed.

In the next step, the full text of the remaining articles was examined using the STORBE checklist, and finally, the articles that received the quorum of the qualitative evaluation score were included in the present study. All the mentioned steps were performed by two independent experts in order to avoid bias. If there was a difference of opinion between the two researchers, the article was reviewed by a third person. The purpose of the STROBE checklist was to provide recommendations to clarify the design of the implementation method and the findings of observational studies. This checklist has six general sections, title and abstract, introduction, methods, results, discussion, and other information. This checklist evaluates different aspects of the methodology, including sampling methods, variable measurement, statistical analysis to adjust for confounders, and the validity and reliability of the used tools and study objectives. Each part of the checklist was given a score, and at the end, two researchers compared the total scores obtained from the articles. The minimum obtainable score was 15.5. The articles that received the quorum of the quality evaluation score were included in the study. The criteria for the inclusion of articles in the study were articles published in English, their content was to investigate the effect of nutrition on women's fertility, and the original research article was refereed. Figure 1 shown the process of searching and selecting articles.

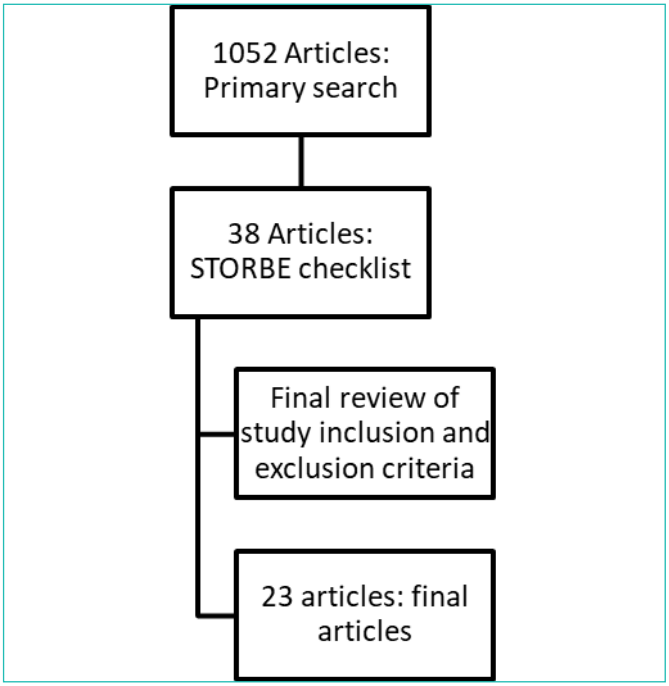

Figure 1. The process of searching and selecting articles

In the beginning, 1052 articles were found, and after reviewing the STROBE checklist, 38 articles were selected. After reviewing the inclusion and exclusion criteria and evaluation, 23 related articles were included in the study.

RESULTS

The results of the reviewed studies are summarized in **Table 1**.

Table 1 lists the results of 23 studies on the relationship between nutrition and infertility in women. The information in this table includes the author(s) of the study, the purpose of the study, the type of study, the sample and location of the study, the method of the study, and the key findings of the studies that examine the relationship between nutrition and infertility.

Table 1. Summary of reviewed studies

Author(s)	Purpose of research	Sample number - location	Method	Results
Mills et al.[11]	Pregnancy loss and iodine levels	347 women - Texas and Michigan	Lab experiment	Low iodine levels associated with highest rate of pregnancy loss
Agrawal et al.[12]	Micronutrient intake in infertile women	104 infertile women - London	Examination of fertility and blood nutrients	Micronutrients during ovulation stimulation can increase ovulation rate
Buhling et al.[13]	Micronutrient intake and female fertility	Germany	Review studies	Micronutrient supplements can increase fertility in infertile women
Paffoni et al.[14]	Vitamin D deficiency and infertility	803 women - Italy	Lab experiment	No negative consequences in surrogate mother's children, regardless of using or not using her eggs
Henmi et al.[15]	Ascorbic acid on serum progesterone levels in women with luteal phase defects	122 women - Japan	Examination of serum progesterone levels	Ascorbic acid and antioxidants can have positive effects on serum progesterone levels
Mills et al.[16]	Fertility delay in women with urinary iodine deficiency	501 Women - United States	FFQ oral frequency questionnaire, lab investigation	Lower urinary iodine-to-creatinine ratio associated with lower likelihood of pregnancy
Lerchbaum et al.[17]	Vitamin D and female fertility	Germany	Review of studies	Vitamin D administration to women with polycystic ovaries increases endometrial lining thickness
Youssef et al.[18]	Oral antioxidants in infertile women with unknown cause	218 women - Egypt	Hormonal examination, ultrasound laparoscopy	Antioxidant intake did not enhance fertility in women without a known cause
Wise et al.[19]	Omega-3 consumption and fertility	1290 women - USA / 1126 women - Denmark	Lab investigation, FFQ oral frequency questionnaire	Decreased omega-3 consumption associated with decreased fertility
Górna et al.[20]	Protein intake and infertility	100 women - Poland	Examination of nutrition and anthropometry	Infertile women had higher intake of calories and protein compared to fertile women
Afeiche et al.[21]	Dairy consumption and in vitro fertilization outcomes	232 women - United States	Questionnaire on eating frequency and examination of ovary and fetus	Increased dairy consumption linked with greater likelihood of achieving live birth
Shishehgar et al.[22]	High protein diet vs standard diet in managing polycystic ovary syndrome	Iran	Review studies	High-protein diet led to improvement of metabolic variables
Phy et al.[23]	Low-starch, low-dairy diet on weight loss among obese individuals	24 women - United States	Measurement of BMI, blood sugar, HbA1, testosterone	Weight loss and testosterone reduction seen with the intervention
Nikokavoura et al.[24]	Very low-calorie diet in promoting weight loss among women	1016 women - England	600 kcal per day with a multivitamin	No significant difference in weight loss outcomes between the two groups
Sadeghi et al.[25]	Differences in food intake, physical activity, and weight between infertile and fertile women	288 women - Iran	Questionnaire and anthropometry	Infertile women had lower physical activity levels and higher intake of certain nutrients compared to healthy women
Ruder et al.[26]	Antioxidant consumption in infertile women with unknown cause	273 women - United States	Food frequency questionnaire	Vitamin E consumption linked with reduction in pregnancy duration in women over 30 years of age. Increased intake of beta-carotene and vitamin C associated with shorter time to conceive in women under 35
Gaskins et al.[27]	Folate intake and fertility in women affected by assisted reproductive treatment	232 women - United States	Food frequency questionnaire	Increased folate intake linked with greater egg replacement and higher chance of achieving live birth
McGrievy et al.[28]	Dietary patterns and nutrient intake of women with polycystic ovary syndrome	46 women - United States	Quality of life, EBI questionnaire, and physical activity questionnaire	Overweight women with polycystic ovary syndrome who were experiencing infertility had a diet lacking in whole grains, fiber, and iron
Rajaeieh et al.[29]	Dairy consumption and polycystic ovary syndrome	400 women - Iran	Food frequency questionnaire	No association between milk consumption and polycystic ovaries
Kazemi et al.[30]	Fat intake and egg quality	236 women - Iran	Food frequency questionnaire	High fat consumption had adverse effect on fetal growth. Eggs low in antioxidants had reduced likelihood of fertilization and egg replacement
Tsai et al.[31]	Dietary habits and nutrient intake in women with polycystic ovary syndrome	206 women - Taiwan	Anthropometry and food consumption questionnaire	Women with polycystic ovary syndrome had lower intake of carbohydrates compared to control group
Twigt et al.[32]	Pre-pregnancy diet and successful continuation of pregnancy in women undergoing IVF/ICSI treatment	199 women - Netherlands	Diet questionnaire	Correlation observed between diet score and probability of achieving pregnancy following IVF/ICSI treatment
Mahoney[33]	Lifestyle modification in overweight and obese women with polycystic ovary syndrome	12 women - United States	Food frequency questionnaire, physical activity survey	Changes in diet and physical activity resulted in regular menstruation

