E-ISSN: 2149-9063

Year: 2023 June Volume: 24 Issue: 2





MEANDROS MEDICAL AND DENTAL JOURNAL

THE OFFICIAL JOURNAL OF ADNAN MENDERES UNIVERSITY FACULTY OF MEDICINE AND DENTISTRY

Citation Abbreviation: Meandros Med and Dental J (Formerly Adnan Menderes Üniversitesi Tıp Fakültesi Dergisi)



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Publishing Date: July 2023 E-ISSN: 2149-9063 International scientific journal published quarterly.



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Periodontal and Periapical Effects of Severity of Fremitus Due to Chronic Occlusal Trauma on Mandibular Incisors

Kronik Okluzal Travmaya Bağlı Fremitus Şiddetinin Mandibular Kesiciler Üzerindeki Periodontal ve Periapikal Etkileri

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Abstract

Objective: Excessive occlusal force is defined as the force that exceeds the repair capacity of periodontal attachment and causes occlusal trauma. Fremitus is an important clinical sign of the tooth exposed to occlusal trauma, defined as functional mobility that can be seen or palpated when subjected to occlusal forces. The aim of this study was to determine the effect of fremitus on the periapical and periodontal tissues of the mandibular incisors.

Materials and Methods: This study evaluated 1,004 mandibular incisors of 251 healthy individuals between the ages of 18–65. The presence or absence of fremitus was determined using the fremitus test by dividing individuals into 3 study groups (severe-fremitus, mild-fremitus, and absence-fremitus). Clinical and radiographic parameters such as gingival recession, tooth mobility, attrition, percussion, thermal hypersensitivity, crowded teeth, deep overbite, indication of root canal treatment, disruption in the lamina dura, periodontal ligament anomalies, and triangulation were recorded. Relationships between these parameters and fremitus groups were compared.

Results: Age and gender distribution, the need for root canal treatment, teeth with percussion, thermal hypersensitivity, mobility, deep overbite, crowded teeth, number of teeth whose periodontal ligament space could not be seen normally, and lamina-dura disruption were significantly higher in the severe-fremitus group compared to the non-fremitus (p<0.05) and mild-fremitus (p<0.05) groups. All three groups were significantly different in terms of triangulation, attrition, the presence of gingival recession, and the amount of gingival recession in mm (p<0.05).

Conclusion: Periodontal, pulpal, and periapical tissues are negatively affected by severe fremitus. Excessive occlusal forces on the mandibular incisors increased the incidence of clinical and radiographic anomalies and pathological findings.

Keywords: Fremitus, occlusal trauma, diagnosis, mandibular incisor

Öz

Amaç: Aşırı oklüzal kuvvetler, periodontal ataçmanın tamir kapasitesini aşan ve oklüzal travmaya neden olan kuvvetlerdir. Fremitus, okluzal travmaya maruz kalan dişin gözle görülebilen veya palpe edilebilen fonksiyonel hareketliliği olarak tanımlanan önemli bir klinik belirtisidir. Bu çalışmanın amacı mandibular kesici dişlerin periapikal ve periodontal dokularına fremitusun etkisini belirlemekti.

Gereç ve Yöntemler: Bu çalışmada 18-65 yaş arasındaki 251 sağlıklı bireyin 1.004 mandibular kesici dişi değerlendirildi. Fremitus testi ile fremitusun varlığı veya yokluğu üç grubuna ayrılarak belirlendi (şiddetli fremitus, hafif fremitus ve fremitus yok). Dişeti çekilmesi, diş mobilitesi, atrizyon, perküsyon, termal aşırı hassasiyet, çapraşıklık, şiddetli derin kapanış, kök kanal tedavisi ihtiyacı, lamina durada bozulma, periodontal ligament anomalileri ve triangulasyon gibi kaydedilen klinik ve radyografik parametreler ile fremitus grupları arasındaki ilişki kıyaslandı.

Bulgular: Yaş ve cinsiyet dağılımı, kanal tedavisi ihtiyacı, perküsyon, termal aşırı hassasiyet, diş mobilitesi, şiddetli derin kapanış, çapraşıklık, periodontal ligament aralığı normal görülemeyen ve lamina dura bozulması olan dişlerin sayısı şiddetli fremitus grubunda

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) fremitus olmayan (p<0,05) ve hafif fremitusu (p<0,05) olan gruplara göre anlamlı olarak daha yüksekti. Üç grup da triangülasyon, atrizyon, dişeti çekilmesi varlığı ve dişeti çekilmesinin miktarı (mm) açısından anlamlı düzeyde farklıydı (p<0,05).

Sonuç: Periodontal, pulpa ve periapikal dokular şiddetli fremitustan olumsuz etkilendi. Mandibular kesici dişler üzerindeki aşırı oklüzal kuvvetler klinik ve radyografik anomalilerin ve patolojik bulguların insidansını artırdı.

Anahtar Kelimeler: Fremitus, oklüzal travma, tanı, mandibuler kesici

Introduction

Excessive occlusal force is defined as the force that exceeds the repairing capacity of periodontal attachment and causes occlusal trauma (1). The injury that causes tissue changes in the attachment supporting the periodontal ligament, alveolar bone and cementum is referred to as occlusal trauma. Some clinical and radiographic indicators used for the diagnosis of occlusal trauma are: Progressive tooth mobility, fremitus, occlusal disharmonies, gingival recession, wear facets, tooth migration, tooth fracture, thermal hypersensitivity, root resorption, and widening of periodontal ligament space (2). It is important to identify these findings with differential diagnoses. For example, pulp vitality tests, tooth brushing habit, severity of periodontal disease and other parafunctional habits should be evaluated.

Fremitus is an important clinical sign of the tooth exposed to occlusal trauma, defined as functional mobility that can be seen or palpated when subjected to occlusal forces (3). As a result of continuous occlusal trauma that exceeds the adaptive capacity of the periodontium, such as the presence of fremitus in a tooth, the density of the alveolar bone decreases while the width of the periodontal ligament space increases. This expansion occurs either in the form of triangulation at the top of the alveolar crest or along the entire width of the periodontal ligament space, in that connection dental mobility also increases (4).

Due to the limitations of the clinical diagnosis and ethical concerns of occlusal trauma, many clinical studies have focused on teeth with occlusal dysharmonies, as in our study. The relationship between the cusps is an important factor in the transmission of occlusal forces to the periodontium (5). In patients with periodontitis who had obvious occlusal trauma symptoms, including fremitus and widening of the periodontal ligament space, it showed greater probing depth, clinical attachment loss and bone loss, (2) and worsening prognosis over time (6). Extreme functional stress can initiate inflammatory changes in the periodontium, thereby increasing destructive bacterial processes (7). Occlusal therapy slows down the progression of periodontitis and can improve prognosis.

There is a consensus that trauma from occlusion may be a co-destructive factor to periodontal destruction, particularly the destruction of the supportive alveolar bone (8). However, its role in influencing marginal gingival tissues is controversial. Gingival recession increases with age (9) and the mandibular anterior region is a frequently affected area. This problem often promotes root caries, poor aesthetics

and increases dentin hypersensitivity (10). Tissue trauma caused by strong toothbrushing, dental malpositions, high muscle attachment, frenum pull, restorative-periodontal treatment procedures, incisor teeth inclination, orthodontic treatments, calculus and iatrogenic factors have been associated with recession of the gingiva (11). Stillman (1) was the first to describe the narrow triangular shaped gingival recession on the facial surface of the tooth. It has been suggested that excessive occlusal forces have been a causal factor in the development of abraction and gingival recession for a long time (1,12,13).

The anatomical and functional presence of the teeth on the arch is important for normal occlusion and healthy dentition. Occlusal trauma leads to changes in the pulp and dentin tissues. The reaction of the pulp to traumatic forces is generally in the form of classical inflammatory responses, similar to the reaction of other connective tissues in the body such as inflammation and fibrous calcification (14). Depending on the intensity and duration of the applied forces and the immune response capacity of each individual, pathological modifications may occur in the pulp tissue or the tissue may be forced to protect itself (14). Nunn and Harrel (6) reported that dentin exposure, dentin hyperhypersensitivity, pulp hyperemia, or pulp necrosis may occur due to abrasions caused by occlusal interferences and paraffunctions. Abnormal occlusal forces accumulate in the apical region of the teeth and cause interruption in circulation of the pulp that leads to pulp necrosis and periapical lesions called sink-like (14-16).

In the present study, it was thought that the mandibular incisors with the lowest root surface areas could be negatively affected under increasing occlusal loads. Therefore, our aim in this clinical study was to investigate the effect of fremitus due to chronic occlusal trauma in the periapical and periodontal tissues of the mandibular incisors.

Materials and Methods

This study was carried out in accordance with the 2002 Helsinki Declaration and "Good Clinical Practices Guide". The purpose of the study and clinical applications were explained to all volunteers. Informed consent forms were signed. The study protocol was approved by the Clinical Research Ethics Committee of Zekai Tahir Burak Women's Health Education and Research Hospital (decision no: 95/2019, date: 25.06.2019). The individuals who participated in the study were selected from among individuals who Beyazit University Faculty of Dentistry, Tepebasi Oral and Dental Health Hospital between June 2019 and February 2020, taking into account the participation criteria detailed below.

Study Population And Design

This cross-sectional study included 251 healthy individuals of both genders (n=126 male, n=125 female), between the ages of 18-65, who had at least 24 teeth. Individuals with periodontitis, treated periodontitis and teeth with reduced attachment level, generalized gingivitis, missing/decayed/ restored tooth in the eight incisor teeth, periodontal and orthodontic treatment or occlusal adjustment history and smokers were not included in the study. The individuals included in the study were patients with a dental relationship of class I and II. Class III was not included in the study. Patients with systemic disease affecting periodontal tissues (diabetes, rheumatoid arthritis, cardiovascular), patients using drugs (antibiotics, anti-inflammatory, calcium channel blockers, etc.) that have an effect on the gingiva in the last six months, patients with active infectious diseases (AIDS, HBV, tuberculosis) and pregnant women were also excluded from the study. In this study, all individuals had good oral hygiene, regular brushing habits and were defined as healthy according to the new periodontal disease classification published in 2018 (17).

Clinical Measurements

Examination of the mandibular incisors of each individual was performed. Clinical and radiographic parameters were measured and recorded. To prevent inter-observer deviation, recordings were made by a single, well-calibrated periodontologist (M.A.T.) (≥85% agreement between recurrent clinical evaluations on non-study patients).

Gingival recession (the distance between the cementoenamel junction and gingival margin) was measured in mm from the midbuccal region of the teeth using a Williams periodontal probe (LM Dental AB, Nynäshamn, Sweden). Dental mobility was recorded for each tooth using Miller's mobility classification (18). Attrition (wear at the enamel or dentin level on the incisal surface of the tooth) and percussion were recorded as present/absent. Dentin hypersensitivity assessment was determined as present/ absent by squeezing cold air spray on the tooth and rubbing the polishing tire. The cold and hot hypersensitivity tests were applied until the patient reacted and for a maximum of 5 seconds. If the pain disappeared immediately after the stimulus was removed, the tested tooth was considered hypersensitive and vital. If the pain persisted after the stimulus disappeared, percussion test, electric pulp test and radiography were evaluated together, and non-vital teeth were referred for root canal treatment.

Presence or absence of fremitus by fremitus test (functional mobility): the index finger was placed on the facial surfaces of the mandibular incisors and the mobility during repeated

centric and eccentric occlusion was monitored visually. According to this: there was 0: non-fremitus in the teeth without mobility, 1: mild-fremitus, which is felt slightly with the finger but not visible with the eye, 2: severe-fremitus, that can be felt and visible with the eye. The diagnosis of occlusal trauma was also supported by articulation paper testing. The markings assigning non-functional occlusion were accepted as occlusal trauma. Representative radiograph and photographs are given in Figure 1.

Periapical films of patients taken with parallel technique were evaluated for radiographic examination. The lamina dura degradation (can be observed interrupted, discontinuous or invisible) was recorded as present/absent. A complete and continuous radiopaque line surrounding the tooth socket and separating the periodontal ligament space from the alveolar bone was evaluated as normal lamina dura imaging. Periodontal ligament anomalies (widening or the disappearance of the periodontal ligament space) were recorded. The physiological periodontal ligament space (approximately 0.2 mm) was defined and expressed as normal. Triangulation, crowded teeth, percussion sensitivity and deep overbite parameters were recorded as present/absent. Vertical defects occur adjacent to a tooth and usually in the form of a triangular area of missing interproximal alveolar bone is defined as triangulation (the base is coronally and the top is apically). The range of 3-4 according to Geiger's classification (19) was determined as deep overbite. The point of contact was from the apical crest of the cingulum, including the palatal mucosa.

Root canal treatment was indicated and performed by an experienced endodontist (E.S.) in non-vital teeth, which responded negatively to electric pulp testing (Gentle Pulse; Parkell Inc., New York, USA), and pathology was detected in the periapical region radiographically. The electric pulp test was measured by placing the electrode of the device perpendicular to the 1/3 incisal of the tooth after the each tooth was isolated and dried.

Statistical Analysis

Data analysis was carried out using the SPSS program (ver 26.0; SPSS Inc., Chicago, IL, USA). Significance was



Figure 1. Number of teeth 31 and 41 have severe fremitus, 42 has mild fremitus, 32 has non-fremitus

defined as p<0.05. Distribution of variables was measured by Kolmogorov-Simirnov test. Analysis of quantitative independent data was carried out with independent sample t-test, Kruskal-Wallis and Mann-Whitney U test. In the analysis of dependent quantitative data, paired sample t-test was used. Qualitative independent data were analyzed by chi-square test. GPower 3.1 program was used in this study for the calculation of the statistical power at 95% confidence interval (CI) (α =0.05) and 90% CI (1- β = 90). The results of this analysis suggested that a total of 984 teeth should be adequate for this study.

Results

Comparing the Non-, Mild- and Severe-fremitus Groups

Table 1 and Figure 2 show the relationship between clinical. radiographic and demographic data between teeth with fremitus (non-fremitus, mild-fremitus and severe-fremitus). The severe-fremitus group was significantly different from the mild-fremitus and non-fremitus groups in terms of age and gender distribution (p<0.05). Severe-fremitus group was significantly different from the other groups (p(0.05)) for the need for root canal treatment; however no significant difference was found between the non-fremitus and the mild-fremitus groups (p<0.05). The teeth with percussion, hypersensitivity, mobility, deep overbite and crowded teeth were statistically significantly higher in severe-fremitus group compared to the other groups (p<0.05). The number of the teeth whose periodontal ligament space and laminadura disruption could not be seen normally were also statistically significantly higher in severe-fremitus group compared to other groups (p<0.05). No significant difference was observed between the non-fremitus and mild-fremitus groups in terms of these parameters (p)0.05). The number of the teeth with gingival recession, attrition and triangulation was the highest in the severe-fremitus group and the lowest in the non-fremitus group (p<0.05). All three groups were significantly different in terms of triangulation, attrition, the presence of gingival recession and the amount of gingival recession in mm (p<0.05).



Figure 2. The relationship between clinical, radiographic and demographic data between teeth with fremitus (non-fremitus, mild-fremitus and severe-fremitus)

Comparing with and Without Anomalies Groups

Age, gender distribution, and attrition rates of patients did not differ significantly between percussion and non-percussion groups (p)0.05). In the percussion group, the rates of root canal treatment, tooth hypersensitivity, gingival recession, tooth mobility, periodontal ligament anomaly, lamina dura disruption, triangulation, deep overbite and crowded teeth were significantly higher than the non-percussion group (p<0.05) (Table 2). Age, gender distribution, attrition and tooth hypersensitivity rate of the patients were not statistically significant between the groups with and without root canal treatment indications (p>0.05). In the group with root canal treatment indication, gingival recession, tooth mobility, periodontal ligament anomaly, lamina dura disruption, triangulation, deep overbite and crowded teeth rate were significantly higher than in the group without root canal treatment indication (p<0.05) (Table 3). The gender distribution and gingival recession millimeter of individuals were not statistically significant between the groups with and without attrition (p>0.05). In the attrition group, patient age, hypersensitivity, gingival recession, tooth mobility, periodontal ligament anomaly, lamina dura disruption, triangulation, deep overbite and crowded teeth rate were significantly higher than the group without attrition (p(0.05)) (Table 4). In the group with gingival recession; patient age, female patient ratio, hypersensitivity, mobility, periodontal ligament anomaly, lamina dura disruption, triangulation, deep overbite and crowded teeth rate were significantly higher than the group without gingival recession (p<0.05) (Table 5). In the group with and without dental mobility, the gender distribution of patients did not differ significantly (p>0.05). Age, hypersensitivity, periodontal ligament anomaly, lamina dura disruption, triangulation, deepbite and crowded teeth rates were significantly higher in the mobility group than the non-mobility group (p(0.05)) (Table 5).