Our results showed that proper nutrition improves women's ovulation parameters and pregnancy outcomes. In general, Table 2 shows the reviewed studies suggestion for diets for women's fertility.

Table 2. Recommended diets for women's fertility

Diet	Description
Balance between protein and carbohydrates	A diet that balances protein and carbohydrates can improve fertility.
Low glycemic index foods	Consuming foods with low glycemic index can improve fertility
High fiber diet	A high fiber diet can improve fertility
Eating five meals a day	Eating five meals a day can improve fertility
Daily consumption of essential fatty acids (omega 3 and 6)	Daily consumption of essential fatty acids, such as omega 3 and 6, can improve fertility
Use of organic materials	Using organic materials can improve fertility
Low-fat proteins	Consuming low-fat proteins can improve fertility
Complex carbohydrates	Consuming complex carbohydrates can improve fertility
Folic acid	Consuming folic acid can improve fertility
Antioxidants and vitamins E, C, and D	Consuming antioxidants and vitamins E, C, and D can improve fertility
Foods that cause regular ovulation	Consuming foods that cause regular ovulation, such as cod liver oil, licorice plant, five-finger plant, thistle, and cinnamon, can improve fertility

DISCUSSION

This study aims to provide a comprehensive survey of articles on preventing infertility in women through proper nutrition. Most of the reviewed studies showed that polycystic ovary is one of the causes of female infertility. Overweight, infertile women with PCOS, who had a diet low in whole grains with fiber and iron,[28,34] and high sugar consumption, along with a low amount of legumes and vegetables,[35] and low consumption of starch and low dairy products were successfully treated for their obesity.[23] Also, weight loss significantly affects the recovery of polycystic ovaries,[22] so moderate weight loss causes recovery in 50% of affected people.[33]

One of the effective foods in treating female infertility is antioxidants and vitamins.[30] Vitamin D was one of the main micronutrients in the studied studies. Vitamin D, also considered a steroid hormone, is received through food in the form of vitamin D2 (ergocalciferol) or vitamin D3 (cholecalciferol) or made in the skin after contact with sunlight. The active form of vitamin D, 1 and 25 dihydroxy vitamin D3 binds to its receptor in different tissues and affects the expression of more than 200 genes, and in this way, it exerts various effects on organs such as parathyroid, pituitary, pancreas, ovary, colon, immune system, and skin.[36]

Vitamin D is present in tissues such as decidua, placenta, ovarian cells, endometrium, and pituitary gland and affects the function of ovarian granulosa cells. It also plays a role in influencing steroidogenesis, fertility, and regulation of the immune system.[37] Recently, the role of vitamin D in the anti-müllerian hormone (AMH) gene expression has been proven in the laboratory environment.[38] In human ovarian tissue, cholecalciferol stimulates the production of progesterone,[13] estradiol,[17] and estrone.[16] Vitamin D is essential in estrogen biosynthesis in female and male gonads.[39-41]

In this regard, the study of Paffoni et al.[14] which aimed to determine vitamin D deficiency and infertility, showed that vitamin D is an influential factor in women's fertility and the outcome of IVF. However, Aleyasin et al.[42] reported no significant relationship between vitamin D levels and IVF outcomes. The study of Anifandis et al.[43] also showed that people with sufficient vitamin D levels are less likely to get pregnant than people with vitamin D deficiency.

German women who had polycystic ovaries and were administered with vitamin D exhibited an increase in their endometrial thickness. Additionally, research has shown that vitamin D has the potential to enhance the ovarian reserve of women in the later stages of their reproductive age.[44] While Rajaeieh et al.[29] found no association between milk consumption and PCOS, other studies.[24,27] have suggested that increased dairy consumption may lead to a greater chance of achieving live birth. The discrepancy between these findings could be due to the differences in the types of low-fat and high-fat dairy products consumed, which were not taken into account during the studies.

Vitamin E was also one of the effective micronutrients in women's fertility in the reviewed studies. Tocopherols, the major forms of vitamin E, protect cell membrane components against oxidation, act as an anti-inflammatory in immunocompromised people, and are neuroprotective. They are fat-soluble, and their deficiency becomes apparent months after the onset of deficiency.[45] In a study, vitamin E supplementation was associated with a shorter time to get pregnant in women over 30 years old, and women under 35 years old had a shorter time to get pregnant with beta-carotene and vitamin C intake.[26]

On the other hand, vitamin C prevents the peroxidation of lipids, revives vitamin E, and protects against DNA damage by H_2O_2 radicals.[46] Ascorbic acid is a crucial component in the biosynthesis of collagen, which is especially important for the growth of ovarian follicles during ovulation and the luteal phase.[47] In ovarian tissue, the concentration of vitamin C is very high.[37] As the study

of Murray et al.[47] showed, the consumption of high doses of ascorbic acid causes a significant increase in tissue-inhibiting metalloproteinases and, as a result, increases the survival of follicles.

According to the results of the Henmi et al.[15] study, which examined the impact of ascorbic acid supplementation on serum progesterone levels in individuals with luteal phase defects, it was observed that the use of ascorbic acid supplements could increase serum progesterone levels among patients with luteal phase defects. Although various micronutrients and antioxidants positively affect infertility treatment.[20] Youssef et al.[18] found that using antioxidants does not increase fertility in infertile women of unknown cause, which may be due to the use of Octatron capsules.

Folate was also another micronutrient of interest in the reviewed studies. Folate, also known as folic acid, is a water-soluble vitamin found in green vegetables, grains, and potatoes. When taken alongside vitamins B6 and B12, folate has been shown to effectively lower blood homocysteine levels.[44] Homocysteine is an amino acid that is indirectly required for protein metabolism. According to studies, low levels of folate and increased levels of homocysteine cause frequent miscarriages. On the other hand, synthetic folic acid folate is oxidized and thus has more stability than folate, which has a bioavailability of nearly 90%.[13] Folic acid is one of the essential and important vitamins for women of childbearing age.[48]

Among the problems associated with folic acid deficiency, we can mention infertility, megaloblastic anemia, increased plasma homocysteine, cancer, and neuropsychiatric disorders.[49] If there is a lack of folic acid during pregnancy, problems such as spontaneous abortion, premature birth, decollement, preeclampsia, and neural tube defects may occur.[50-52] Higher folate levels are associated with higher implantation and live birth.[27]

Iodine was another significant micronutrient in the reviewed studies. Iodine is a rare element that is necessary for the synthesis of thyroid hormones. Iodine exists in various forms of sodium iodide and potassium salts in nature. Any change in thyroid function can reduce sexual activity and fertility.[53] The effect of hypothyroidism on the hypothalamus, pituitary axis, gonads, peripheral metabolism, and sex hormones is undeniable.[54] A prospective cohort study by Mills et al.[11] aimed at pregnancy loss and iodine levels showed that urinary iodine levels were associated with pregnancy loss, and the lower the urinary iodine level, the higher the pregnancy loss rate. Also, the results of a population-based prospective study by Mills et al.[16] which was conducted to examine delaying pregnancy in women with low urinary iodine concentration, showed that improving urinary iodine concentration can increase the fertility of women.

Omega-3 fatty acids were also one of the significant micronutrients in the studies. The three main omega-3 fatty acids are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Because these fatty acids are vital and necessary for the body's regular metabolism, they are considered essential fatty acids.[55] Unsaturated fatty acids are needed as a dietary supplement throughout a person's life, especially during pregnancy, breastfeeding, and old age. Many recommendations exist to include these two fatty acids EPA and especially DHA, in the human diet.[44] Polyunsaturated fatty acids (PUFAs) have been found to possess anti-inflammatory properties that can be attributed to a range of mechanisms, such as eicosanoid metabolites, thromboxane, prostaglandins, leukotrienes, and prostacyclin. Wise et al.[19] study, which aimed to determine dietary fat intake and fertility in two Danish and American women groups, showed a positive and significant relationship between omega-3 fatty acid consumption and fertility. Nevertheless, in the study of Stanhiser et al.[56] the average ratio of omega-3 to omega-6 and omega-6 to omega-3 was not significantly different between fertile and infertile groups. Moreover, there was no relationship between pregnancy and the concentration of but-3 fatty acids, including alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, or omega-6 fatty acids linoleic acid (LA). Also, there was no significant relationship between the individual's serum concentration of omega fatty acids and the chance of miscarriage adjusted for age.

While physical activity and a healthy diet have been shown to enhance fertility outcomes, it is crucial to identify an appropriate dietary regimen for the management of fertility-related issues.[16] Certain foods have been recommended to improve women's fertility, indicating that infertile women should incorporate a proper diet into their treatment plans.

Our study contributes to the literature by comprehensively reviewing the evidence regarding the role of nutrition in women's fertility. The findings indicate that diet interventions focused on adequate intake of key micronutrients can benefit clinical practice for infertility prevention and management. The limitation of the present study is that due to the simultaneous consumption of food, it was not possible to investigate the effect of individual foods on women's fertility.