The parameters in the group with periodontal ligament anomaly compared to the non-anomaly group were as follows: the age of the patients ($40.4\pm10.9-33.6\pm9.9$), nonnormal lamina dura disruption (33.1-1.3%), triangulation (58.5-11.2%), deep overbite (30.2-8.6%) and crowded teeth (56.9-25.5%). These parameters were significantly higher than in the non-anomaly group (p(0.05).

Discussion

In this study, clinical and radiographic data of individuals in mandibular incisors were examined and the effects of fremitus on teeth and related tissues were investigated. Accordingly, all evaluated clinical and radiographic parameters were found to be significantly higher in teeth with severe-fremitus.

Dental mobility has been described as one of the common clinical signs of occlusal trauma (1,20). However, increased mobility may be due to inflammation and/or bone loss or connective tissue loss (21). Mobile teeth with widening of periodontal ligament space has increased probing pocket

Fremitus				Absent (n=169,	16.8%)	Mild (n=60	Mild (n=609, 60.7%) (2.5%)	p-value
Age	Median (min-max)		:		33 (18-63) ^A		3-63) ^a	38 (18-65) [₿]		0.000 ^ĸ
Gingival recession (mm)	Median (min-max)		1		1 mm (1-3) ^A		(1-4) ^B	2 mm (2-6) ^c		0.000 ^ĸ
		n	%	n	%	n	%	n	%	р
	Female	500	49.8	72	42.6	295	48.4	133	58.8	
Gender	Male	504	50.2	97	57.4 ^A	314	51.6 ^A	93	41.2 ^B	0.003*
	(-)	544	54.2	112	66.3	347	57.0	85	37.6	
Attrition	(+)	460	45.8	57	33.7^	262	43.0 ^B	141	62.4 ^c	0.000**
<u>р</u> .	(-)	985	98.1	169	100	606	99.5	210	92.9	0.00 ^{x²}
Percussion	(+)	19	1.9	0	0.0 ^A	3	0.5 ^A	16	7.1 ^B	
D	(-)	995	99.1	169	100	607	99.7	219	96.9	0.0001
Root canal treatment	(+)	9	0.9	0	0.0 ^A	2	0.3 ^A	7	3.1 [₿]	0.000**
Hypersensitivity (cold-	(-)	729	72.6	137	81.1	464	76.2	128	56.6	0.000 ^{x²}
hot)	(+)	275	27.4	32	18.9^	145	23.8 ^A	98	43.4 ^B	
Gingival recession	(-)	676	67.3	153	90.5	430	70.6	93	41.2	0.0001
	(+)	328	32.7	16	9.5 ^A	179	29.4 ^B	133	58.8 ^c	0.000*
	(-)	840	83.7	159	94.1	551	90.5	130	57.5	0.000 ^{x²}
Mobility	(+)	164	16.3	10	5.9 ^A	58	9.5 [^]	96	42.5 ^B	
	1	147	14.6	10	5.9	57	9.4	80	35.4	
Mobility	П	13	1.3	0	0.0	1	0.2	12	5.3	
		4	0.4	0	0.0	0	0.0	4	1.8	
	Normal	756	75.3	151	89.3 ^A	509	83.6 ^A	96	55.8 [₿]	
Periodontal ligament Space	Narrow- disappear	123	12.3	15	8.9	76	12.5	34	19.8	0.000 ^{x²}
	Wide	121	12.1	3	1.8	78	12.8	42	24.4	
	Normal	912	90.8	166	98.2	599	98.4	147	65.0	
Lamina dura	Disruption	92	9.2	3	1.8 ^A	10	1.6 ^A	79	35.0 ^в	0.000**
-	(-)	774	77.1	157	92.9	528	86.7	89	39.4	0.0001
Iriangulation	(+)	230	22.9	12	7.1^	81	13.3 ^B	137	60.6 ^c	0.000*
	(-)	864	86.1	153	90.5	555	91.1	156	69.0	
Deep overbite	(+)	140	13.9	16	9.5 [^]	54	8.9 ^A	70	31.0 ^B	0.000**
a	(-)	669	66.6	137	81.1	445	73.1	88	38.9	0.0001
Crowded teeth	(+)	334	33.3	32	18.9^	164	26.9 ^A	138	61.1 ^B	0.000x²

Table 1. The relationship between clinical, radiographic, demographic data and fremitus (non-fremitus, mild fremitus and severe fremitus)

Table 2. The relationship between clinical, radiographic, demographic data and groups with and without percussion							
		Percuss	ion (-)	Percussion	(+)	p-value	
Age	Median (min-max)	34	(18-65)	36	(18-52)	0.596 ^m	
Gingival recession (mm)	Median (min-max)	2 mm	(1-5)	3 mm	(1-6)	0.000 ^m	
		n	%	n	%	р	
Gondor	Female	490	49.7	10	52.6	0 803%	
	Male	495	50.3	9	47.4	0.003~	
Attrition	(-)	537	54.5	7	36.8	0.126 ^{x²}	
	(+)	448	45.5	12	63.2	0.126%	
Post canal treatment	(-)	985	100.0	10	52.6	0 000x²	
	(+)	0	0.0	9	47.4	0.000*	
Hypersensitivity	(-)	724	73.5	5	26.3	0.00072	
(cold-hot)	(+)	261	26.5	14	73.7	0.000*	
Cincipal responsion	(-)	672	68.2	4	21.1	0.000**	
	(+)	313	31.8	15	78.9	0.000^	
N de la Titas -	(-)	836	84.9	4	21.1	0.000x²	
мориту	(+)	149	15.1	15	78.9		
	1	137	13.9	10	52.6		
Mobility	П	11	1.1	2	10.5		
	Ш	1	0.1	3	15.8		
	Normal	753	76.4	3	15.8		
Periodontal ligament space	Narrow-disappear	127	12.9	0	0.0	0.000x²	
	Wide	105	10.7	16	84.2		
Lamina dura	Normal	907	92.1	5	26.3	0.000y²	
	Disruption	78	7.9	14	73.7	0.000	
Triongulation	(-)	771	78.3	3	15.8	0.000 ²	
	(+)	214	21.7	16	84.2	0.000*	
	(-)	857	87.0	7	36.8		
	(+)	128	13.0	12	63.2	0.000	
Crowdod tooth	(-)	665	67.5	5	26.3		
	(+)	320	32.5	14	73.7	0.000	

^mMann-Whitney U test, ^{x²}Chi-square test, min-max: Minimum-maximum

depth, clinical attachment loss and bone loss compared to teeth without mobility (2). There are also studies that could not find a relationship between mobility and occlusal forces (22). In our study, mobility was detected in teeth with severe-fremitus at 35.4% first degree, 5.3% 2nd degree, and 1.8% 3rd degree according to the classification of Miller (18). In the teeth with mild-fremitus and nonfremitus, only 1st degree mobility was determined as 5.9% and 9.4%, respectively. Thus, dental mobility was found to be significantly increased in teeth with traumatic occlusal forces. The researchers also found a strong correlation between the quantitative values of the periotest and Miller's original mobility index (23).

Many types of occlusion including premature contacts or parafunctional habits in centric relation or centric occlusion are significantly associated with deeper probing pocket

Table 3. The relationsh	nip between clinical radi	ographic der	nographic data a	nd groups w	ith and without ro	oot canal treatment
		Root cana	al treatment (-)	Root can	al treatment (+)	p-value
Age	Median (min-max)	35	(18-65)	38	(18-52)	0.590 ^m
Gingival recession (mm)	Median (min-max)	2 mm	(1-5)	4 mm	(1-6)	0.008 ^m
		n	%	n	%	p-value
Canadaa	Female	496	49.8	4	44.4	0.747*2
Gender	Male	499	50.2	5	55.6	0.747*
A.L. 11	(-)	542	54.5	2	22.2	0.050%
Attrition	(+)	453	45.5	7	77.8	0.053*
Hypersensitivity	(-)	725	72.9	4	44.4	0.057~2
(cold-hot)	(+)	270	27.1	5	55.6	0.057*
<u> </u>	(-)	673	67.6	3	33.3	0.000**
Gingival recession	(+)	322	32.4	6	66.7	0.029%
Mobility	(-)	839	84.3	1	11.1	0.000**
	(+)	156	15.7	8	88.9	0.000*
	I	143	14.4	4	44.4	
Mobility	Ш	12	1.2	1	11.1	
	Ш	1	0.1	3	33.3	
	Normal	755	75.9	1	11.1	
Periodontal ligament	Narrow-disappear	127	12.8	0	0.0	0.000 ^{x²}
space	Wide	113	11.4	8	88.9	
	Normal	910	91.5	2	22.2	0.000**
Lamina dura	Disruption	85	8.5	7	77.8	0.000*
T · · · ·	(-)	772	77.6	2	22.2	0.000**
Irlangulation	(+)	223	22.4	7	77.8	0.000*
	(-)	861	86.5	3	33.3	0.000**
Deep overbite	(+)	134	13.5	6	66.7	0.000*
	(-)	667	67.0	3	33.3	0.000%
Crowded teeth	(+)	328	33.0	6	66.7	0.033**
™Mann-Whitney U test. ^{x²} C	hi-square test					

depth, clinical attachment loss, increased mobility and poor prognosis in individuals with periodontitis, and their elimination can improve clinical periodontal conditions (24,25). According to the results of our study, the width of the periodontal ligament space increased significantly depending on the severity of fremitus. Similarly, deterioration in the radiographic image of the lamina dura and triangulation findings were significantly increased in individuals in the severe-fremitus group. According to the results of this study, the rate of triangulation detected radiographically in individuals with severe-fremitus was 60%, which was significantly higher than the other groups. These findings are supported by previous studies as findings of traumatic occlusal forces (4,20). In this study, only mandibular incisors under fremitus were evaluated. The root surface area was the smallest in these teeth group, and it was thought that these teeth could be more affected under occlusal forces rather than maxillary incisors. Furthermore,

Table 4. Relationship between c	linical, radiographic, demogr	aphic data ar	nd groups with	h and without	ut attrition	
		Attrition	(-)	Attrition	(+)	p-value
Age	Median (min-max)	30	(18-59)	41	(18-65)	0.000 ^m
Gingival recession (mm)	Median (min-max)	2 mm	(1-5)	2 mm	(1-6)	0.990 ^m
		n	%	n	%	p-value
Candar	Female	261	48.0	239	52.0	0.000*2
Gender	Male	283	52.0	221	48.0	0.209%
	(-)	431	79.2	298	64.8	0.000**
Hypersensitivity (cold-hot)	(+)	113	20.8	162	35.2	0.000*
	(-)	451	82.9	225	48.9	0.000+2
Gingival recession	(+)	93	17.1	235	51.1	0.000*
	(-)	491	90.3	349	75.9	0.000 y2
Mobility	(+)	53	9.7	111	24.1	0.000*
Mobility	1	53	9.7	94	20.4	
	Ш	0	0.0	13	2.8	
	III	0	0.0	4	0.9	
	Normal	470	86.4	286	62.2	
Periodontal ligament space	Narrow-disappear	44	8.1	83	18.0	0.000 ^{x²}
	Wide	30	5.5	91	19.8	
	Normal	513	94.3	399	86.7	0.000**
Lamina dura	Disruption	31	5.7	61	13.3	0.000*
T · · · · ·	(-)	457	84.0	317	68.9	0.000**
Irlangulation	(+)	87	16.0	143	31.1	0.000*
	(-)	502	92.3	362	78.7	0.000**
Deep overbite	(+)	42	7.7	98	21.3	0.000*
	(-)	423	77.8	247	53.7	0.000x2
Crowded teeth	(+)	121	22.2	213	46.3	0.000**
^m Mann-Whitney U test, ^{x²} Chi-square t	test, min-max: Minimum-maximu	ım				

the four incisors of a patient could have different results on examination, therefore, the teeth were evaluated regardless of the patients.

Nine of 251 patients that had symptoms of fremitus received root canal treatment indication for any mandibular incisors. None of these teeth had caries or restorations that would require root canal treatment. However, individuals who needed root canal treatment also had a significantly increased level of crowded teeth and deep overbite. The absence of a classification that reveals the severity of crowded teeth can be expressed as the limitation of this study. All of the teeth received root canal treatment after being diagnosed as nonvital. 88.9% of the teeth were accompanied by a periapical lesion and all had percussion sensitivity. In addition, 77.8% of the lamina dura was disrupted and 77.8% had severefremitus. Root canal treatment was indicated in 9 (47.4%) of 19 teeth with percussion sensitivity. In a finite element analysis study, it has been reported that hyperfunctional occlusal forces accumulate in the alveolar bone and mostly in the periapical region (26). Therefore, teeth exposed to abnormal occlusal stresses due to deep-bite and crowding may experience pulp necrosis by interrupting pulpal circulation in the apical foramen (14,15). These findings increased with age.

Excessive occlusal loads are supported by finite element analysis studies (12,27) which can demonstrate cervical stress and induce the formation of non-carious cervical lesions (such as abfraction) (28). However, these studies

mobility							
	T	Gingival	recession (-)	Gingival re	cession (+)	p-value	
Age	Median (min-max)	32	(18-63)	42	(18-65)	0.000 ^m	
	1	n	%	n	%	p-value	
Gender	Female	306	45.3	194	59.1		
	Male	370	54.7	134	40.9	0.000	
Hypersensitivity (cold-	(-)	567	83.9	162	49.4		
hot)	(+)	109	16.1	166	50.6	0.000	
Mobility	(-)	628	92.9	212	64.6		
Mobility	(+)	48	7.1	116	35.4	0.000*	
	1	46	6.8	101	30.8		
Mobility	11	0	0.0	13	4.0		
	Ш	2	0.3	2	0.6		
	Normal	584	86.4	172	52.4	0.000×2	
Periodontal ligament	Narrow-Disappear	63	9.3	64	19.5		
space	Wide	29	4.3	92	28.0		
	Normal	650	96.2	262	79.9	0.000**	
Lamina dura	Disruption	26	3.8	66	20.1	0.000*	
-	(-)	610	90.2	164	50.0	0.000	
Irlangulation	(+)	66	9.8	164	50.0	0.000	
	(-)	623	92.2	241	73.5	0.000	
Deep overbite	(+)	53	7.8	87	26.5	0.000*	
	(-)	536	79.3	134	40.9	0.000**	
Crowded teeth	(+)	140	20.7	194	59.1	0.000*	
		Mobility	(-)	Mobility (+)	p-value	
Age	Median (min-max)		33.5 (18-63)		39 (18-65)	0.000 ^m	
		n	%	n	%	p-value	
Gondor	Female	412	49.0	88	53.7		
Gender	Male	428	51.0	76	46.3	0.200*	
Hypersensitivity (cold-	(-)	646	76.9	83	50.6		
hot)	(+)	194	23.1	81	49.4	0.000^	
	Normal	702	83.6	54	32.9		
Periodontal	Narrow-disappear	119	14.2	8	4.9	0.000x²	
ingament space	Wide	18	2.1	103	62.8		
Lensing down	Normal	825	98.2	87	53.0	0.000 x²	
Lamina dura	Disruption	15	1.8	77	47.0		
T	(-)	733	87.3	41	25.0	0.000	
Irlangulation	(+)	107	12.7	123	75.0	0.0002	
	(-)	752	89.5	112	68.3	0.000**	
Deep overbite	(+)	88	10.5	52	31.7	0.000*	
	(-)	596	71.0	74	45.1	0.000	
Crowded teeth	(+)	244	29.0	90	54.9	0.000%	
™Mann-Whitney U test, ^{x²} Ch	ii-square test, min-max: l	Minimum-m	aximum				

Table 5. The relationship between clinical, radiographic, demographic data and groups with and without gingival recession and mobility

did not reflect a clinical situation. The relationships between bruxism and the presence of occlusal wear facets has been shown, however, the causal relationship has not been confirmed (29). Although there are studies that did not find a correlation between non-carious cervical lesions and the degree of occlusal abrasions (30), there are also studies that found an increase in the frequency of non-carious cervical lesions related to the presence of occlusal wear facets (attrition) (31) and bruxism (32). In this study, fremitusinduced occlusal forces were significantly associated with attrition and facial marginal gingival recession.

It has been suggested that excessive occlusal forces may be an etiological factor in gingival recession, facial clefts such as "Stillman's cleft" and gingival loss (1). Supportingly, in our study, gingival recession was observed in 58.8% of teeth with severe-fremitus and severity of gingival recession was significantly associated with fremitus-induced occlusal trauma. In a study that examined patients with gingival recession, there was no significant relationship between fremitus test and gingival recession (33). In another study, labial gingival recession of mandibular incisors was found to be associated with linguoversion (34) Stillman (1), Box (35) and Miller (18) argued that gingival recession can be caused by heavy occlusal contacts. Harrel and Nunn (36) reported that more than 70% of teeth with dysfunction are associated with gingival recession. However, Gorman (37) could not associate the presence of gingival recession with occlusal trauma. The available data provide contradictory results regarding the effects of occlusal forces on gingival recession (36-38) Jin and Cao (2) also reported more clinical attachment loss and less bone support in teeth with obvious fremitus. Teeth with significant occlusal wear facets also had less clinical attachment levels. In this current study, the age of patients in the group with gingival recession also increased significantly compared to the group without gingival recession, as stated in previous studies (9).

Most researchers have concluded that the prevalence of dentin hypersensitivity ranges from 3% to 57% (39). In one review, dentin hypersensitivity was reported to affect a guarter of the adult population (40). In our study, individuals with hypersensitivity were 27.4%. In an other study, the prevalence of dentin hypersensitivity was about 25-30%, regardless of age (41). Dentin hypersensitivity is mostly associated with dentin on the buccal surface of permanent teeth (42). Addy and Pearce (43) attributed this to powerful tooth brushing, and this etiology was confirmed by another review (44). The cervical dentin tubules exposed by erosion or gingival recession are thought to cause pain by inducing thermal or tactile stimuli (45). The combination of erosion and abrasion are ideal etiological risk factors for dentin hypersensitivity (46). In our study, only teeth with severefremitus had significantly higher hypersensitivity (43.4%) than teeth with mild-femitus and non-fremitus. At the same time, significantly increased attrition, gingival recession and mobility were detected in teeth with thermal hypersensitivity.

However, as a limitation of our study, we did not detect gingival recession and thermal hypersensitivity because of powerful brushing.

Researchers reported that excessive occlusal forces cause periodontitis (1) and should be controlled to treat periodontitis successfully (20). According to the results of animal studies (47) occlusal trauma does not cause irreversible bone loss or connective tissue loss without plaque-induced inflammation, and an increase in mobility and a decrease in bone density are reversible. In addition, the researchers created experimental periodontitis using rat models and combined inflammation with occlusal trauma. These studies have demonstrated that connective tissue loss and bone loss due to periodontitis were increased (48). Consequently, occlusal trauma is considered as a cofactor that accelerates periodontitis and loss of attachment has not been associated with trauma on a healthy tooth. However, in terms of the short-term use of extreme forces in animal studies, it could not mimic human tissues in all aspects.

In this current study, individuals diagnosed with periodontitis were excluded from the study. It may be considered as a limitation of our study to exclude periodontal disease and related clinical and radiographic symptoms and not to investigate the combined effects of occlusal trauma with periodontal disease. The whole analysis in this study does not take into account how long has this overload already existed and this can be considered as the limitation of the study as well. In addition, this study only examined the clinical and radiographic findings of mandibular incisors. Further studies are needed to examine the maxillary incisors and posterior teeth, to develop treatment methods against traumatic occlusal forces and to determine the effectiveness of these methods.

Conclusion

Periodontal, pulpal and periapical tissues are negatively affected by severe-fremitus. Excessive occlusal forces on the mandibular incisors increased the incidence of clinical and radiographic anomalies and pathological findings, thus worsening the prognosis of the teeth. Fremitus was also found to be associated with deep overbite and crowded teeth. It is necessary to diagnose and correct problems caused by damaging occlusion in order to maintain oral and dental health.

Ethics

Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of Zekai Tahir Burak Women's Health Education and Research Hospital (decision no: 95/2019, date: 25.06.2019).

Informed Consent: Informed consent forms were signed.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A.T., E.S., Concept: M.A.T., E.S., Design: M.A.T., E.S., Data Collection or Processing: M.A.T., E.S., Analysis or Interpretation: M.A.T., E.S., Literature Search: M.A.T., Writing: M.A.T.

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

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

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Effects of Peri-implant Mucosal Characteristics on Clinical Peri-implant Parameters: A Retrospective Study

Peri-implant Mukoza Özelliklerinin Klinik Peri-implant Parametreler Üzerine Etkileri: Retrospektif Bir Çalışma

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Abstract

Objective: This study aimed to examine the effects of peri-implant keratinized mucosa (KM) width and thickness on peri-implant soft tissue health in functionally loaded implants and evaluate the factors affecting peri-implant health.

Materials and Methods: A total of 645 implants from 169 patients were evaluated in this study. The duration of the implant followup ranged from 12 months to 46 months. Clinical measurements such as plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), clinical attachment loss (CAL), KM width, mucosal phenotype (MP), and buccal mucosal recession (MR) of peri-implant sites were performed. Peri-implant mucosa was classified as healthy, peri-implant mucositis, and peri-implantitis.

Results: It was determined that the thickness and width of the KM around the implant did not affect the peri-implant soft tissue health and PI, GI, and BOP scores. PD was found to be significantly lower in peri-implant regions with KM width <2 mm and thin MP. CAL was found to be significantly higher in peri-implant regions with KM width <2 mm and thin MP. Significantly less buccal MR was detected in peri-implant regions with KM width ≥2 mm and thick MP. The peri-implant regions treated with the overdenture were significantly less healthy.

Conclusions: It was determined that the CM width and thickness around the implant did not have a significant effect on soft tissue health in patients who could achieve plaque control. It is important to provide sufficient KM width and thickness in the peri-implant soft tissue, especially in esthetic areas.

Keywords: Keratinized mucosa width, mucosal phenotype, mucosal recession, peri-implantitis, peri-implant mucositis

Öz

Amaç: Çalışmanın amacı, fonksiyonel yükleme yapılmış implantlarda implant çevresi keratinize mukoza (KM) genişliğinin ve kalınlığının peri-implant yumuşak doku sağlığı üzerindeki etkilerini incelemek ve peri-implant yumuşak doku sağlığını etkileyen faktörleri değerlendirmektir.

Gereç ve Yöntemler: Çalışmada 169 hastaya ait toplam 645 implant değerlendirildi. İmplant takip süresi 12 ay ile 46 ay arasında değişmektedir. Peri-implant bölgelerin plak indeks (PI), gingival indeks (GI), sondalamada kanama (SK), sondalama derinliği (SD), klinik ataşman kaybı (KAK), KM genişliği, mukozal fenotipi (MF) ve bukkal mukozal çekilme (MÇ) miktarı gibi klinik ölçümleri yapıldı. Peri-implant mukoza sağlıklı, peri-implant mukozitis ve peri-implantitis olarak sınıflandırıldı.

Bulgular: İmplant çevresindeki KM kalınlığının ve genişliğinin peri-implant yumuşak doku sağlığını ve PI, GI, SK skorlarını etkilemediği belirlendi. SD değerleri, KM genişliği <2 mm ve MF'si ince olan peri-implant bölgeleride anlamlı düzeyde daha düşük bulundu. KAK değerleri, KM genişliği <2 mm ve MF'si ince olan peri-implant bölgelerde anlamlı düzeyde daha yüksek bulundu. KM genişliği ≥2 mm ve MF'si kalın olan peri-implant bölgelerde anlamlı olarak daha az bukkal MÇ tespit edildi. Overdenture ile tedavi edilen peri-implant bölgeleri anlamlı düzeyde daha az sağlıklıydı.

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) **Sonuç:** Plak kontrolünü sağlayabilen hastalarda implant çevresi KM genişliği ve kalınlığının yumuşak doku sağlığı üzerine belirgin bir etkisinin olmadığı tespit edildi. Özellikle estetik bölgelerdeki peri-implant yumuşak dokuda yeterli miktarda KM genişliğinin ve kalınlığının sağlanması önemlidir.

Anahtar Kelimeler: Keratinize mukoza genişliği, mukozal fenotip, mukozal çekilme, peri-implantitis, peri-implant mukozitis

Introduction

The presence of adequate keratinized tissue width is thought to be essential for maintaining gingival health and preventing periodontal disease progression (1). However, studies evaluating the effect of peri-implant keratinized mucosa (KM) width on long-term implant success have encountered different clinical results (2,3). Costa et al. (3) claimed that the insufficiency of the keratinized tissue band causes mucositis, peri-implantitis and even implant loss, respectively. Similarly, Ueno et al. (4) emphasized that clinical parameters such as bleeding on probing (BOP), plaque index (PI) and probing depth (PD) increase in areas with peri-implant KM width <2 mm. However, there are also studies reporting that peri-implant KM width does not affect gingival index (GI) or BOP scores (5-8). While Bengazi et al. (9) reported that the presence of KM did not affect soft tissue recession in their observations 6-24 months after prosthetic loading, many clinical studies (2,5-7) showed that the amount of mucosal recession (MR) increased significantly in implant sites with narrow KM.

It is seen in the literature that there are conflicting results between peri-implant tissue health and the amount of keratinized tissue. There is only one study evaluating the effect of peri-implant mucosal phenotype (MP) on the amount of peri-implant MR and clinical attachment loss (CAL). Therefore, the main purpose of this retrospective study is to examine the effects of peri-implant KM width and thickness on peri-implant soft tissue health in functionally loaded implants and evaluate the factors affecting periimplant health.

Materials and Methods

Approval was obtained from the İnönü University Scientific Research and Publication Ethics Committee for this study (protocol number: 2020/844, date: 14.07.2020). Written and verbal information about the purpose and method of the study were given to all patients, the information forms were read and consent forms were signed by the patients.

Patient Sample

Patients who underwent implant surgery at İnonu University Faculty of Dentistry, Department of Periodontology between March 2016 and December 2019 were included in this retrospective clinical study. All of the implants were placed by the same surgeon (M.Ö.U.) using a non-traumatic surgical technique. The inclusion criteria were as follows: patients older than 18 years and with complete radiographic and clinical records during implant placement, implants that have been in function for at least more than one year.

Pregnant women, those who used systemic antibiotics in the 6 weeks before the study, patients who needed prophylactic antibiotics, smokers and those with any systemic disease that may affect soft tissue and hard tissue health (such as hyperthyroidism, hyperparathyroidism and uncontrolled diabetes mellitus) were excluded from the study.

Clinical Measurements

PI, GI, BOP, PD and CAL measurements of all periimplant sites were measured using a probe (Hu-Friedy Colorvue Periodontal Probe, Chicago, IL, ABD) at six points (mesiobuccal, mid-buccal, distobuccal, distolingual/ palatinal, mid-lingual/palatinal and mesiolingual/palatinal) of each implant. PD was measured as the distance between the mucosal margin and the bottom of the pocket or sulcus. CAL was defined as the distance between the abutmentimplant junction and the bottom of the pocket. BOP and suppuration were recorded as present or absent (+\-).

KM width, MP and MR amount were measured from the midbuccal point using a Williams probe (Hu-Friedy, Chicago, IL, USA). KM width was determined as the distance between the mucosal margin and the mucogingival line. MP was measured for each dental implant from the buccal surface using the "probe method" (10). After the periodontal probe was placed in the peri-implant sulcus, it was checked whether the color of the probe was reflected from the periimplant mucosa. The mucosa was recorded as thin when the color of the probe was reflected and thick when it did not. The amount of MR was determined as the distance between the abutment-implant junction and the mucosal margin. All clinical measurements were performed by a single investigator. PI, PD and MP measurements obtained from 10 patients who were not included in the study for intraexaminer calibration were repeated 1 week later, resulting in 95%, 90% and 90% agreement, respectively.

Standard periapical radiographs were taken from the patients using the parallel technique. Soft tissue around the implant was grouped as healthy, peri-implant mucositis and peri-implantitis (11). Implants were grouped according to their superstructure as a single crown, implant-supported crown-bridge restoration and overdenture. The implanted area and the duration of the implants in function were recorded. Straumann (Waldenburg, Switzerland), Astra Tech (Astra Tech AB, Mölndal, Switzerland), Nobel (Branemark, Sweden), Zinedent (Batı Group and Instradent, Turkey), MIS (MIS Implants Technologies Ltd, Shlomi, Israel) branded dental implants were included in this study.

Statistical Analysis

IBM SPSS Statistics 22 (IBM SPSS, USA) program was used for evaluation of statistical analyzes. The suitability of the parameters to the normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilks tests and it was determined that the parameters did not show normal distribution. While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation, frequency), Mann-Whitney U test was used to compare quantitative data. Chi-Square test and Fisher-Freeman-Halton Exact test were used to compare qualitative data. Significance was evaluated at the p<0.05 level.

Results

In the study, 645 peri-implant area was evaluated in a total of 169 individuals, 88 (52.1%) men and 81 (47.9%) women. The duration of implant follow-up ranged from 12 months to 46 months, with a mean of 25.86±8.74 and a median of 26 months. The distribution of general information about the participants and implants is shown in Table 1.

Table 1. Distribution of general information on participants and implants						
Distribution of information on participants		(n=169)	%			
	Male	88	52.1			
Gender	Female	81	47.9			
	18-29	6	3.6			
	30-39	18	10.7			
A	40-49	46	27.2			
Age	50-59	54	32			
	60-69	35	20.7			
	70 and older	10	5.9			
	≥2 mm	496	76.9			
Keratinized tissue width	<2 mm	149	23.1			
	Thick	479	74.3			
Mucosal phenotype	Thin	166	25.7			
	Healthy	433	67.1			
Peri-implant tissue	Peri-implant mucositis	138	21.4			
nealth	Peri-implantitis	74	11.5			
	No	627	97.2			
Suppuration	Yes	18	2.8			
	Single crown	104	16.1			
T () I	Crown-bridge	500	77.5			
type of restoration	Overdenture	41	6.4			

The rate of being healthy with peri-implants treated with overdenture was found to be statistically significantly lower than those treated with a single crown and crown bridge (p1=0.000; p2=0.000; p $\langle 0.05 \rangle$). There was no statistically significant difference in terms of healthy rates between peri-implant sites treated with a single crown and crown bridge (p $\rangle 0.05$) (Table 2).

There was no statistically significant difference in periimplant tissue health, PI, GI and BOP levels between periimplant sites with KM width ≥ 2 mm and peri-implant sites with KM width $\langle 2 \text{ mm and } (p \rangle 0.05)$ (Table 2, 3). There was no statistically significant difference in peri-implant tissue health PI, GI and BOP levels between the peri-implant regions with thick MP and those with thin MP (p $\rangle 0.05$) (Table 2, 3).

Peri-implant sites with KM width ≥ 2 mm had significantly higher PD values and lower CAL and buccal MR amounts (p<0.05) (Figure 1). In peri-implant regions with thick MP, PD was significantly higher, and CAL and buccal MR were lower (p<0.05) (Figure 2).

Keratinized Mucosa Width 5 4,5 4 3.5 3 Mean±55 2,5 2 1.5 1 0.5 0 Probing Depth Clinical Attachment Loss Mucosal Recession KDG≥2 mm KMG<2 mm

Figure 1. The relationship between peri-implant keratinized mucosa width and probing depth, clinical attachment loss and amount of mucosal recession



Figure 2. The relationship between peri-implant mucosal phenotype and probing depth, clinical attachment loss and amount of mucosal recession

Discussion

There are different opinions about the effect of the width and thickness of the keratinized tissue around the dental implant on the peri-implant soft tissue health. According to our study results, it was seen that the insufficiency in the thickness and width of the keratinized tissue around the implant did not negatively affect the clinical periodontal parameters such as PI, GI and BOP and the peri-implant soft tissue health. It is known that good oral hygiene is an important factor in reducing the risk of peri-implant disease and maintaining peri-implant health. It has been shown by some researchers that plaque accumulation, GI and BOP scores are higher in peri-implant areas with a narrow width of KM (2,5,12). However, some studies have reported that peri-implant keratinized mucosa width did not affect PI, GI and BOP scores (6,7). In their study, Buyukozdemir Askin et al. (13) found that the PI scores of the peri-implant regions with narrow KM were higher than the regions with wide KM and they showed that there was a significant improvement in PI scores after the gingival grafting procedure in the periimplant regions with narrow KM. As a result of the study conducted by Schrott et al. (5), it was shown that the width of the KM in the buccal peri-implant regions has no effect on plague accumulation. However, they found statistically significant higher plaque accumulation and bleeding scores in the lingual region with narrow KM (5). In their study, Schrott et al. (5) followed the patients for 60 months at 6-month intervals. During each visit, implant care was performed, which included debridement and oral hygiene instructions. In this study, the amount of KM in the buccal region may not have affected the PI scores, since plaque control was performed at regular intervals. We think that it is more important to provide plaque control rather than the

Table 2. Evaluation of peri	-implant tissue health						
		Peri-implant tis	Peri-implant tissue health				
		Healthy	Peri-implant mucositis	Peri-implantitis	p-value		
		n (%)	n (%)	n (%)			
Type of restoration	Single crown	74 (71.2%)	16 (15.4%)	14 (13.5%)			
	Crown-bridge	345 (69.0%)	105 (21.0%)	50 (10.0%)	0.000*		
	Overdenture	14 (34.1%)	17 (41.5%)	10 (24.4%)			
Kanatining tingun width	≥2 mm	338 (68.1%)	104 (21%)	54 (10.9%)	0.540		
Keratinized tissue width	<2 mm	95 (63.8%)	34 (22.8%)	20 (13.4%)	0.362		
Mucosal phenotype	Thick	327 (68.3%)	100 (20.9%)	52 (10.9%)	0544		
	Thin	106 (63.9%)	38 (22.9%)	22 (13.3%)	0.546		
Chi-square test *n(0.05							

Table 3. Relationship between mucosal phenotype, keratinized tissue width and plaque index, gingival index and bleeding on probing

probilig							
			Diagua index	Cincinal index	Bleeding on probing, n (%)		
				Gingivat index	No	Yes	
	>2 mm	Min-max	0-3	0-3			
Keratinized	22 11111	Mean ± SD (median)	0.63±0.68 (1)	0.64±0.88 (0)	325 (65.5%)	171 (34.5%)	
tissue width	(2 mm	Min-max	0-3	0-2			
		Mean ± SD (median)	0.58±0.69 (0)	0.69±0.87 (0)	94 (63.1%)	55 (36.9%)	
		p-value	¹ 0.432	¹ 0.416	² 0.585		
Mucosal	Thiak	Min-max	0-3	0-3			
phenotype	THICK	Mean ± SD (median)	0.60±0.67 (1)	0.64±0.88 (0)	314 (65.6%)	165 (34.4%)	
	Thin	Min-max	0-3	0-2			
		Mean ± SD (median)	0.66±0.71 (1)	0.69±0.86 (0)	105 (63.3%)	61 (36.7%)	
		p-value	¹ 0.455	¹ 0.381	² 0.592		

¹Mann-Whitney U test, ²Chi-square test, SD: Standard deviation, min-max: Minimum-maximum

amount of KM to protect the peri-implant soft tissue health. Therefore, in patients with good oral hygiene, it can be said that the soft tissue around the implant may be clinically healthy even when there is no keratinized tissue.