CONCLUSION

Many studies reported a positive effect of micronutrients on women's fertility and stated that insufficient micronutrients could affect women's fertility adversely. Also, it seems that correct nutritional planning effectively supports and provides nutritional needs and has favorable effects on women's reproductive health in the short and long term. On the other hand, providing education and training on proper nutrition and providing advisory programs and nutritional counseling for women of childbearing age could be effective in preventing women's infertility. For this reason, since infertility treatments are costly and many people are not covered by insurance for this type of treatment, it seems that preventing fertility problems and achieving reproductive health with a correct diet and the use of complementary medicines containing micronutrients can have a positive and beneficial effect on women's reproductive health. More controlled clinical trial studies in this regard are recommended to achieve more robust and reliable results.

ETHICAL DECLARATIONS

Referee Evaluation Process: Externally peer reviewed.

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Review

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Does melatonin as an antioxidant and anticancer agent potentiate the efficacy of curcumin?

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ABSTRACT

Melatonin is a hormone that exhibits many bioactivities such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic, neuroprotective, and anti-aging activity. In addition, melatonin has been used to strengthen the effect of drugs or agents used for treatment in many different disease models such as cancer, tumor, bacterial infection, autoimmune diseases, and gastrointestinal diseases. According to recent studies, curcumin is used as a pigment and powerful antioxidant and anti-inflammatory agent with therapeutic potential against various cancers. Melatonin and curcumin have been used in different models of disease or injury: such as Alzheimer's disease treatment, testicular tissue preservation, bladder cancer treatment, gastric mucosal damage, and prevention of nephrotoxicity. This review covers published literature studies on the effects of comparison or combined use of melatonin and curcumin as anticancer and antioxidant agents and it reveals whether melatonin potentiates the effect of curcumin when used together.

Keywords: Melatonin, curcumin, antioxidant, anticancer, apoptosis

INTRODUCTION

Melatonin

Melatonin is a hormone with strong antioxidant properties secreted from the cells of the pineal gland called pinealocytes, effective in determining biorhythm and the circadian rhythm.[1] Apart from its antioxidant activity, it displays many bioactivities such as anti-inflammatory properties, enhancing immunity, creating anti-carcinogenic effects, cardiovascular protection, protection against diabetes and obesity, and neuroprotective and anti-aging activity.[2] It has been noted that melatonin is also found in the leaves, fruits, and seeds of plants. Melatonin, whose secretion is adjusted according to the length of the night, is the hormone responsible for giving the body information about the light and dark cycle. The main physiological functions of melatonin are hormonal however, it may also exhibit autocrine or paracrine properties in the retina or intestine.[3] Today, the role of melatonin in human physiology and the treatment of many diseases has been proven. However, many functions and effects of melatonin still await discovery. According to the study by Reiter et al.[4] melatonin seems to reduce the toxic effect on the tissue and increase the effectiveness of the active substance with which it is used together. In addition, melatonin has been used to strengthen the effect of a drug or agent used for treatment in many different disease models such as cancer, tumor, bacterial infection, autoimmune diseases, and gastrointestinal diseases. As a result of the research of Qi et al.[5] it was noted that melatonin had an inhibitory effect on ferric nitrilotriacetate Fe-NTA-induced oxidative DNA damage. Liu et al.[6] reported in their study that melatonin was also effective on cerebral I/R. Melatonin prevents the harmful effects of free oxygen radicals formed in the tissue during reperfusion in the renal ischemia-reperfusion model. Apoptosis is seen especially in proximal tubule epithelial cells in renal ischemia-reperfusion injury, and melatonin has been shown to have a protective effect on renal tubular dysfunction.[7] Guzel Tanoglu et al.[8] reported that in an experimental chronic pancreatitis rat model, melatonin has shown to have protective properties, improving inflammation, oxidative stress, and pancreatic fibrosis. Another study shows that melatonin modulates by reversing the adverse effects of diabetes on NK-cell activity, which has a protective function in inflammatory and immunological processes.[9] Sapmaz et al.[10] investigated the effects of melatonin, oxytetracycline, and N-acetylcysteine on ovarian follicle reserves and surface epithelium in autologous intraperitoneal ovarian transplantation in

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rats. It was concluded that oxytetracycline and melatonin may be more effective than N-acetylcysteine in protecting against oxidative stress during ovarian transplantation. Melatonin is used for the prevention and treatment of several cancers e. g. breast cancer, gastric cancer, prostate cancer, colorectal cancer.[11]

Curcumin

Curcumin turmeric is a compound obtained from the rhizome of the Curcuma longa plant, which is widely used as a spice in Asia and is also used as a pigment.[12] Curcumin is an easily available antioxidant substance, inexpensive and available all over the world. It is considered a therapeutic agent used in a variety of foods in traditional and complementary medicine. Curcumin, which is widely consumed in traditional recipes in the Indian Subcontinent, South Asia, and Japan, is a potent anti-inflammatory agent against various cancers, according to recent studies.[13]

Curcumin has been used in many different studies as an antioxidant, anti-inflammatory, and anticancer agent. In the review prepared by Trujillo et al.[14] it was shown in the chronic kidney failure model that curcumin exerts a therapeutic effect. In this study, the renoprotective effect of curcumin was collected under the following headings: It helps inhibition of mitochondrial dysfunction, weakening of the inflammatory response, protection of antioxidant enzymes, and prevention of oxidative stress. The information presented in this article identifies curcumin as a promising molecule against kidney damage. In a review on curcumin doses prepared by Shoba et al.[15], it was stated that curcumin is safe at high doses e. g. 12 g/day in humans, 2 g/kg in rats and does not show any undesirable effects. As a result of all these studies, curcumin is a substance suitable for use as an antioxidant and anticancer agent in many diseases because it is not toxic even at high doses and is an easily accessible active substance.