Studies have reported that soft tissue recession is common in implant-supported prostheses (14). In particular, the stability of the marginal mucosa of the implant restoration in the maxillary anterior region is important in determining the aesthetics. In most clinical studies, it has been shown that the amount of MR increased significantly in implant sites with narrow keratinized mucosa (2,5-7). However, in the study by Bengazi et al. (9), it was found that the lack of keratinized mucosa did not significantly affect the amount of marginal soft-tissue recession. In addition to KM width, soft-tissue phenotype affects the marginal mucosal level of implants. In a study evaluating the effect of peri-implant MP on periodontal parameters around the dental implant, it was reported that MR was twice as high in peri-implant regions with thin phenotype compared to peri-implant regions with the thick phenotype (6). Similar observations have been previously shown around teeth. Similar to the previous study, in our study, significantly higher CAL and MR were observed in areas where peri-implant MP was thin. Based on these findings, it is seen that insufficient keratinized mucosa in the peri-implant sites increases the amount of CAL and MR. Therefore, it is important to provide sufficient KM width and thickness in the peri-implant soft tissue in the aesthetic zone.

To our knowledge, there is only one study evaluating the relationship between peri-implant MF and CAL. In this study by Zigdon and Machtei (6), they reported that they found statistically significantly higher CAL scores in the peri-implant regions with thin MP. Mericske-Stern et al. (15) found significantly higher CAL scores in lingual periimplant sites with narrow KM. However, they showed that the amount of peri-implant KM in the buccal region did not affect the CAL level. In our study, statistically significantly higher CAL scores were found in peri-implant regions with a KM width of <2 mm and a thin MP. In terms of this finding, the results of our study and the study by Zigdon and Machtei (6) are similar. However, the methods used in the determination of peri-implant MP in the study by Zigdon and Machtei (6) and our study are different. Zigdon and Machtei (6) used the camera to calculate the distance measured with a 27G needle while determining the MP in the buccal periimplant region. In our study, the probe method was used to determine peri-implant MP.

In our study, 5 implants with 4 different surface properties were used. These are Straumann (Waldenburg, Switzerland) and Zinedent (Batı Group and Instradent, Turkey) roughened with sandblasted and large grit acid etched (SLA), Astra Tech (Astra Tech AB, Mölndal, Switzerland) roughened with fluorine modified, Nobel (Branemark, Sweden) roughened by anodization and MIS (MIS Implants Technologies Ltd, Shlomi, Israel) roughened with airborne-particle abraded-and-etched. As a result of a study, it has been shown that

implant surface properties and design affect peri-implant soft tissue health and marginal bone level (7). Polizzi et al. (16) compared bone loss rates between minimally rough implants and moderately rough implants. They found no statistical difference in bone loss between the two surfaces. John et al. (17) investigated the initial biofilm formation on four titanium discs with different surface properties or chemical modifications. Samples were evaluated 48 hours after intraoral contact. It was concluded that discs with a machined surface showed slower biofilm formation and slower plaque maturation than a rough SLA surface. On the other hand, five-year clinical follow-up of partially roughened surfaces did not show an increased risk of peri-implantitis (18). However, studies investigating the effect of implant surface on peri-implantitis are limited and contradictory. While some studies have found a positive association between smooth surface and peri-implant health (19), some studies have found no correlation between the type of implant surface and marginal bone loss (20). Therefore, we think that direct comparisons should be made in order to determine the effect of the implant surface on the peri-implant tissues. Many factors such as previous periodontitis history, diabetes and smoking affect periimplant soft tissue health.

Various methods have also been used in studies to determine peri-implant MP (14,21,22). Although none of these methods are seen as the gold standard today, each method has its advantages and disadvantages Gharpure et al. (21) used 12 color-coded probes to determine peri-implant MP. Periimplant MP was categorized as thin and thick according to the visibility of the probe from the mucosa. In one study, an endodontic reamer was used to measure soft tissue thickness in the buccal region of implant-supported restorations (22). The endodontic reamer with a rubber stopper was inserted perpendicular to the gingiva until its contact with the alveolar bone was felt. Peri-implant mucosa thickness was determined by measuring the distance between the tip of the endodontic reamer and the rubber stopper with a caliper. Cardaropoli et al. (14) used a calibrated ultrasonic device to determine peri-implant mucosal thickness. The disadvantages of transgingival probing and endodontic reamer methods are that they are invasive procedures and require anesthesia. In addition, it is recommended to wait 20 minutes after the application of anesthesia in the transgingival probing method. In addition, it has been shown that reliable results are obtained with the probing method (10). De Rouck et al. (10) reported that the probe method is simple and reproducible. In our study, we preferred to use the probe method, which is a reliable and minimally invasive method that does not require the use of anesthesia, reproducible results can be obtained, in determining the phenotype. In addition, another advantage of my use of the probe method is that the required equipment is inexpensive.

In our study, the rate of the health of peri-implant areas restored with overdenture was found to be significantly lower than those of peri-implant areas restored with a single crown and crown bridge. According to the results of a review, it was reported that the mean marginal bone loss in the mandible was 0.9 mm 10 years after rehabilitation of implants with a fixed prosthetic and 1.2 mm after 15 years (23). Another study found that the mean bone loss after 12 years was 1.7 mm around implants treated with overdentures in the mandible (24). These findings indicate that more bone loss occurs in implants treated with overdenture in the long term. It is known that overdenture restorations are preferred in completely edentulous advanced age group patients. In addition, studies have reported that the possibility of peri-implantitis is higher in patients older than 65 years of age (25). These results can be explained by the decrease in tooth brushing cooperation of patients due to aging. It is seen that the probability of developing the peri-implant disease is high with advancing age. Therefore, it is important to apply supportive periodontal and peri-implant treatment to patients in the advanced age group.

This study had some important limitations. The amount of peri-implant MR is affected by various factors such as KM width and thickness, crestal bone level, depth of the implant platform and buccal position of the implant. In our study, only the effect of peri-implant KM width and thickness on the marginal mucosal level was evaluated. The effect of the oral hygiene habits of the patients on the peri-implant tissues was not evaluated.

Conclusion

Within the limits of our study, it was found that KM width and thickness around the implant are not necessary for soft tissue health in patients who can provide plaque control. However, to reduce the risk of developing the peri-implant disease in patients who cannot provide adequate plaque control, the amount of keratinized soft tissue should be evaluated during implant planning.

Ethics

Ethics Committee Approval: Approval was obtained from the İnönü University Scientific Research and Publication Ethics Committee for this study (protocol number: 2020/844, date: 14.07.2020).

Informed Consent: Written and verbal information about the purpose and method of the study were given to all patients, the information forms were read and consent forms were signed by the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.B., M.Ö.U., Concept: M.Ö.U., Design: M.Ö.U., Data Collection or Processing: E.B., Analysis or Interpretation: E.B., Literature Search: E.B., Writing: E.B., M.Ö.U. **Conflict of Interest:** No conflict of interest was declared by the authors.

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

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The Impact of COVID-19 Outbreak on the Pediatric Dentists and the Pediatric Dental Practice Across Turkey

Türkiye'de COVID-19 Pandemisinin Çocuk Diş Hekimleri ve Çocuk Diş Hekimliği Uygulamaları Üzerindeki Etkisi

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Abstract

Objective: The coronavirus disease-2019 (COVID-19) pandemic has had a significant impact on pediatric dentists and the clinical practice of pediatric dentistry. The aim of this study was to investigate the effect of the COVID-19 pandemic among pediatric dentists in Turkey by evaluating protective measures, attitudes, and perception levels regarding COVID-19, clinical protocols, and professional approaches of pediatric dentation dentitioners.

Materials and Methods: A descriptive cross-sectional survey was conducted on a sample of pediatric dentists in Turkey. The questionnaire consisted of 34 questions in five domains: personal data, health conditions, precautionary measures and working conditions, knowledge and self-perceived risk of infection, attitudes and perception, and COVID-19 pandemic impact on clinical practice.

Results: A total of 221 pediatric dentists participated in the survey. 33.9% of the participants (n=75) perform non-emergency procedures during the pandemic. Almost half of the participants considered atraumatic restorative treatment option for teeth with dentin caries (n=120, 54.3%). Moreover, the participants reported that they would treat a traumatized tooth [a tooth with luxation (n=210, 95%)].

Conclusion: Pediatric dentists can limit their dental procedures to emergency treatments and select biological, noninvasive, or minimally invasive methods to reduce the risk of COVID-19 transmission.

Keywords: COVID-19, dental practice, infection control, pediatric dentistry

Öz

Amaç: Koronavirüs hastalığı-2019 (COVID-19) pandemisinin çocuk diş hekimleri ve çocuk diş hekimliği klinik uygulamaları üzerinde önemli bir etkisi olmuştur. Bu çalışmanın amacı, çocuk diş hekimlerinin COVID-19 ile ilgili aldıkları koruyucu önlemleri, tutum ve algı düzeylerini, klinik uygulamalarını ve profesyonel yaklaşımlarını değerlendirerek, Türkiye'deki çocuk diş hekimleri arasında COVID-19 pandemisinin etkisini araştırmaktır.

Gereç ve Yöntemler: Türkiye'deki çocuk diş hekimlerinin bir bölümüne tanımlayıcı, kesitsel bir anket gönderildi. Anket beş alanı kapsayan 34 sorudan oluşmaktadır: kişisel veriler, sağlık durumları, koruyucu önlemler ve çalışma koşulları, bilgi ve algılanan enfeksiyon riski, tutum ve algı düzeyleri ve COVID-19 pandemisinin klinik uygulamalar üzerindeki etkisi.

Bulgular: Araştırmaya toplam 221 çocuk diş hekimi katıldı. Katılımcıların %33,9'u (n=75) pandemi döneminde, acil olmayan tedavileri uygulamaktadır. Katılımcıların neredeyse yarısı dentin çürüğü olan dişler için atravmatik restoratif tedavi seçeneğini tercih etmektedir (n=120, %54,3). Ayrıca, katılımcılar travmatize olmuş bir dişi [luksasyonlu bir dişi (n=210, %95)] tedavi edeceklerini bildirdi.

Sonuç: Çocuk diş hekimleri, diş tedavilerini acil tedavilerle sınırlayabilir ve COVID-19 bulaşma riskini azaltmak için biyolojik, invazif olmayan veya minimal invaziv yöntemler seçebilirler.

Anahtar Kelimeler: COVID-19, diş hekimliği uygulamaları, enfeksiyon kontrolü, çocuk dişhekimliği

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Introduction

The coronavirus disease-2019 (COVID-19) outbreak is a public health crisis for which diagnostic and treatment efforts present challenges for the healthcare communities all around the world (1). As the world fights the COVID-19 pandemic, dentists have had to alter routine clinical actions. The nature of dental procedures that can create an aerosol cloud makes dental work environments among the highest risk categories for COVID-19 transmission (2). As such, routine dental care was suspended to help reduce transmission of infection during the COVID-19 outbreak (3).

Pediatric dentists who treat children in the pandemic must implement universal infection control implementations at the highest level. As opportunities to encourage preventive dental practice have emerged in this pandemic situation, they must be seized. Moreover, contemporary, minimally invasive procedures that lessen or wipe out aerosol generation must be used during the pandemic (4). When using restorative or endodontic treatment, chemomechanical caries removal e.g., Carisolv and papain gel is a suitable alternatives (5,6). Additionally, silver diamine fluoride and glass ionomer cements can be preferred (6). In some cases, analgesics and/or antibiotics may be considered as therapeutic agents (6).

It is widely accepted that standard personal protective equipment (PPE) is not sufficient for airborne infections (e.g., COVID-19). With this in mind, some more effective measures can be taken. Protective equipment e.g., safety glasses, mask, glove, face shield, and protective outerwear to create a barrier against the virus is highly recommended to dentists because transmission of airborne droplet infection is mentioned as the main spread route in dental practice (5). However, considering that all PPE that must be worn as recommended in all general guidelines are also valid for pediatric dentists, wearing all of this equipment at the same time helps create a dental anxiety-enhancing situation, especially for children in the younger age group. In addition to the possibility of increasing anxiety in the pediatric patient as a result of wearing PPE, another situation experienced is the difficulty of verbal communication. This situation may cause an increase in dental treatments under sedation and general anesthesia in children during the pandemic period.

Pediatric dentistry aims to protect children's well-being and safety by redesigning, reassessing and reflecting dental care and treatment practices during the pandemic, staying up to date with existing evidence-based guidance and recommendations for child oral health care. Therefore, a risk assessment of the application must be conducted to minimize the risk of COVID-19 transmission and to determine the necessary precautions. The purpose of this study was to assess the knowledge, thoughts and behaviors of pediatric dentists about the measures to be taken against COVID-19 disease caused by the severe acute respiratory syndrome coronavirus-2 as they actively carry out their duties. It is also the intention to evaluate the effects of the COVID-19 outbreak on clinical practice in pediatric dentistry as well as to raise awareness on the importance of these changes.

Materials and Methods

This study was based on a questionnaire applied between November 3 and December 3, 2020, among Turkish pediatric dentists including those doing post-graduation studies, working in government, private and other health sectors. The total number of pediatric dentists in Turkey was estimated to be about 1,500 in total, including the ones who work in public, private, and nonprofit organizations. Based on simple random sampling method, the minimum number of participants needed for the study was found as 211. A questionnaire was designed with guidance from the relevant sources and based on experts' opinions (attending pediatric dentists). Ethical permission required for the study to be carried out was obtained from Gaziosmanpasa Training and Research Hospital, Medical Research Local Ethics Board (number: 179, date: 21.10.2020); and 221 individuals who responded to the survey were included.

For this study, Google forms was used to create the survey. Before starting the study, in order to test the comprehensibility and consistency of the questionnaire within the scope of the study, the questions were sent to 3 specialists, 2 pediatric dentists and 1 biostatistician. With their guidance, biased and confusing questions were omitted. The questionnaire consisted of six domains and included 46 guestions. The domains included in the questionnaire were personal characteristics (age, gender, working status), health status (symptoms of the COVID-19), precautionary measures and working conditions, knowledge and self-perceived infection risk, attitudes and perception and COVID-19 pandemic impact on clinical practice. A brief introduction was presented at the beginning of the survey to inform the respondents of the purpose and content of this study, and electronic informed consent was obtained if they agreed to complete the questionnaire. The dentists were asked to participate in the study online (e-mails/social media) and to distribute the survey to colleagues. A total of volunteering 221 Turkish pediatric dentists answered the survey.

Statistical Analysis

All data were transferred from Google forms into Microsoft Excel (Microsoft Corp, Redmond, WA) and analyzed with IBM SPSS Statistics 22 (SPSS IBM, Turkey). The suitability of the parameters to the normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilks tests, and it was found that the parameters did not show a normal distribution. To evaluate the data, descriptive statistical methods (mean scores, SD, frequencies), and Kruskal-Wallis test were used in the comparison of more than two groups of parameters in comparison of quantitative data and Dunn's test was used to determine the group that caused the difference. Mann-Whitney U test was used to compare parameters between groups. To compare qualitative data, chi-square test, Fisher's exact chi-square test, and Fisher-Freeman-Halton test were used. Significance was assessed at the p<0.05 level for this study.

Results

Overall, 221 pediatric dentists filled the questionnaire. Among all the participants, 38 (17.2%) were male, and 183 (82.8%) were female. The average age was 31.66±7.88 years. 52.5% of the participants work in a state university, 17.6% in a private dental polyclinic, 13.1% in a foundation university, 8.1% in a private dental office, 5.4% in a goverment oraldental health center, and 3.2% in a private dental hospital.

The distribution of the participants' answers given to questions about dental practices which were done during the pandemic is presented in Table 1. 57.9% of the participants stated that there was no change in the number of patients who wanted to be treated with sedation or general anesthesia when compared with the pre-pandemic period, while 28.5% of the participants stated that the number of patients who wanted to be treated with sedation or general anesthesia increased and 13.6% of the participants stated that there was a decrease in this respect.

Table 1. Distribution of answers given to questions about dental practices which were done during pandemic						
Questions	Options	n	%			
	Root canal treatment	107	48.4			
Treatment option considered for the treatment of a deciduous tooth with acute pulpitis	Tooth extraction	90	40.7			
	Giving antibiotics and follow up	68	30.8			
	Atraumatic restorative treatment	85	38.5			
	Pit and fissure sealant	72	32.6			
The treatment option considered for enamel caries	Treatment with chemomechanical caries removal agents	22	10			
	Conservative treatment	102	46.2			
	Atraumatic restorative treatment	120	54.3			
	Pit and fissure sealant	7	3.2			
The treatment option considered for teeth with dentin caries	Treatment with chemomechanical caries removal agents	28	12.7			
	Caries sealing technique	50	22.6			
	Conservative treatment	140	63.3			
	Hall technique	41	18.6			
	Yes	40	18.1			
Application of hall technique	No	173	78.3			
	I don't know this technique	8	3.6			
	Yes	32	14.5			
The use of topical silver diamine fluoride application	No	181	81.9			
	I don't know this technique	8	3.6			
During the pandemic, treatment of a tooth with enamel-	Yes	193	87.3			
dentin fracture as a result of trauma	No	28	12.7			
During the pandemic, treatment of a tooth avulsed as a	Yes	207	93.7			
result of trauma	No	14	6.3			
During the pandemic, treatment a tooth with luxation as	Yes	210	95.0			
a result of trauma	No	11	5.0			
The preferred imaging method when the patient wants	Intraoral imaging	124	56.1			
to make a radiographic diagnosis of a single tooth in the first examination	Extraoral imaging	97	43.9			

Table 1. Continued						
Questions	Options	n	%			
Continuing to work as before the COVID-19 pandemic	Yes	58	26.2			
	No	163	73.8			
	Yes	75	33.9			
Emergency patients only	No	142	64.3			
	I do not accept any patients	4	1.8			
Knowing the definition of emergency patient in	Yes	215	97.3			
paediatric dentistry	No	6	2.7			
COVID-19: Coronavirus disease-2019						

Precautions were taken by dentists continuing to work in the pandemic. PPE and devices used by pediatric dentists are presented in Table 2.

A statistically significant difference was detected between the rates of only taking care of dental emergency patients according to institution (p=0.000; p<0.05). In governmental oral and dental health centers and in state universities, the rates of treating only emergency patients (66.7%, 49.1%) are significantly higher than it is in private dental offices (5.6%), private dental polyclinic (7.7%) and foundation universities (13.8%) (p1=0.001; p2=0.001; p3=0.002; p<0.05). There is no significant difference between other institutions (p>0.05).

The treatment modalities applied by dentists during the pandemic period also changed according to the institution they work in (Table 3).

Discussion

While 64.3% of the pediatric dentists treated all of the pediatric dental patients, 33.9% only cared for dental emergency patients. Unlike, in the studies by Bekes et al. (7) and Ahmadi et al. (8), the majority of the participants (respectively 78.6%, 70%) refrained from performing non-

emergency procedures while the pandemic continues. This suggests that dental practice must be performed with more infection control measures and non-emergency treatments must be delayed until the pandemic is over (9).

For patients suffering from severe toothache and intense caries, pathogenic tooth extraction may be considered instead of a restorative treatment because this may shorten the treatment time and reduce infection risk (10). In case of emergency, the American Dental Association COVID-19 Dental Emergency document (10) suggests that chemomechanical caries removal and handpieces must be preferred to rotary systems. In symptomatic irreversible pulpitis, pain reduction with pulpotomy and pulpectomy is recommended over conventional root canal treatments, if possible (11). In this study, the percentage of tooth extraction option considered for the treatment of a deciduous tooth with acute pulpitis is 40.7%, while conventional root canal treatment option is 48.4%. 54.3% of the participants considered atraumatic restorative treatment option for teeth with dentin caries. Only 12.7% of the participants considered treatment with chemomechanical caries removal agents for teeth with dentin caries.

During the pandemic, the percentages of treatment option for a tooth with enamel-dentin fracture, the treatment option

Table 2. Answers to questions about personal protective equipment, devices and aerosol				
		n	%	
	Surgical mask	199	90.0	
	Filtering facepiece 2 or filtering facepiece 3 masks	214	96.8	
	Disposable headset		62.9	
	Sterile microfiber disposable gown	174	78.7	
Personal protective equipment	Disposable gown	173	78.3	
	Safety glasses or visor	152	68.8	
	Sterile disposable gloves	68	30.8	
	Disposable gloves	215	97.3	
	Rotating instrument with anti-retraction valve	14	6.3	

Table 3. Evaluations of clinical practices during COVID-19 pandemic according to the institution being worked at								
		Private dental office	Private dental polyclinic	Private dental hospital	State university	Foundation university	Govermental oral and dental health center	
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	p-value
Treatment option in the	Root canal treatment	15 (83.3%)	22 (56.4%)	4 (57.1%)	45 (38.8%)	17 (58.6%)	4 (33.3%)	² 0.005*
treatment of a deciduous	Tooth extraction	2 (11.1%)	9 (23.1%)	3 (42.9%)	63 (54.3%)	10 (34.5%)	3 (25%)	¹ 0.000*
tooth with acute pulpitis	Giving antibiotics and follow up	3 (16.7%)	13 (33.3%)	3 (42.9%)	31 (26.7%)	9 (31%)	9 (75%)	¹ 0.014*
	Atraumatic restorative treatment	1 (5.6%)	7 (17.9%)	5 (71.4%)	57 (49.1%)	9 (31%)	6 (50%)	¹ 0.000*
Treatment	Pit and fissure sealant	6 (33.3%)	10 (25.6%)	2 (28.6%)	41 (35.3%)	11 (37.9%)	2 (16.7%)	¹ 0.715
tooth with enamel caries	Treatment with chemomechanical caries removal agents	0 (0%)	3 (7.7%)	0 (0%)	17 (14.7%)	1 (3.4%)	1 (8.3%)	¹ 0.291
	Conservative treatment	13 (72.2%)	22 (56.4%)	3 (42.9%)	46 (39.7%)	17 (58.6%)	1 (8.3%)	² 0.004*
	Atraumatic restorative treatment	1 (5.6%)	10 (25.6%)	5 (71.4%)	78 (67.2%)	14 (48.3%)	12 (100%)	² 0.000*
	Pit and fissure sealant	0 (0%)	0 (0%)	0 (0%)	6 (5.2%)	1 (3.4%)	0 (0%)	² 0.734
Treatment option for a tooth with dentin caries	Treatment with chemomechanical caries removal agents	0 (0%)	5 (12.8%)	0 (0%)	16 (13.8%)	4 (13.8%)	3 (25%)	¹ 0.364
	Caries sealing technique	0 (0%)	6 (15.4%)	0 (0%)	33 (28.4%)	4 (13.8%)	7 (58.3%)	¹ 0.001*
	Conservative treatment	17 (94.4%)	30 (76.9%)	3 (42.9%)	61 (52.6%)	26 (89.7%)	3 (25%)	¹ 0.000*
	Hall technique	0 (0%)	3 (7.7%)	3 (42.9%)	26 (22.4%)	7 (24.1%)	2 (16.7%)	¹ 0.021*
	Yes	1 (5.6%)	3 (7.7%)	3 (42.9%)	26 (22.4%)	7 (24.1%)	0 (0%)	² 0.013*
Applying the	No	15 (83.3%)	34 (87.2%)	4 (57.1%)	88 (75.9%)	22 (75.9%)	10 (83.3%)	
hall technique	l don't know this technique	2 (11.1%)	2 (5.1%)	0 (0%)	2 (1.7%)	0 (0%)	2 (16.7%)	
The use of	Yes	3 (16.7%)	4 (10.3%)	1 (14.3%)	20 (17.2%)	4 (13.8%)	0 (0%)	² 0.150
topical silver diamine	No	12 (66.7%)	34 (87.2%)	6 (85.7%)	92 (79.3%)	25 (86.2%)	12 (100%)	
fluoride application	l don't know this technique	3 (16.7%)	1 (2.6%)	0 (0%)	4 (3.4%)	0 (0%)	0 (0%)	
¹ Fisher-Freeman-	Halton test, ² Chi-square	e test, *p<0.05,	COVID-19: Coroi	navirus-2019				

of a tooth avulsed, and the treatment option for a tooth with luxation as a result of trauma were 87.3%, 93.7%, 95% respectively, which means that pediatric dentists care about trauma treatment.

Wearing filtering facepiece 2 or filtering facepiece 3 masks was among the most frequent (96,8%) measure. Duruk et al. (12) indicated that wearing an N95 mask was one of the measures taken at least by 12.36% of Turkish dentists. That sudy was published at the begining of the pandemic so this low rate may be related to the difficulties of finding an N95 mask in the early days of the pandemic.

The rate of applying for root-canal treatment in private dental offices (83.3%) is significantly higher than those in private dental polyclinics (56.4%), state universities (38.8%) and governmental oral and dental health centers (33.3%) (p1=0.048; p2=0.001; p3=0.009; p(0.05). Moreover, the rate of applying for conservative treatment in private dental offices (72.2%) is significantly higher than in state universities (39.7%) (p=0.010; p<0.05). Although it was reported by Ahmadi et al. (8) that 86% of dental professionals will emphasize preventive care, not perform unneeded treatment and cut back on the treatment sessions as much as they can in the future, urgent dental cases need treatment, and dentists must adopt strict infection control measures and use minimally invasive methods, and avoid equipment generating aerosol (13).

It is seen that the rate of applying for tooth extraction in the treatment of a deciduous tooth with acute pulpitis at state universities (54.3%) is significantly higher than in private dental offices (11.1%) and private dental polyclinics (23.1%) (p1=0.001; p2=0.001; p<0.05). There is a statistically significant difference between the administration of antibiotics and follow-up rates in the treatment of a primary tooth with acute pulpitis according to the type of institution (p=0.000; p<0.05). The rate of giving antibiotics and follow up in govermental oral and dental health centers (75%) is significantly higher than in private dental offices (16.7%), private dental polyclinics (33.3%), state universities (26.7%) and foundation universities (31%) (p1=0.002; p2=0.011; p3=0.001; p4=0.010; p(0.05). It was discovered that caries sealing technique and the hall technique, both of which are minimally invasive techniques, in the treatment of a tooth with dentin caries has not been used in any of the private dental offices.

All in all, during the COVID-19 pademic, there has been some restrictions on non-emergency dental care. In this respect, reopening dental practices will probably present some unique challenges and will certainly provide opportunities to have more focus on prevention and procedures that are nonaerosol-generating.

This study aims to contribute to the literature by raising awareness among pediatric dentists about infection control measures, prevention and non-aerosol-generating procedures.

Conclusion

Since the symptoms of the COVID-19 disease are less common in children, pediatric dentists are at high risk of contracting COVID-19. For this reason, pediatric dentists must assume that every person is potentially infected and follow unquestionably universal infection control procedures. In the current scenario, it is important to prioritize dental procedures labeled by World Health Organization as emergencies to reduce the COVID-19 transmission risk. Pediatric dentists in Turkey must encourage the use of minimally invasive procedures that greatly reduce or even eliminate aerosol formation while the pandemic continues to exist.

Ethics

Ethics Committee Approval: Ethical permission required for the study to be carried out was obtained from Gaziosmanpasa Training and Research Hospital, Medical Research Local Ethics Board (number: 179, date: 21.10.2020).

Informed Consent: A brief introduction was presented at the beginning of the survey to inform the respondents of the purpose and content of this study, and electronic informed consent was obtained if they agreed to complete the questionnaire.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

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Effects of Ozone Threapy as an Adjunct to Non-Surgical Periodontal Treatment on Clinical Periodontal Parameters and Inflammatory Markers in Periodontitis Patients

Periodontitisli Bireylerde Cerrahi Olmayan Periodontal Tedaviye Ek Olarak Uygulanan Ozonun Klinik Periodontal Parametrelere ve Enflamatuvar Belirteç Seviyelerine Etkileri

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Abstract

Objective: Ozone therapy (OT) is known to eliminate pathogens in the periodontal pocket and accelerate wound healing. This study aimed to investigate the clinical and biochemical efficacy of OT as adjunctive scaling and root planning (SRP) in the treatment of periodontitis.

Materials and Methods: Fifty systemically healthy individuals with periodontitis were included in this study. Gingival crevicular fluid (GCF) samples were taken from the patients before and 8 weeks after starting the treatment, and gingival index (GI), plaque index (PI), bleeding on probing (BOP), pocket depth (PD), and clinical attachment level (CAL) were obtained from all subjects. Non-surgical periodontal treatment was applied to the test and control groups, and gaseous ozone treatment was applied in addition to the test group. TNF- α , IL-1 β , IL-6, IL-10, and MMP-9 values in GCF samples before and after treatment were determined by the ELISA method and evaluated statistically.

Results: Both groups presented significantly decreased clinical periodontal parameters at 8-week from baseline (p<0.05). Clinical periodontal parameters were found to be significantly lower in the test group than In the control group, there was a decrease in TNF- α levels after non-surgical periodontal treatment (p<0.05). Both groups demonstrated significantly decreased IL-1 β , IL-6, IL-10, and MMP-9 levels at 8-week (p<0.05). The test group showed a significant decrease in MMP-9 levels compared with the control group at 8-week (p<0.05).

Conclusion: As a result of the close relationship observed between clinical periodontal measurements and biochemical parameters, it was observed that clinical periodontal healing affects biochemical parameters, and in addition to non-surgical periodontal treatment, ozone treatment improves clinical periodontal measurements and affects biochemical parameters by reducing the GCF volume.

Keywords: Periodontitis, cytokines, inflammation, matrix metalloproteinases, ozone, periodontal debridement

Öz

Amaç: Medikal ozon tedavisinin, periodontal cepteki patojenleri elimine ettiği ve yara iyileşmesini hızlandırdığı bilinmektedir. Bu çalışmanın amacı, periodontal hastalıklı bireylerde ozon tedavisinin klinik ve biyokimyasal olarak etkilerinin değerlendirilmesidir.

Gereç ve Yöntemler: Bu çalışmaya sistemik olarak sağlıklı ve periodontitisli 50 birey dahil edildi. Tedaviye başlamadan önce ve 8 hafta sonra hastalardan dişeti oluğu sıvısı (DOS) örnekleri alınmış, tüm bireylerden gingival indeks, plak indeksi, sondalamada kanama, sondalamada cep derinliği ve klinik ataçman seviyesini içeren klinik periodontal ölçümler elde edilmiştir. Test ve kontrol grubuna cerrahi olmayan periodontal tedavi yapılmış, test grubuna ek olarak gaz ozon tedavisi uygulanmıştır. Tedavi öncesi ve sonrası, DOS örneklerinde Tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-10 ve MMP-9 değerleri ELİSA metoduyla saptanmış ve istatistiksel olarak değerlendirilmiştir.

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) **Bulgular:** Hem test hem de kontrol grubunda cerrahi olmayan periodontal tedavi sonrası klinik periodontal ölçümlerde başlangıç değerlerine göre azalma saptanmıştır ($p\langle 0,05$). Test grubunda, kontrol grubuna göre, klinik periodontal ölçümlerde azalma tespit edilmiştir ($p\langle 0,05$). Her iki grupta da DOS IL-1 β , IL-6, IL-10 ve MMP-9 seviyelerinde başlangıç değerlerine göre, cerrahi olmayan periodontal tedavi sonrası azalma gözlenmiştir ($p\langle 0,05$). Kontrol grubunda DOS TNF- α seviyesinde başlangıç değerlerine göre, cerrahi olmayan periodontal tedavi sonrası azalma gözlenmiştir ($p\langle 0,05$). Kontrol grubunda DOS TNF- α seviyesinde başlangıç değerlerine göre, cerrahi olmayan periodontal tedavi sonrasında azalma saptanmıştır ($p\langle 0,05$). Test grubunda kontrol grubuna göre, DOS MMP-9 seviyesinde azalma gözlenmiştir ($p\langle 0,05$).

Sonuç: Klinik periodontal ölçümler ve biyokimyasal parametreler arasında gözlenen yakın ilişki, klinik periodontal iyileşmenin biyokimyasal parametreleri etkilediği, cerrahi olmayan periodontal tedaviye ek olarak ozon tedavisinin DOS hacmini azaltarak klinik periodontal ölçümleri iyileştirdiği ve biyokimyasal parametrelere etki ettiği de gözlenmiştir.