ANTIOXIDANT POTENTIALS OF MELATONIN AND CURCUMIN

Nervous System

Alzheimer's disease: Melatonin is known as the sleep-regulating hormone and has a neuroprotective effect. Because of this neuroprotective feature, melatonin has been used as a therapeutic agent in many diseases of the brain Alzheimer's disease AD, Huntington's Disease HD. AD is a neurodegenerative disease that is seen in memory and cognitive impairments and develops with a sleep disorder. Sleep disturbance SD is a disease also seen in AD patients and has been shown to increase memory, behavioral and cognitive complications.[16] AB peptide, which is one of the important factors in the etiology of AD, accumulates in the brain of AD patients as a result of production and clearance imbalance. Researchers speculate that this leads to the formation of neurotoxic Aβ oligomers.[17] Melatonin hormone levels have been observed to decrease in the preclinical stages of AD, so it can be said that melatonin deficiency occurs in AD.

Studies are carried out to prevent or delay the onset of neurodegenerative diseases with the use of curcumin. These diseases include Alzheimer's disease, stroke, Huntington's disease, Parkinson's disease, Multiple Sclerosis, Prion disease, Down syndrome, anxiety, autism, Amyotrophic lateral sclerosis, depression, and aging. In one study, Z-CM-I-1, one of the curcumin and melatonin hybrid compounds with potential therapeutic effects for AD, was used in an APP/PS1 transgenic AD model. As a result of this in vivo study, it was shown that this hybrid compound exhibited functional properties on AD pathologies.[18] In another study, Chojnacki et al.[19] designed hybrid compounds of two natural products, curcumin, and melatonin, these hybrid compounds were synthesized and biologically characterized. It has been stated that optimization of a new hybrid structure named 5-4-hydroxyphenyl-3-oxo-pentanoic acid 2-5-methoxy-1H-indol-3-yl-ethyl-amide can be effective on AD by strengthening it.

In another study, unsaturated anionic membranes made of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine POPC and 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine DMPS in Alzheimer's disease used. In this way, it is aimed to delay the progression of Alzheimer's disease. As a result of this study, in which melatonin, acetylsalicylic acid ASA, and curcumin substances were used to examine the membrane active molecule, researchers think that the progression of Alzheimer's disease can be delayed by changing the anionic unsaturated lipid membrane properties.[20] In one study, Espargaró et al.[21] reported an in vitro cell-based assay to evaluate the potential anti-aggregation activity of putative Aβ aggregation inhibitors. In this study, which analyzed the anti-Aβ aggregation activity of rosmarinic acid, melatonin, o-vanillin, curcumin, apigenin, and quercetin it was stated that curcumin had an inhibitory effect.

Huntington's disease: Huntington's disease HD is seen as a result of the disorder in the sleep-wake order and the lack of motor ability accompanying the disturbance of circadian behavior. Changes in melatonin levels have been noted in diseases Huntington's disease, multiple sclerosis, and cerebral ischemia in which severe ischemia occurs and, as a result, neuronal cells die within minutes. This shows the potential of melatonin to be therapeutic for these diseases. Neurodegenerative diseases cannot be completely cured with any drug today, treatments are often used to reduce the severity of disease symptoms. As an example, curcumin is preferred in HD to stop the progression of neurodegeneration. This antioxidant works

by targeting the reduction of oxidative and inflammatory stresses, metal ion chelation, and transcriptional changes. In a study looking at the effect of melatonin and curcumin in a Drosophila model of HD, mRNA expression of the genes of the transcriptional feedback TF loop, which creates rhythmicity for approximately 24 hours, was examined to examine the circadian timing. As a result of this study, researchers thought that melatonin and curcumin could be potential therapeutic agents for the treatment of HD in humans.[22]

Hippocampus: The hippocampus is the brain region that manages various functions of the body. It has been shown in various studies such as Alzheimer's Disease, and Parkinson's Disease that the lower region of the hippocampus is particularly affected by ischemia.[23] In vivo studies have reported that curcumin has the potential to increase the expression of various detoxifying enzymes e. g. glutathione-S-transferase, superoxide dismutase, GSH reductase and decrease glutathione in the brain.[23] In a study conducted by creating a chronic Gulf War Illness rat model, the effectiveness of melatonin was examined to improve cognitive and mood function with antioxidant, anti-inflammatory, and pro-cognitive effects. In this study, it was shown that melatonin increases neurogenesis in the hippocampus and decreases synapse loss.[24]

In light of these studies, it can be said that melatonin and curcumin have neuroprotective effects. However, there is a need for further studies on the mechanisms of neuroprotective effects in the brain. In a study where melatonin and curcumin were used together, the effects of treatment with these antioxidant substances on oxidative stress parameters, SIRT2, Bcl-2, and Bax expression in the hippocampus were investigated. It has been stated that melatonin can reduce cell apoptosis in the hippocampus by decreasing oxidative stress and SIRT2 expression and increasing Bcl-2 expression.[25]

Sciatic nerve crush injury: The aim of another study examining peripheral nerve injury, which is one of the acute clinical problems, in rats was to compare the effects of melatonin and curcumin on sciatic nerve crush injury repair. As a result of the study, it was stated that these two antioxidant substances accelerate nervous recovery and help treat nerve injury.[26]

Fetal rhombencephalic neurons: Antonio et al.[27] examined the effects of --epigallocatechin-3-gallate, polyphenol, curcumin, resveratrol, melatonin, and α-lipoic acid against ethanol-related apoptosis in fetal rhombencephalic neurons in vitro and investigated the potential neuroprotective effects of these antioxidant substances. As a result of this study, the researchers stated that these antioxidant substances can exert neuroprotection against ethanol-related apoptosis in fetal romencephalic neurons.