Anahtar Kelimeler: Periodontitis, sitokinler, enflamasyon, matriks metalloproteinaz, ozon, periodontal debridman

Introduction

Periodontal disease stems from the reaction of the host defense mechanisms with the plaque microorganisms, which destroy the supporting tissues of the teeth (1). The basis of non-surgical periodontal therapy is the mechanical elimination of microbial plaque and calculus by scaling and root (SRP). However, SRP alone may be insufficient to destroy the pathogenic bacteria, due to their location in the gingival and dental tissues or in other sites that are not easily accessed by periodontal instruments (2). Local or systemic antibiotics and antiseptics are suggested to improve the results of periodontal therapy (3).

Ozone therapy (OT), widely used in dentistry, has been proven *in vitro* and *in vivo* studies to be effective in the removal of pathogenic microorganisms in periodontal disease (4,5). Medical ozone has very high oxidation power, it is recognized as "active oxygen" in the medical field (6). OT also stimulates the immune system by releasing cytokines such as interferon and interleukin (IL) by leukocytes (7,8). Studies are stating that OT as an adjunct to SRP contributes to the improvement in clinical parameters in patients with periodontitis (9,10). This study was aimed to evaluate the clinical and biochemical efficacy of gas OT as an adjunctive to SRP in patients with periodontitis.

Materials and Methods

This study was a randomized-controlled, double-blind clinical trial, conducted between October 2014 and October 2015 in a single centre, and each individual was followed up for 8 weeks. The study protocol was confirmed by the Ethics Committee of Atatürk University, Erzurum, Turkey (decision code: 24/2014, date: 13.10.2014), and managed according to the principles outlined in the Declaration of Helsinki on experimentation involving human individuals. The signed consent form was obtained from all participants.

Selection of Patient

A total of 50 healthy, non-smoking patients with periodontitis (19 women and 31 men) between 31 to 57 were selected. All patients presented stage III periodontitis, based on clinical parameters [plaque index (PI), gingival index (GI), probing pocket depth (PD), clinical attachment level (CAL)]. Patients of each group had a minimum of 20 teeth and at least two pocket site with a probing depth 5 mm or more in each quadrant. Patients with periodontitis were classified according to the 1999 classification at the time of treatment, but were revised according to the 2017 periodontal disease classification defined by the European Federation of Periodontology and the American Academy of Periodontology (11) classification criteria. Patients with stage III periodontitis according to current classification were included in the study. All individuals were generally healthy, non-smoking, and none had undergone periodontal therapy and/or antibiotic therapy in the past 6 months. Pregnant or breastfeeding women and tobacco users were excluded from the study. Depending on the treatment plan, the patients were divided into 2 groups.

Control group: This group included 25 patients with stage III periodontitis (9 women and 16 men) aged between 31 to 57 who were applied SRP.

Test group: Test group consisted of 25 patients stage III periodontitis (10 women and 15 men), aged between 33 to 55, who were applied OT as an adjunctive to SRP.

Power Analysis

The sample size was determined by showing regard to α =0.05 and power (1- β)=85%. Because of the variability value (σ =SD), a 0.05 mm change in the clinical attachment gain was used. The clinically allowable mean value (δ) was taken as 0.5 mm. Relying on these data, the minimum number of patients required for this study was computed as 22 for each group

Study Design

The study design summerized Figure 1. Before treatment was initiated, the randomized determination of the test or control groups was performed using a coin toss. Two calibrated rearchers (A.D., S.S.) conducted the study. Only one researcher (S.S.) conducting clinical evaluation was blinded to the treatment undergone by patients. The treatment groups were encoded, therefore only the operator (A.D.) was informed of the protocol and the examiner maintained blinded during the study. The clinical examination in both groups was performed two times: baseline and eight weeks after treatment. The parameters were calculated by a periodontal probe (Williams; Hu-Friedy). The following parameters was assesed: PI, GI, PD, CAL were measured at six sites of each tooth (mesialbuccal, buccal, distalbuccal, mesiolingual, lingual and distolingual). Only periodontal pockets depth of 5 mm and above were included in the assessment. Before SRP, patients were given oral hygiene training including toothbrush and dental floss and/or interdental brushes were called for control 1 week later and gingival crevicular fluid (GCF) samples were taken.

Examiner Calibration

The investigator (S.S.) conducting clinical evaluation was calibrated for intra-examination repeatability before the study initiation. Five patients with 10 defects were included as this reason. Duplicate parameters of PD were gathered with a period of 48 hours among the first and the second record. Standardization was approved whereas parameters at baseline and at 48 h were within a milimeter (mm) at \geq 90% of the time.

Treatment Procedure

The study was performed in the Department of Periodontics, Faculty of Dentistry, Ataturk University, Erzurum, Turkey.

Test Group: Each of the patients received SRP. Mechanical debridement was applied with ultrasonic piezoelectric scalers (EMS, Mini-Piezon) and hand instruments (Gracey curettes, Hu-Friedy). Following SRP in the test group, gas ozone application into the periodontal pockets was carried out by the capillary tip (KPX) for 60 sec at 26 µg power (16,600 ppm at 60% power) accordance to the parameters



Figure 1. Study desing

GCF: Gingival crevicular fluid, SRP: Saling and Root Planing, OT: Ozone therapy specified by the manufacturer company (Ozonytron XP, MYMED Gmb H). The application of each tooth are made in equal time careffully. Gas ozon was applied two times for three consecutive days. Aspiration was performed to prevent ozone inhalation during application.

Control Group: Only SRP was applied.

Sample Collection

GCF samples were collected from four affected teeth using paper strips (PeriopaperTM, Oraflow Inc). The procedure was continued for 30 seconds at the gingival sulcus for each tooth. The sample volumes were measured with a calibrated specific device (Periotron 8000®, Oraflow Inc). The strips stored at -80 °C prior to use. GCF sampling and clinical periodontal parameters were obtained before SRP for both groups and after 8 weeks period.

Biochemical Analysis

Biochemical analyses were evaluated with the "Enzyme Linked-Immuno-Sorbent Assay" (ELISA) method using a diagnostic tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), Interleukin-6 (IL-6), Interleukin-10 (IL-10) (eBioscience, Bender Medsystems) and matrix metalloproteinases-9 (MMP-9) (Quantikine®, R&D Systems) ELISA kit.

Statistical Analysis

Statistical analyses was performed by using SPSS 20.0 program.

The results were described as the mean ± standard deviation (SD). The normal distribution suitability of the parameters was determined by the Kolmogorov-Smirnov test. Because all parameters were normalized, pre-and post-treatment values, which were assessed by t-test (paired samples t-test) for dependent samples, are used to compare the mean of numerical data from two dependent groups. The student t-test for dependent samples were done with differences over time in the related groups. Pearson correlation analysis was applied with regard to correlation analysis. The value of p<0.05 was taken into account to be statistically significant.

Results

The test group consisting of 10 females and 15 males with a mean age of 42.16 ± 6.32 with periodontitis was carried out on the control group consisting of 9 females and 16 males with a mean age of 40.16 ± 8.04 . There was no significant difference between the groups in terms of age and gender distribution (p>0.05).

The mean and SDs of the clinical periodontal parameters and GCF volumes obtained from baseline and after 8-week are shown in Table 1. There was no statistically significant difference between groups baseline clinical periodontal parameters and the GCF volume (p>0.05).

There was no statistically significant difference between the test and control groups in the GCF volume after 8-week (p>0.05), but there was a statistically significant reduction in the clinical periodontal parameters (p<0.05).

In the test and control groups, a statistically significant decrease in all clinical periodontal parameters and GCF volumes after baseline and after 8-week were observed (p<0.05).

The mean values of biochemical markers between baseline and after 8-week and statistical comparison of these values between the test and control groups are given in Table 2.

There was no statistically significant difference between the mean values of baseline levels of biochemical marker between the test and control groups (p>0.05)

In the test and control group, the baseline levels of IL-1 β , IL-6, IL-10 and MMP-9 decreased after 8-week and this decrease was statistically significant (p(0.05).

Comparasion between the test and control group after therapy, a significant decrease in the GCF MMP-9 levels was found in the test group (p=0.01).

Discussion

As a result of our study; there was a significant decrease in clinical periodontal parameters and GCF volume in patients treated with gas ozone after SRP. A statistically significant reduction was observed IL-1 β , IL-6, IL-10 and MMP-9 levels in groups, while MMP-9 levels were significantly decreased in the test group compared to the control group at 8-week.

In this study, it was observed that clinical periodontal parameters showed improvement after SRP according to baseline values after gas OT. In several studies, it has been determined that ozonized water treatment is more effective in improving clinical periodontal parameters compared to SRP alone in periodontitis therapy (9,10,12,13). In a study, Hayakumo et al. (9) reported that ozonized water

as an adjunctive to SRP, improved the clinical periodontal parameters in patients with periodontitis after 4-week. Ranjith et al. (10) found that adjunctive ozone water irrigation resulted in significant advancement in all clinical parameters, outside of PD. Also a study performed by Kshitish et al. (12) showed that ozonized water as an adjunctive to SRP significantly improved the clinical periodontal parameters on the 18th day in patients with periodontitis. Moreover, Katti et al. (13) demonstrated that ozonized water as an adjunctive to SRP lead to a significant reduction in clinical periodontal parameters in 30-day evaluation. The results of our study is consistent with their studies.

There are also studies showing that OT has no effect on clinical periodontal parameters in addition to SRP (4,8,14-17). The results of these studies are not compatible with the results of our study. The differeces between our results and those of similar studies can be explained partially by the different types and concentration of ozone used, duration of application, presence or absence of control and type of control. Further randomized, double-blind and well-controlled clinical trials are required to obtain remarkable conclusions.

Ozonized water, ozonized oil and gas ozone are applied at different doses and times in *in vivo* and *in vitro* studies until now (5,8,16). In the present study, gas ozone was applied each tooth two times for consequtive three days for 60 sec, each tooth at 26 µg power (16,600 ppm at 60% power). Uraz et al. (15) reported that the split mouth study gas ozone application was 3 times during 30 s, 2100 ppm with 80%. Tasdemir et al. (16) in their study were applied gas ozone twice a week for 2 weeks, 75% power for 30s.

In the literature search, Uraz et al. (15) evaluated TNF- α , IL-1 β , IL-6 in the GCF of patients with periodontitis for SRP + OT and SRP-alone. Following SRP-alone group TNF- α levels were significantly reduced at 4 week. IL-6 concentration

Table 1. Comparison of clinical parameters between groups, baseline and 8-week				
	SRP + OT (n=25) (Mean + SD)	Between groups p* values	SRP alone (n=25) (Mean + SD)	
PI baseline	2.26±0.38	NS	2.15±0.34	
Within the group 8-week	p=0.000, 1.33±0.37	p<0.05	p=0.000, 1.62±0.37	
Gl baseline	2.50±0.44	NS	2.32±0.46	
Within the group 8-week	p=0.000, 1.53±0.45	p<0.05	p=0.000, 1.86±0.42	
PD (mm) baseline	3.88±0.48	NS	4.07±0.70	
Within the group 8-week	p=0.000, 2.33±0.28	p<0.05	p=0.000, 2.60±0.43	
CAL (mm) baseline	5.61±0.95	NS	6.23±1.26	
Within the group 8-week	p=0.000, 4.12±0.39	p<0.05	p=0.000, 5.03±0.96	
GCF volume (pl) baseline	0.65±0.20	NS	0.69+0.16	
Within the group 8-week	p=0.000, 0.09±0.05	NS	p=0.000, 0.11±0.05	

p^{*}- Comparison of 8-weeks values of groups (t-student test). GCF: Gingival crevicular fluid, SRP: Scaling and root planing, OT: Ozone therapy, PI: Plaque index, GI: Gingival index, PD: Probing depth, BOP: Bleeding on probing, SD: Standard deviation, NS: Non-significant

Table 2. Comparison of biochemical parameters between groups, baseline and 8-week				
	SRP + OT (n=25) (mean + SD)	Between groups p* values	SRP alone (n=25) (mean + SD)	
TNF- α (pg/mL)				
Baseline	1.63±0.32	NS	1.73±0.35	
Within the group	NS		NS	
8-week	1.87±0.51	NS	1.51±0.19	
IL-1β (pg/mL)				
Baseline	131.52±53.49	NS	148.57±68.29	
Within the group	p<0.05		p<0.001	
8-week	1.53±0.45	p>0.05	59.64±40.35	
IL-6 (pg/mL)				
Baseline	3.35±0.46	NS	3.51±1.18	
Within the group	p<0.001		p<0.001	
8-week	1.08±0.15	p>0.05	0.97±0.45	
IL-10 (pg/mL)				
Baseline	1.40±0.58	NS	1.41±0.59	
Within the group	p<0.05		p<0.05	
8-week	0.71±0.21	p>0.05	0.82±0.35	
MMP-9 (pg/mL)				
Baseline	93.73±48.90	NS	98.47±43.47	
Within the group	p<0.05		p<0.05	
8-week	14.37±5.91	p<0.05	45.30±14.61	

p^{*}- Comparison of 8-weeks values of groups (t-student test). SRP: Scaling and root planing, OT: Ozone therapy, TNF- α : Tumor necrosis factoralpha, IL-1 β : Interleukin-1 beta, IL-6: Interleukin-6, MMP-9: Matrix metalloproteinases-9, SD: Standard deviation, NS: Non-significant

significantly decreased at 3-month follow-up in the SRP alone group. Tasdemir et al. (16) investigated reduction in IL-1 levels after both SRP + OT group and SRP alone group after 3-month, but the decrease was not statistically significant. No clinical studies were found topical application of ozone in the treatment of periodontitis to evaluate levels of IL-10 and MMP-9 in GCF, except for the study investigating the effects of ozonized water on salivary MMP-9 level. Skurska et al. (14) after treatment with ozonized water in patients with periodontitis, saliva was increased in MMP-9 level and saliva in MMP-9 level decreased in patients with aggressive periodontitis.

OT influences biomolecules that contain structures cysteine, methionine or histidine residues via ozone's oxidation power (18). Cysteine residue, in the homology region in domain-1 of MMPs, regulates the preservation of latency of the enzyme proform. The shift from the inactive into active form is subjected by degradation of cysteine thiol group that is caused by ozone-dependent reactions proteolytic dissociation or action of free radicals (18-20). It is considered that OT effects the structure of MMP-9 molecules, due to significant decrease in MMP-9 levels in the test group compared to the control group (p=0.01).

The present study has some limitations. First of all, ozone was determined to be toxic to human oral epithelial cells and gingival fibroblast. Secondly, each study was conducted under a different protocol as stated in the manufacturer's instructions for ozone application. Thus, the timing and power of ozone application were based on the manufacturer's suggestion, and not on scientific evidence. The last limitations of this study may be considered to have a small sample size and a short follow up period. Future studies with larger samples and longer follow-up periods to contrast various applications ozone agents for the treatment of periodontitis will give exciting results.

Conclusion

Medical ozone can be used as an adjunctive to SRP in the treatment of periodontal diseases. The most important issue in OT is the need for scientific studies to examine its long and short term effects by considering the parameters such as strength, duration and frequency of administration with all three ozone types.
Acknowlodgements

This investigation was supported by Atatürk University Research Grant PN-2014/160. This study was presented as an oral presentation at the 46. Scientific Congress, Turkish Society of Periodontology, İzmir, Turkey. The authors report no conflicts of interest related to this study.

Ethics

Ethics Committee Approval: The study protocol was confirmed by the Ethics Committee of Atatürk University, Erzurum, Turkey (decision code: 24/2014, date: 13.10.2014).

Informed Consent: The signed consent form was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.N.S.G., A.D., Concept: S.N.S.G., Design: A.D., Data Collection or Processing: S.N.S.G., N.Ö., M.A.G., Analysis or Interpretation: A.D., N.Ö., M.A.G., Literature Search: S.N.S.G., Writing: S.N.S.G., A.D.

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

Financial Disclosure: This investigation was supported by Atatürk University Research Grant PN-2014/160.

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Meandros Med Dent J doi:10.4274/meandros.galenos.2022.32650 Meandros Med Dent J 2023;24(2):125-130

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Root Dentin Remineralization by Arginine and Sodium Fluoride

Arginin ve Sodyum Florür ile Kök Dentin Remineralizasyonu

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Abstract

Objective: The aim of this study was to investigate the possible effects of arginine and sodium fluoride on the microhardness of demineralized root dentin.

Materials and Methods: Forty anterior teeth were collected. Disc specimens with 2 mm thickness were prepared. Specimens were divided into 4 groups (10 specimens/group). Microhardness measurements and scanning electron microscopy/energy dispersive X-ray spectrometry analysis were performed before and after the treatment. Statistical analyses were conducted with the software SPSS 19.0.

Results: There was a significant difference between mean values of microhardness (p<0.05). There were statistically significant differences among the groups in terms of phosphorus (p<0.05) while the difference in calcium was not statistically significant (p>0.05).

Conclusion: No effects on microhardness and remineralization were observed by arginine application.

Keywords: Arginine, dental caries, energy dispersive X-ray spectroscopy, fluoride, microhardness, scanning electron microscopy

Öz

Amaç: Bu çalışmanın amacı, arginin ve sodyum florürün demineralize kök dentinin mikrosertliği üzerindeki olası etkisini araştırmaktır.