Neurodegeneration and depression: Olfactory bulbectomized is an established disease of depression and cognitive decline. In the study by Borre et al.[28] an experimental method consisting of zinc, melatonin, curcumin, piperine, eicosapentaenoic acid EPA, 20:5, n-3, docosahexaenoic acid DHA, 22:6, n-3, uridine, and choline, the diet, was administered to olfactory bulbectomized rats. The findings in rats on this diet were as follows: reduction in glutamate excitotoxicity, potent antioxidant/anti-inflammatory effect, and improved synaptogenesis. In rats fed this diet, decreased cognitive and behavioral deficits were noted. At the end of this study, in which more than one disease etiology was investigated at the same time, Borre et al.[28] say that the development of neurodegenerative and depressive disorders and accompanying cognitive deficits can be prevented with this diet.

Kidney

Kidney ischemia-reperfusion injury: Ischemia is a condition in which oxygen cannot reach an organ in the body for any reason. With the disappearance of ischemia, the blood flow entering the organ is called reperfusion. Renal ischemia/reperfusion injury increases the risk of mortality and morbidity and causes permanent damage to the organ, especially due to the oxidative stress it causes during the reperfusion phase. In a study investigating how renal ischemia-reperfusion damage affects the ovaries as a distant organ in rats and the effects of melatonin, curcumin, and combined melatonin-curcumin treatments on ischemia-reperfusion; It has been stated that all treatments partially preserve the histological structure. However, especially melatonin treatment appears to be more effective than curcumin treatment.[29]

Nephrotoxicity: In a study investigating the effect of the combination of melatonin and curcumin on cisplatin-induced nephrotoxicity in rats, the following results were found: According to the report of Ali et al.[30] when curcumin and melatonin were given together, tumor necrosis factor-alpha, cystatin C, uric acid, phosphorus in plasma and creatinine in urine and creatinine clearance values improved. It has been stated that the renal platinum concentration decreases much more when these two active substances are given together than when they are given separately.

Acute kidney injury: In another study investigating the effects of cisplatin on the kidney, they stated that thanks to the antioxidant properties of amifostine, curcumin, and melatonin, they could be a promising agent in the treatment of malignant tumors and the prevention of acute kidney disease caused by cisplatin.[31]

Renal oxidative damage: In another study investigating the antioxidant effect of curcumin, quercetin, melatonin, and resveratrol on ferric nitrilotriacetate Fe-NTA

induced oxidative kidney damage in rats, the amount of lipid peroxidation induced by Fe-NTA was found to be significantly suppressed by these antioxidant substances.[32]

Testis

Testicular tissue preservation: In the study by Muratoglu et al.[33] the effect of melatonin and curcumin on testicular tissue in rats was investigated. According to the results of this study, the total testes/body weight ratio of the group given melatonin in rats decreased significantly compared to the control group. In addition, a significant increase in glutathione levels was noted in rats treated with curcumin compared to rats in the control group. It was noted that the number of apoptotic cells determined by the TUNEL method decreased significantly in the group treated with melatonin and curcumin. However, it was stated that melatonin and curcumin treatment significantly increased the Johnsen score.

Bone

Rapid maxillary expansion: In another study, researchers used the rat model to investigate the effects of curcumin and melatonin on new bone formation following rapid maxillary enlargement. According to the results of this study, the researchers stated that new bone formation was accelerated in rats treated with curcumin and melatonin.[34]

Liver

Age-related liver injury: In a study of the age-related carbonyl content of the liver modeled in mice, the effects of melatonin and curcumin were examined. The results of this study, which was performed separately in young 1 month and old 18 months mice, showed that protein carbonyl formation in the liver of old mice increased compared to young mice, but the use of melatonin and curcumin decreased this value.[35]

Cold hepatic ischemia-reperfusion: In another study on the effect of multi-drug donor preconditioning MDDP: Curcumin, simvastatin, N-acetylcysteine, erythropoietin, pentoxifylline, melatonin, glycine, and methylprednisolone on cold ischemia-reperfusion injury in a rat liver perfusion model, MDDP inhibited inflammation. and it is stated that it can completely prevent parenchymal damage. Researchers say that this MDDP, in which melatonin and curcumin are used together, can be used in clinical liver transplantation.[36]

Stomach

Gastric mucosal damage: In gastric mucosal damage, a disease associated with extracellular matrix degradation in which matrix metalloproteinases play a role, conditions such as remodeling of connective tissues and loss of tissue integrity are seen. The same situation is observed in gastric ulcers, which is one of the inflammatory diseases. In gastric ulceration, reactive oxygen species are formed and transcription and translation of metalloproteinases are decreased.[37] In a study to determine the mechanism of suppression of the activity of metalloproteinases by reactive oxygen species in acute ulceration disease and also to examine some antioxidants including melatonin and curcumin during healing, it was noted that melatonin blocked the formation of reactive oxygen species, protein oxidation, mucosal cell disruption and down-regulation of metalloproteinases. In vitro studies have shown that suppression of metalloproteinase activity by H_2O_2 is blocked by melatonin and curcumin. As a result of these studies, the researchers emphasized that melatonin and curcumin inhibit H_2O_2-mediated inactivation and down-regulation of the expression of metalloproteinases at the onset of ulceration.[37]

ANTICANCER POTENTIALS OF MELATONIN AND CURCUMIN

Nervous system

Pc12 cells: Cellular oxidative stress and changes in redox metabolisms play a role in the etiology and pathology of many diseases, including cancer. Antioxidant therapies have proven beneficial in controlling these diseases. 4-hydroxynonenal 4-HNE, a byproduct of lipid peroxidation, induces oxidative stress in PC12 cells by damaging mitochondrial redox metabolism. In a study investigating the effects of 4-HNE on mitochondrial respiratory functions and apoptosis in the PC12 cell line, the effects of curcumin and melatonin were also compared. As a result of this study, researchers stated that melatonin and curcumin have a protective effect on mitochondrial functions.[38]

Bladder

Bladder cancer: Melatonin has been used in many different studies to increase the effect of the active substance it is applied together. In one study, researchers examined whether the effect of curcumin on bladder cancer cells would be altered when used with melatonin. According to the results of the research, melatonin increased the anti-proliferation, anti-migration, and pro-apoptotic activities of curcumin. The combination of melatonin and curcumin has been noted to exert an inhibitory effect against the growth of bladder cancer.[39]

Prostate

Prostate cancer: Rodriguez-Garcia et al.[40]'s study, in which curcumin, resveratrol, melatonin, and silibinin were used, investigated their effects on prostate cancer cells. As a result of the study, the researchers noted that

melatonin and silibinin inhibited cell growth. In addition, curcumin and resveratrol have been shown to induce apoptosis in prostate cancer cells.