Gereç ve Yöntemler: Kırk adet anterior diş toplandı. İki mm kalınlığında disk örnekler hazırlandı. Örnekler 4 gruba ayrıldı (10 örnek/ grup). Her örneğin mikrosertlik ölçümleri ve taramalı elektron mikroskobu/enerji dağıtıcı X-ışını spektrometresi analizleri, test öncesi ve sonrası yapıldı. İstatistiksel analizler SPSS 19.0 programı ile yapıldı.

Bulgular: Ortalama mikrosertlik değerleri arasında anlamlı fark vardır (p<0,05). Gruplar arasında fosfor açısından istatistiksel olarak anlamlı fark bulunurken (p<0,05), kalsiyum açısından fark istatistiksel olarak anlamlı değildir (p>0,05).

Sonuç: Arjinin uygulamasının mikrosertlik ve remineralizasyon üzerinde herhangi bir etkisi gözlenmemiştir.

Anahtar Kelimeler: Arjinin, diş çürüğü, enerji dağılımlı X-ışını spektroskopisi, florür, mikrosertlik, taramalı elektron mikroskobu

Introduction

Dental caries is still one of the most prevalent and common chronic disease in the world (1). Anti-caries agents and products prevent the development of dental caries by inhibiting the acid production of bacteria or by changing the remineralization balance of the hard tissues of the tooth. In the remineralization process, calcium (Ca) and phosphate are incorporated into the demineralized tooth structure, resulting in a net mineral recovery (2). Many remineralizing agents and remineralizing techniques have been investigated and most are used clinically with positive results (3). Remineralization agents should give Ca and phosphate without causing any side effects or increase the remineralization potential of saliva (4). Fluoride (F) is an effective anti-caries agent. F inhibits enamel and dentin

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) demineralization and enhances remineralization. By topical application of high concentration F, before the subsurface of the tooth is remineralized, enamel surface may also be sealed with F. Therefore, F is widely used in studies that examined remineralization (5).

Dentin hypersensitivity due to exposure to dentin in response to thermal, vaporizing, tactile, osmotic, chemical stimuli is the most common problem characterized by short, sharp pain after tooth preparation (6). Altered fluid flow in the dentinal tubules in response to temperature, drying, or osmotic balance fluctuations is the source of the pain. As a result, nociceptor activation occurs in the pulp/dentin border region. The application of sodium F to exposed dentin surfaces allows calcium F crystals to precipitate by saturating the dentin fluid with Ca and phosphate ions, and the canal diameters are narrowed and the impulse transmission stops due to the mechanical occlusion of the dentin canals (7). Osmari et al. (8) concluded that F varnish had an immediate desensitising effect after one single application. Another study showed that sodium F is effective in reducing cervical dentin hypersensitivity (9). All these results support the efficiency of sodium F on dentin hypersensitivity.

Arginine is an amino acid found in saliva and is responsible for raising the pH of saliva (10). Due to its desensitizing effect, in recent years, arginine has been added as an additive to toothpaste and other F-containing dental care products such as mouth rinses, topical F preparations and dental devices (11). Today, protective approaches gained great importance in caries control. Sodium F and arginine are among the agents used in dentin hypersensitivity treatment (12). These applications are economical because they have lower cost and they are easier to use. For this reason, F and arginine are widely used in order to relieve the dentin hypersensitivity, to treat anterior ventilation caries and to provide early remineralization of caries.

The aim of this study was to investigate the potential effects of arginine and sodium F on dentin remineralization by using micro-hardness and scanning electron microscopy (SEM)/energy dispersive X-ray spectroscopy (EDS) analyses of demineralized root dentin. The null hypothesis tested for present study was that application of arginine on root surface did not affect the remineralization efficiency of sodium F.

Materials and Methods

Specimen Preparation

Forty freshly extracted caries free straight single-rooted anterior teeth with similar dimension and morphology were collected from adult patients. Teeth were examined under a stereomicroscope and teeth with root caries, cracks and endodontic treatment were excluded from the study. The selected teeth were cleaned with gauze and a fine brush and soft and/or hard attached tissues were removed with a scaler. After ultrasonically cleaning, teeth were stored in 0.5% thymol solution at 4 °C. The teeth were cut with a slow-speed diamond-saw sectioning machine (Isomet, Buehler, Lake Bluff, IL, USA) under cooling water and disc dentin specimens were removed from cervical onethird of the root. All samples were embedded into acrylic molds and polished with a series of silicon carbide abrasive papers from 600 to 1,200 grit to provide a flat surface. Ethical permission required for the study to be carried out was obtained from the Ethic Committee of Artvin Çoruh University (decision number: 5, date: 16.11.2017).

Preparation of Demineralizing and Remineralizing Solutions

Artificial saliva solution was prepared fresh before the tests according to the same composition described by Ten Cate et al. (13). The solution contains 1,28568 g NaCl, 0,0320 g MgCl₂.6H₂O, 0,07945 g CaCl₂.2H₂O, 0,29857 g KCl, 0,897 g KOH and 472 μ l H₃PO₄.

Remineralization solution was prepared fresh before the tests according to the same composition described by Ten Cate et al. (13) with 1.0 mM CaCl₂, 2 mM KH₂PO₄, 150 mM KCl. It was preserved by adding 0.01% NaN₃, pH was adjusted to 7.0 using 1M KOH and kept at room temperature.

The demineralization solution was prepared as a mixture of 100 mmol/L sodium hydroxide and 100 mmol/L lactic acid at pH 5.0. To achieve a viscosity of 100 cp, 0.2 g/L carboxymethyl cellulose sodium salt was added to the solution. The demineralized teeth were treated twice a day in all different mouthwash solutions for one month, as prescribed by each manufacturer.

Dentin disc specimens were immersed in demineralizing solution (DS) for 7 days in capped containers containing 10 mL of demineralization solution for each sample. The DS was changed every 24 h. After artificial incipient caries-like lesion formation, the specimens were removed from DS and thoroughly rinsed with deionized water. For this experimental study, the specimens were randomly divided into 4 groups (10 specimens/group): Group remineralizing solution (RS): control RS, group NaF: 5% NaF, group Arg: 8% Arg, group NaF-Arg: 5% NaF-8% Arg. The samples of all groups were then kept in artificial saliva at 37 °C for 7 days.

Microhardness Testing and SEM/EDS Analysis

The microhardness measurements were performed with a micro-hardness tester (Future Tech FM 800e, Future Tech Corp, Tokyo, Japan) using a Vickers diamond indenter at three different points, 0.5 mm distance from the root canal. The indentations were made equally, perpendicular to the surface of the specimen and each indentation being no closer than 0.5 mm to the other, using a 100 g load with a dwell time of 15 s. The three hardness values for each specimen were averaged and reported as a single value.

SEM (EVO LS 10, Carl Zeiss NTS, Germany), as well as EDS analysis were performed by using a scanning electron

microscope in connection with EDS X-ray detector (EVO LS 10, Carl Zeiss NTS, Germany). SEM images were obtained under 2,000x, 5,000x magnifications, at 20 kV voltage and a working distance of 10 mm. After SEM analysis, the rates of Ca, phosphorus (P), Oxygen (O), and F elements on the root dentin surface were determined in percentages by EDS-X ray detector and Ca/P ratios were calculated.

Statistical Analysis

Statistical analyses were performed with the software SPSS 19.0 (IBM Corporation, Armonk, USA). The Shapiro-Wilk test was used to assess the normality of the distributions. As the micro-hardness measurements before the treatment were not normally distributed the nonparametric Wilcoxon signed-ranked test was used to assess the difference before and after the treatment. Kruskal-Wallis tests were conducted to compare the differences caused by the treatment groups and the Mann-Whitney U test was performed to test the significance of pairwise differences of the treatment groups. One-way ANOVA and pairwise post-hoc tests of Bonferroni and Tamhane's test were used to compare the parameters among the groups for SEM/EDS statistics. A significant level of α =0.05 was set for comparison between the groups.

Results

Microhardness

Table 1 describes the comparison of microhardness values before and after treatment. By Wilcoxon signed-ranked test there is a significant difference between mean values of root dentin microhardness (p(0.05). All DSs, except for Group Arg, increased the root dentin microhardness.

Chemical analysis of the demineralized root dentin using EDS showed that it had predominantly O, phosfporus (P), Ca, and F. There were significant differences in O, P, F atomic weight percentages and Ca/P weight percentage

(Wt%) ratio except for Ca after the treatment (p<0.05). Comparison of the mineral content obtained in each test groups after treatment are shown in Table 2. According to the results of this study, there was statistically significant difference among the groups in terms of P (p<0.05) while the difference in Ca atomic percentages was not statistically significant (p>0.05). Similarly, there was statistically significant difference between group NaF and group NaF-Arg (p<0.05). When compared with group NaF, in group NaF-Arg, the atomic weight percentage of O and P increased while F and Ca/P level decreased.

The SEM micrographs of the remineralized root dentin surfaces for control, NaF and Arg groups in 5,000x magnification are shown in Figures 1-3. Specimens showed some remineralization and crystal structure formation on the surface of the sections. Dentin surface was covered with a homogeneous and dense mineral content and the boundaries of the crystal structure are clearly observed in the SEM micrographs of the control group. Specimens treated with NaF showed a surface layer in which irregular and porous minerals are deposited, while in samples treated with arginine, the surface layer is much more homogeneous and denser than the NaF group.

Discussion

In the present study, the potential effects of arginine and sodium F on root dentin remineralization was investigated. The null hypothesis that application of arginine on root surface did not affect the remineralization efficiency of sodium F, was accepted.

In this *in vitro* study, SEM combination with EDS elemental analysis have been used for the investigation of root dentin surfaces before and after the treatments in the same specimens. Thus, each specimen served as its own control (14). All specimens demonstrated a similar pattern of root dentin surfaces. Several studies have used SEM-

Table 1. Comparison of microhardness values obtained in each test groups before and after treatment using Wilcoxon analysis (p<0.05)						
	Group 1 (control)	Group 2 (5% NaF)	Group 3 (8% Arg)	Group 4 (5%NaF + 8%Arg)		
Before	61.24±3.09	61.82±1.43	59.24±3.77	61.77±1.43		
After	86.75±20.56	110.54±21.90	54.87±14.26	64.02±13.10		

Table 2. Comparison of the mineral content obtained in each test groups after treatment n=40 Ca/P Oxygen Phospforus Calcium Fluorine Control 67.27±2.49 10.05±0.80 20.08±1.80 2.13±0.86 2.00±0.14 NaF 43.37±6.58* 8.36±1.32* 20.28±1.13 27.64±7.34* 2.49±0.52* Arg 11.05±0.92 19.65±0.80 2.70±0.87 1.79±0.09 66.27±2.21 NaF + Arg 61.01±6.55* 10.21±1.10* 19.97±1.24 8.52±7.23* 1.97±0.15* *Statistically significant difference (p<0.0)

EDS to evaluate the demineralized tooth surfaces (15-17). The combined use of SEM with EDS elemental analysis is a good tool for investigating demineralization and remineralization. Several studies have been carried out on the tooth demineralization process and the contribution of home oral care products containing specific substances that can remineralize and/or repair the tooth surface in this process (18). It was reported that enamel caries lesions and root caries lesions are quite different because of their morphology and pathogenesis (19). In this study, roots of natural teeth were cut horizontally and the surface of the test specimens was mechanically removed. As a result, root dentin was always at the surface zone. Thus, demineralization process allowed to obtain standardized and comparable root caries lesions.

Microhardness tests are widely used to measure the hardness of teeth. There is no standard condition for enamel and dentin microhardness testing; therefore, selection depended on the researcher's decision. Because of the difference between enamel and dentin microstructure, hardness values depend upon indentation loads or times. In the current study, Vickers microhardness test was used and 100 g load with a dwell time of 15 s was applied. Although the Vickers hardness value for dentin was shown to be between 46 and 53 (20), higher values were obtained in this study. While NaF solution significantly increased the microhardness values, 8% Arg solution significantly decreased the microhardness values as compared to the control group and the baseline. This difference may be due to factors such as sample preparation, diagonal length reading error, variation in chemical composition, age, and location in the tooth.

The remineralizing effect of F was demonstrated by analyzing the microhardness of the enamel surface. In this study, there was no statistically significant difference between the study groups in terms of baseline microhardness values. However, a significant increase in root dentin microhardness was shown after topical application of F in the NaF group in comparison to the baseline values and the control group. It was reported in the literature that a continuous F support is needed to ensure its contribution to mineralization, while it is rapidly removed by saliva (21). Rosin-Grget et al. (22) reported that the samples treated with topical F were different from the control group while globular and crystalline formations occurred on the enamel surface after topical F application. In the present study too, formation of crystalline structures of various shapes and densities were observed in the samples of F group. The crystal structures that cannot be removed from the surface indicate that these formations cannot be taken as ordinary superficial deposits, but are actually adhered to the enamel surface.



Figure 2. SEM image of NaF group at 5,000x magnification after treatment

SEM: Scanning electron microscopy



Figure 1. SEM image of control group at 5,000x magnification after treatment

SEM: Scanning electron microscopy



Figure 3. SEM image of Arg group at 5,000x magnification after treatment

SEM: Scanning electron microscopy

Dentifrice products containing arginine/bicarbonate and Ca carbonate, with or without F, have been shown to be significantly effective in reducing dentin sensitivity by plugging and sealing dentinal tubules. Hsua et al. (23) reported that toothpaste containing 8% arginine and Ca carbonate reduced the dentin hypersensitivity. It was demonstrated in clinical studies comparing 8% argininecontaining toothpaste with F-containing toothpastes in the prevention of dentin hypersensitivity, that the sensitivity can be effectively relieved after 8 weeks and natural protective benefits can be achieved for oral care (24). Unlike the literature, 8% arginine application decreased the microhardness level in this study. We could not obtain compatible results with the literature. However, the microhardness level decreased in the NaF + Arg group in comparison to both baseline values and control group. The fact that such a result obtained in the NaF + Arg group suggested the question that arginine might have inhibited the effect of F and eventually reduced its effectivity. In addition, the effect of 8% arginine application on remineralization was not statistically significant different in comparison to the control group. In the present study, it was not observed any positive effects of 8% arginine application on both microhardness and remineralization. Moreover, SEM results of this group were found to be more homogeneous and more intense than the other groups.

There are some limitations of this *in vitro* study. The origin of the human teeth that were used for this experimental study was unknown. Because, age, sex, date of the extraction and how long they were stored in solution was not recorded. This may explain the significant differences of this study results when compared with the previous similar studies.

Conclusion

Although there is a wide range of products available in the treatment of dentin hypersensitivity, further studies should be conducted to develop more effective products or to increase the effectiveness of existing products. Within the limitations of this study strengthening of dentin surfaces was provided by 5% NaF while use of arginine did not make any contribution.

Ethics

Ethics Committee Approval: Ethical permission required for the study to be carried out was obtained from the Ethic Committee of Artvin Çoruh University (decision number: 5, date: 16.11.2017).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.S.B., Concept: S.S.B., F.B., Design: S.S.B., F.B., Data Collection or Processing: S.S.B., Analysis or Interpretation: S.S.B., E.D., Literature Search: S.S.B., E.D., Writing: S.S.B., E.D. **Conflict of Interest:** No conflict of interest was declared by the authors.

Financial Disclosure: The support of Artvin Çoruh University Scientific Research Projects Coordinatorship was received in the research (project no: 2019.M80.02.01.).

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Does Temporomandibular Joint Pain have a Predictive Value for Temporomandibular Joint Internal Derangement in Fibromyalgia Patients? Magnetic Resonance Imaging Role in Diagnosis

Fibromyaljili Hastalarda Temporomandibular Eklem Ağrısı, Temporomandibular Eklem iç Düzensizlikleri için bir Belirteç Olabilir mi? Manyetik Rezonans Görüntülemenin Tanıdaki Rolü

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Abstract

Objective: The purpose of this study was to evaluate magnetic resonance imaging (MRI) findings of the temporomandibular joint (TMJ) in patients with temporomandibular disorders (TMDs) with and without fibromyalgia syndrome (FMS).

Materials and Methods: There 3 groups of TMJ included in this study: 1) patients diagnosed with FMS, 2) healthy patients with pain, and 3) healthy patients without pain. The MRI variables were disc/condyle relation (normal, anterior disc displacement with reduction, and anterior disc displacement without reduction) and joint effusion (absent, moderate, and severe). Pain was assessed using the visual analog scale (VAS). Significance was evaluated at the level of p<0.05.

Results: There was a significant relationship between disc/condyle relation and the prevalence of effusion in all groups (p(0.05). There was also a significant relationship between pain and the disk/condyle relationship and the grade of the effusion (p(0.05). The incidence of more severe effusion and anterior disc displacement without reduction in painful joints was significantly higher than in painless joints (p(0.05). In terms of VAS values, there was no significant difference between groups 1 and 2 (p>0.05). The MRI findings in group 2 were more dramatic than in the other groups.