CONCLUSION

Unlike many substances with antioxidant properties, melatonin has a high inducible capacity to scavenge free radicals, even under stressful conditions. Melatonin has secondary and tertiary metabolites, which can neutralize too many toxic oxygen derivatives. An antioxidant substance can usually scavenge 1 reactive oxygen derivative, while melatonin can scavenge 10 reactive oxygen derivatives. In addition, melatonin has been used as a second active substance in many studies, because melatonin strengthens the antioxidant, anti-inflammatory, and anti-carcinogenic effects of the other active substance. Melatonin is used as a therapeutic agent against many diseases due to its properties and a substance naturally found in the body.

Like melatonin, curcumin is an active ingredient with antioxidant properties. In addition, it is frequently used in various chronic inflammatory diseases with its anti-inflammatory properties. In addition, curcumin, which has anti-carcinogenic properties, is also used to prevent/treat cancer. If we look at curcumin from a biological point of view, its non-toxicity even at high doses and long-term use ensures its safe use in in vivo studies. In addition, curcumin, which releases the active free thiol group in the target tissue, inhibits the growth of cells. This ensures that fast-growing tissues such as cancer are kept under control. In addition to these properties, the low half-life of curcumin can be considered an obstacle to its use. Researchers continue to work towards overcoming this obstacle. In light of all this information, we can say that the treatment of curcumin for various chronic inflammatory diseases or cancer is promising.

The ability of melatonin to potentiate the effect of a second antioxidant has also been shown in various studies when used together with curcumin (**Tables 1**, **2**, and **3**). In this review, studies in which melatonin and curcumin, which have antioxidant and anticarcinogenic properties, were compared or used together, were compiled. As a result of all studies, it can be said that these two active substances are more effective when used together, creating a synergistic effect, compared to their separate use. However, the articles published in the literature on the combined use of these two active substances are limited. We think more studies should be done with the combination of melatonin and curcumin, and this combination has a clear path to preventing and treating diseases.

Table 1. The antioxidant effects of melatonin and curcumin in various in vitro and in vivo nervous system studies at IRI

Antioxidant potentials	Type of tissue/ Disease	Dose	Outcome	In vivo/ In vitro	Study
Nervous system	Alzheimer's Disease	Concentrations of 5 mol% (drug-to-peptide ratio)	Melatonin did not change the structural parameters of the membranes and did not impact the size or extent of peptide clusters Curcumin made membranes softer and thinner Curcumin reduced the volume fraction of cross-β sheets by ~70%	Unsaturated anionic membranes	Khondker et al.[20]
	Huntington's Disease	Melatonin (50, 100, or 150 µg) or curcumin (10 µM) in the diet commencing from the larval stage	Both melatonin (100 µg) and curcumin reestablished the 24-h pattern in mRNA expression of Period and Timeless to normal (control) levels, and significantly improved both locomotion ability and eclosion behavior of HD flies	the Drosophila model	Khyati et al.[22]
	Hippocampus	Melatonin (10 mg/kg/day, s.c. for 30 days), Curcumin (30 mg/kg/day, i.p. for 30 days)	Melatonin and curcumin significantly decreased MDA and SIRT2 expression in the hippocampus ($p < 0.05$) A significant increase in the GSH levels of curcumin-treated group and melatonin-treated group Melatonin, but not curcumin, significantly increased the Bcl-2 expression of the hippocampal region. There was a significant correlation between SIRT2 and MDA levels ($p < 0.05$).	Rats	Keskin-Aktan et al.[25]
	Sciatic Nerve Crush Injury	IP injections of curcumin (100 mg/kg) and melatonin (10 mg/kg) over two periods of light (9:00 a.m.) and dark (9:00 p.m.) for 4 weeks	No statistically significant difference was identified between dark and light curcumin groups while curcumin groups displayed better results than did melatonin groups Dark melatonin group displayed better results than the light melatonin	Rats	Moharrami Kasmaie et al.[26]
	Fetal Rhombencephalic Neurons	1 µM melatonin, 1 µM curcumin	Co-treatment of these cultures with melatonin and curcumin prevented ethanol-associated apoptosis	Cultures of fetal rhombencephalic neurons	Antonio et al.[27]
	Neurodegeneration and Depression	Dietary treatment (containing melatonin and curcumin) started 2 weeks before olfactory bulbectomized surgery, continuing for 6 weeks in total	The experimental diet reduced hippocampal atrophy and decreased the peripheral immune activation in the olfactory bulbectomized rats The ameliorating effects of the diet on the olfactory bulbectomized-induced changes were comparable to those of the NMDA receptor antagonist, memantine, a drug used for the management of Alzheimer's disease	Rats	Borre et al.[28]

Table 2. The antioxidant effects of melatonin and curcumin in various in vitro and in vivo studies at IRI

Antioxidant potentials	Type of tissue/ Disease	Dose	Outcome	In vivo/ In vitro	Study
Kidney	Nephrotoxicity	Curcumin (200 mg/kg) or melatonin (10 mg /kg) given singly by oral gavage for eight consecutive days prior to CP injection and four days thereafter	Curcumin and melatonin were given together, the ameliorative effect was augmented in some of the measured indices e.g. tumor necrosis factor alpha, cystatin C, uric acid, phosphorus in plasma and, urine creatinine and creatinine clearance Renal platinum concertation was reduced more with curcumin than that with melatonin, while the reduction was maximized when both melatonin and curcumin were given.	Rats	Ali et al.[30]
	Acute Kidney Injury	Oral CMN at 200 mg/kg/day dissolved in freshly prepared corn oil over a total of 5 days, 5 mg/ kg/day melatonin dissolved with saline solution IP for 5 days	Curcumin and melatonin reduced the increases in serum urea and serum creatinine levels following cisplatin administration ($p < 0.05$) Curcumin and melatonin reduced the levels of TNS, HPS, NF-κB/p65, 8-OHdG, and caspase-3 expressions ($p < 0.05$)	Rats	Mercantepe et al.[31]
Testis	Testicular Tissue Preservation	Melatonin (10 mg/kg, s.c.); Curcumin (30 mg/kg, i.p.)	Melatonin treatment for aged rats significantly decreased paired total testicular/body weight ratio compared to aged control group ($p < 0.05$) Curcumin treatment for aged rats significantly increased GSH level compared to the aged control group ($p < 0.05$)	Rats	Muratoğlu et al.[33]
Bone	Rapid Maxillary Expansion (RME)	Melatonin 75 mg/d/kg and Curcumin 150 mg/d/kg by intraperitoneal injection during the whole study period	Serum bone alkaline phosphatase levels in the melatonin group were statistically (P=.007) higher than in the expansion group Serum glutathione peroxidase and catalase activities in the curcumin and melatonin groups were significantly higher than in the expansion group (P=.007 and P=.021, respectively) Inflammatory cell infiltration, new bone formation and capillary intensity parameters did not demonstrate statistically significant differences between the groups (P=.865, P=.067 and P=.055, respectively) The immunohistochemical findings revealed that IL-1, IL-6 and TNF-α H scores showed considerable differences between the groups (all P < .001) The highest IL-1, IL-6 and TNF-α H scores were found in the expansion groups rather than in the other groups (P < .001)	Rats	Cesur et al.[34]
Liver	Age-related Liver Injury	Melatonin (10 mg/kg body weight) and curcumin (90 mg/ kg body weight) in dimethyl sulfoxide intraperitoneally.	Protein carbonyls of liver have been found to be significantly higher in 18-month-old mice as compared to 1-month-old mice The carbonyl content in 1- and 18-month-old mice decreases significantly upon administrations of melatonin and curcumin	Mice	Dkhar et al.[35]
Stomach	Gastric Mucosal Damage	Melatonin (60 mg/kg bw) curcumin (60 mg/kg bw) intraperitoneally (ip) 30 min before indomethacin treatment	Melatonin and curcumin offered gastroprotection in vivo by upregulation of suppressed MMP-2 expression and activity at the level of secretion and synthesis Antioxidants reversed the suppression of MMP-2 expression by upregulation of MT1-MMP and downregulation of TIMP-2	Rats	Ganguly et al.[37]

Table 3. The anticancer effects of melatonin-curcumin in various in vitro and in vivo studies at IRI

Anticancer potentials	Type of tissue/ Disease	Dose	Outcome	In vivo/ In vitro	Study
Nervous system	PC12 cells	25 μM curcumin for 16 h / 100 μM melatonin for 16 h.	Curcumin and melatonin treatments maintained the mitochondrial redox and respiratory functions without a marked effect on ROS production and cell viability	PC12 cell line	Raza et al.[38]
Bladder	Bladder Cancer	Appropriate dosage	Combinational treatment enhanced the repression of nuclear translocation of NF-κB and their binding on COX-2 promoter via inhibiting IKKβ activity, resulting in inhibition of COX-2 expression Combined treatment with curcumin and melatonin induced cell apoptosis in bladder cancer through enhancing the release of cytochrome c from the mitochondrial intermembrane space into the cytosol	Human bladder cancer cell lines T24, UMUC3 and 5637	Shrestha et al.[39]
	Bladder Cancer	10 mg/kg melatonin and 30 mg/ kg curcumin by intraperitoneal injection each day	Combinational treatment enhanced the repression of nuclear translocation of NF-κB and their binding on COX-2 promoter via inhibiting IKKβ activity, resulting in inhibition of COX-2 expression. Combined treatment with curcumin and melatonin induced cell apoptosis in bladder cancer through enhancing the release of cytochrome c from the mitochondrial intermembrane space into the cytosol	Mice	Shrestha et al.[39]
Prostate	Prostate Cancer	Curcumin (1.6–25 μM), melatonin (0.062–1 mM)	These compounds affect differently one of the main intracellular redox regulator, the thioredoxin system. Exposure to curcumin promoted TRX1 oxidation and altered its subcellular location. Conversly, melatonin only worked as cytostatic agents, reducing ROS levels and showing preventive effects against TRX oxidation.	Human androgen-dependent epithelial prostate cancer cells (LNCaP)	Rodriguez-Garcia et al.[40]

HIGHLIGHT KEY POINTS

Melatonin has been used as a second active substance in many studies because melatonin strengthens the antioxidant, anti-inflammatory, and anti-carcinogenic effects of the other active substance.

The treatment of curcumin for various chronic inflammatory diseases or cancer is promising.

The ability of melatonin to potentiate the effect of a second antioxidant has also been shown in various studies when used together with curcumin (**Tables 1**, **2**, and **3**).

Melatonin and curcumin are more effective when used together, creating a synergistic effect, compared to their separate use.

ETHICAL DECLARATIONS

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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