Conclusion: This study demonstrated that based on the MRI findings of patients presenting with fibromyalgia, effusion severity increased as disc/condyle compatibility deteriorated, which is similar to patients presenting without fibromyalgia. In addition, it was found that the severity of pain did not indicate a more dramatic internal derangement and effusion.

Keywords: Fibromyalgia, temporomandibular disorders, magnetic resonance imaging

Öz

Amaç: Bu çalışmanın amacı, ağrı şikayeti bulunan ve temporomandibular eklem (TME) bozukluğu görülen fibromyalji teşhisi konmuş ve sağlıklı bireylerdeki manyetik rezonans görüntülüme (MRG) bulgularını değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmaya 3 grup TME dahil edilmiş olup; 1) fibromyalji teşhisi konmuş hastalar, 2) ağrı şikayeti olan sağlıklı bireyler ve 3) ağrı olmayan sağlıklı bireylerde olarak belirlenmiştir. Disk/kondil ilişkisi (normal, redüksiyonlu ve redüksiyonsuz disk deplasmanı) ve efüzyon (yok, orta ve ciddi) MRG ile değerlendirilmiş olup ağrı bulgusu ise vizual analog skala (VAS) ile skorlanmıştır. İstatistiksel olarak anlamlılık değeri p<0,05 olarak belirlenmiştir.

Bulgular: Tüm gruplarda disk/kondil ilişkisi ile efüzyon prevalansı arasında anlamlı bir ilişki olduğu görülmüştür (p<0,05). Bunun yanı sıra ağrı ile disk/kondil ilişkisi ve efüzyon seviyesi arasında anlamlı bir ilişki bulunmaktadır (p<0,05). Ağrı görülen eklemlerde redüksiyonsuz disk deplasmanı ile şiddetli efüzyon görülme sıklığının ağrı görülmeyen bireylere göre anlamlı derecede fazla olduğu

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) belirlenmiştir (p<0,05). Grup 1 ile grup 2 VAS değerleri arasında anlamlı bir farklılık görülmemektedir (p>0,05). Grup 2 MRG bulguları diğer gruplara göre daha dramatiktir.

Sonuç: Fibromyalji teşhisi konmuş bireylerin MRG bulgularına göre disk/kondil uyumu bozuldukça efüzyon şiddetinin de arttığı görülmüş olup bu durum, sağlıklı bireylerin TME MRG bulguları ile benzerlik göstermektedir. Bu çalışma ile fibromyalji görülen bireylerde ağrı şiddetinin; eklem içi bozukluk ve efüzyon şiddeti hakkında bir belirleyici olmadığı sonucuna varılmıştır.

Anahtar Kelimeler: Fibromyalji, temporomandibular eklem bozuklukları, manyetik rezonans görüntüleme

Introduction

Fibromyalgia syndrome (FMS) is a chronic musculoskeletal disorder characterized by generalized fatigue, pain, morning stiffness, sleep disturbances, psychological distress, a low pain threshold, and tender points (1,2). The prevalence of FMS ranges from 2 to 8%, and it is seen more frequently with advanced age (3,4). Pain in the craniofacial area has not traditionally been considered in the diagnosis of FMS. Nevertheless, FMS patients often have orofacial pain, including temporomandibular disorders (TMDs) (5).

TMD is defined as "an umbrella" term that embraces a number of clinical problems that involve the masticatory muscles, the temporomandibular joints (TMJ) internal structures, and the associated structures" (6). Symptoms in patients with TMD can include pain, joint clicking, and limited mouth opening, or there may be no complaints.

Evaluation of TMD symptoms in patients with and without FMS has been discussed in previous studies (7,8), but the correlating magnetic resonance imaging (MRI) findings of TMJ in patients with and without FMS have not been fully examined. The aims of this study were: 1) evaluating the MRI findings of TMJ in TMD patients with and without FMS, and 2) comparing MRI findings of TMJ between the painful and nonpainful sides of each individual in the two groups.

Materials and Methods

This study was approved by the Clinical Research Ethics Committee of Uşak University (decision number: 173-01, date: 15.05.019). A total of 590 patients' data records were evaluated retrospectively. As a result, 295 of the patients with information from completed Diagnostic Criteria (DC) for TMD questionnaires and MRIs were available bilaterally (reference number: 173-01).

The inclusion criteria consisted of unilateral or bilateral painful TMD based on DC/TMD, patients aged 18 and over, and a diagnosis of FMS by the same physiatrist.

Clinical assessment of the patients was carried out according to the DC/TMD guidelines. The visual analog scale (VAS 0-10) was used to quantify pain. There were three groups:

Group 1: TMJ among patients diagnosed with FMS

Group 2: Painful TMJ among otherwise healthy patients

Group 3: Pain-free TMJ among otherwise healthy patients

The study by Koca et al. (9) guided the MRI application and evaluation protocol of this study (Figures 1, 2).

Statistical Analysis

The Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) program was used for statistical analysis. The Mann-Whitney U test was used for two-group comparisons. The chi-square test was used to identify the relationships among qualitative data. Significance was evaluated at levels of p<0.05.

Cohen's kappa tests were used to determine the agreement between each analysis.

Results

Evaluation of the Relationship Between the Intragroup Disc Position and Effusion

There was a significant relationship between the disc/ condylar relation and the prevalence of effusion in the patients in group 1, group 2 and group 3 (Table 1). In group 1, in cases presenting with severe effusion, a normal disc/ condylar relationship was observed in four (2.3%) joints, anterior disc displacement with reduction (ADDwR) in 17 (10%) joints, and anterior disc displacement without reduction (ADDwoR) in 33 (19.5%) joints. A significant difference was found between those with a normal disc/ condylar relationship with ADDwR and those with ADDwoR (p<0.05) (Table 1).

In group 2, in cases presenting without effusion, a normal disc/condylar relationship was observed in 18 (6.7%) joints, ADDwR in 20 (7.4%) joints, and ADDwoR in 16 (5.9%) joints. In cases presenting with severe effusion, a normal disc/ condylar relationship was observed in five (1.9%) joints, ADDwR in 25 (9.3%) joints, and ADDwoR in 73 (27%) joints. While there was no statistically significant difference between the normal disc/condyle relationship and ADDwR (p>0.05), a significant difference was observed between the normal disc/condyle relationship and ADDwoR (p<0.05) (Table 1).

In group 3, in cases presenting without effusion, a normal disc/condylar relation was observed in 60 (40%) joints, ADDwR in 17 (11.3%) joints, and ADDwoR in three (2%) joints. In joints presenting with severe effusion, a normal disc/condylar relationship was observed in seven (4.7%) joints, ADDwR in 10 (6.7%) joints, and ADDwoR in 12 (8%) joints, and there were significant differences among the groups (p<0.05) (Table 1).

Effusion Comparison Among the Groups

No effusion was observed in 65 (11%) joints in group 1, in 54 (9.2%) joints in group 2, and in 80 (13.5%) joints in group 3. There was a statistically significant difference among the groups (p<0.05) (Table 2).

Moderate effusion was observed in 51 (8.6%) joints in group 1, 113 (19.1%) joints in group 2, and 41 (6.9%) joints in group 3. There was a statistically significant difference between group 2 and the other groups (p<0.05) (Table 2).

Severe effusion was observed in 54 (9.1%) joints in group 1, in 103 (17.4%) joints in group 2, and in 29 (4.9%) joints in group 3. There was a statistically significant difference between group 3 and the other groups (p(0.05) (Table 2).



Figure 1. Moderate effusion image in the anterior joint space, it was taken with the mouth closed and T2 weighted. Indicated by a red arrow



Figure 2. Severe effusion image in the lower and upper joint space, it was taken with the mouth closed and T2 weighted. Indicated by a red arrow

Comparison of the Disc/condyle Relationship among the Groups

A normal disc/condylar relation was observed in 35 (5.9%) joints in group 1, in 40 (6.8%) joints in group 2, and in 90 (15.2%) joints in group 3. There was a statistically significant difference between group 3 and the other groups (p<0.05) (Table 2).

VAS Comparisons among the Groups

No statistically significant difference was observed in terms of VAS scores between group 1 and group 2 (p>0.05) (Table 3).

Discussion

TMD is one of the major causes of nondental pain in the orofacial area (6). Pain involving the masticatory muscles in particular is considered a common symptom in FMS patients (5). Although the craniofacial area is not taken into consideration in the diagnosis of FMS, it has been shown that pain in the orofacial area usually originates from TMD (7,8). There are studies in the literature that have investigated the prevalence of FMS among TMD patients and reported different rates in this regard (5,7). A study by Leblebici et al. (7) reported that FMS was observed in 52% of patients diagnosed with TMD. Velly et al. (10) on the other hand, reported that 11% of patients with TMD had a diagnosis of FMS.

Numerous studies have reported that FMS is more prevalent in women (11,12). The present study revealed that all patients diagnosed with FMS were women, which is consistent with the literature (1,8). In addition, to eliminate the differences between sexes, the other groups only included women, because all individuals in group 1 were women.

Many studies in the literature have evaluated the connection between the disc/condylar relationship and effusion in cases with TMD and have offered different views on this subject. A relationship between disc position and effusion is hypothesized since the TMD etiopathogenesis remains unclear (13). The cause of an effusion may be a response to trauma or an inflammatory reaction (14). Many studies have argued that there is a significant relationship between anterior disc displacement and the prevalence of effusion (15,16). Regarding the relationship between effusion and disc position, Larheim et al. (17), Manfredini et al. (18), and Orlando et al. (19) reported that the mechanical pressure occurring due to disc position causes the release of inflammatory mediators, which in turn may result in effusion. In the present study, when an internal assessment independent of pain was made in all groups, it was found that as the effusion severity increased, the normal disc/ condylar relationship deteriorated, and the prevalence of ADDwoR increased. A significant relationship was observed between ADDwoR and the prevalence of severe effusion. The study conducted by Hoşgör showed an increase in the

prevalence of ADDwoR with increasing effusion severity, and it is safe to say that the findings of the present study are compatible with the previous literature (16). In other words, the relationship between effusion and the disc/condyle relationship has symptoms in joints with fibromyalgia that are similar to joints without fibromyalgia.

In TMD studies, the relationship between disc position and effusion has promoted the argument that pain may be a clinical symptom. In the present study, group 1 and group 2 was significantly less common than that in group 3, while the prevalence of ADDwoR was significantly higher. The absence of effusion was significantly less common than in group 1 and group 2 than in group 3, while the prevalence of severe effusion was significantly higher. A study conducted by Pinto et al. (20) evaluated the relationship between pain factors and disc position and effusion. It was found that an excessive mechanical load caused by disc displacement can result in the release of inflammatory mediators into the articular disc and retrodiscal spaces and form effusions in the synovial membrane, which in turn can cause pain. Many studies have evaluated the disc position, effusion, and pain, and they suggested that the presence or severity of effusion has a clinically significant relationship with pain (16,21). Conversely, many studies in the literature argue

Table 1. Relationship between disc/condyle relation and effusion values in group 1, group 2 and group 3						
Effusion in groups	Disc/condyle relati	on			p-value	
		Normal	ADDwR	ADDwoR		
	No	20ª (11.7%)	39ª (22.9%)	6 ^b (3.5%)		
Group 1	Moderate	11ª (6.5%)	23ª (13.6%)	17ª (10.1%)	0.001**	
	Severe	4ª (2.4%)	17ª (10.1%)	33⁵ (19.5%)		
	No	18ª (6.7%)	20 ^b (7.4%)	16 ^b (5.9%)		
Group 2	Moderate	17ª (6.3%)	45ª (16.7%)	51ª (18.9%)	0.001**	
	Severe	5ª (1.9%)	25ª (9.3%)	73 [⊳] (27%)		
	No	60ª (40%)	17 ^b (11.3%)	3 ^b (2%)		
Group 3	Moderate	23ª (15.3%)	13ª (8.7%)	5ª (3.3%)	0.001**	
	Severe	7ª (4.7%)	10 ^b (6.7%)	12º (8%)		

^{a.b.c}Lettering of post-hoc tests, Chi-square ^{**}p<0.01, Normal: Normal disc/condyle relation, ADDwR: Anterior disc displacement with reduction, ADDwoR: Anterior disc displacement without reduction

Table 2. Comparison of effusion value and disc/condyle relation among groups

	Groups	p-value			
		Group 1	Group 2	Group 3	
Effusion	No	65° (11%)	54ª (9.2%)	80 ^b (13.5%)	0.001*
	Moderate	51 ^b (8.6%)	113ª (19.1%)	41 ^b (6.9%)	
	Severe	54ª (9.1%)	103ª (17.4%)	29 ^b (4.9%)	
Disc/condyle relation		Group 1	Group 2	Group 3	0.001*
	Normal	35ª (5.9%)	40ª (6.8%)	90 ^b (15.2%)	
	ADDwR	79ª (13.3%)	90ª (15.2%)	40 ^b (6.8%)	
	ADDwoR	56° (9.4%)	140ª (23.7%)	20 ^b (3.4%)	

abcLettering of post-hoc tests, Chi-square **p<0.01, Normal: Normal disc/condyle relation, ADDwR: Anterior disc displacement with reduction, ADDwoR: Anterior disc displacement without reduction

Table 3. Comparison of VAS values between group 1 and group 2					
Groups	n	mean ± SD	min-max (median)		
Group 1	170	6.84±0.59	6-8 (7)		
Group 2	270	6.84±1.28	4-9 (7)		

Mann-Whitney U test, *p<0.05, **p<0.01, SD: Standard deviation, min-max: Minimum-maximum

that there is no significant relationship between effusion, the disc/condyle relationship, and pain (22,23). The findings of the present study found significant relationships among the disc/condyle relation, effusion, and, pain which is compatible with some of the literature.

Although there was no significant difference in VAS scores between groups, a more dramatic picture was seen in joints presenting with pain in group 2. In other words, in group 2, the MRI findings were more dramatic than group 1. Among the possible causes of this condition are the lower pain thresholds of FMS patients, which may result in higher pain expression on the VAS. In addition, since the patients presenting with FMS did not have ample joint movement due to sensitive points in their masticatory muscles, the fact that a destructive effect on the joint does not occur as frequently in non-FMS patients can also be listed among the possible causes (24).

There are numerous studies in the literature evaluating the relationship between FMS and TMD. They reported a substantial prevalence of TMD in FMS patients, and the most relevant reason for this was muscular factors (25). However, these studies used only RDC/TMD for the diagnosis and evaluation of TMD. The use of MRI in the present study allowed for observations of changes in the disc/condyle relation and synovial fluid. This also allowed the present study to observe the effusion and internal derangement in cases that were asymptomatic in terms of pain. For that reason, identifying the cause of TMD as being of muscular origin in individuals presenting with FMS in previous studies that did not use MRI may have resulted in a failure to detect the presence of intra-articular internal derangements and effusion. As a result, asymptomatic cases may have gone undetected. In the literature, there is only one study conducted by Leblebici et al. (7) in which cases of TMD presenting with FMS were evaluated with MRI. They concluded that among the causes of TMD in FMS patients, those of muscle and arthrogenic origin were significantly more relevant than those of arthrogenic origin only. However, they only evaluated 52 patients and, unlike the present study, did not address the disc position and effusion or evaluate any cause and effect relationships.

Conclusion

The present study has shown that based on the MRI findings of patients presenting with FMS, the effusion severity increased as the disc/condyle compatibility deteriorated, which is similar to patients presenting without fibromyalgia. Additionally, it can be said that the severity of pain did not indicate more dramatic internal derangement and effusion in FMS individuals. The fact that the prognosis of intraarticular findings in patients presenting with fibromyalgia was better than that of healthy patients is another important finding of this study.

The authors of this study believe that future studies evaluating synovial fluid in terms of inflammatory mediators

and proteins and discussing it along with the clinical symptoms in cases presenting with and without fibromyalgia will light the way for other studies to be conducted on this subject.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Uşak University (decision number: 173-01, date: 15.05.019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.G.K., G.P., Concept: C.G.K., G.P., Design: C.G.K., G.P., Data Collection or Processing: C.G.K., M.K., Analysis or Interpretation: C.G.K., M.K., Literature Search: C.G.K., M.K., Writing: C.G.K., M.K.

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

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

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Fractal Dimension and Lacunarity Analyses of Root Canal Dentin with or without Smear Layer

Smear Tabakası Varlığı veya Yokluğunda Kök Kanal Dentininin Fraktal Boyut ve Lakünarite Analizi

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Abstract

Objective: To investigate the fractal dimension (FD) and lacunarity of dentin in the presence or absence of a smear layer using scanning electron microscopy (SEM) images at various magnifications.

Materials and Methods: Extracted human mandibular premolar teeth were divided into two groups (n=5). After decoronation, the root canals were prepared. While the smear layer was left intact in the first group, it was removed with 5% EDTA and 2.5% NaOCI irrigation in the second group. The roots were split longitudinally and one half was prepared for SEM. Four images at 500 and 1000 magnifications were obtained from the middle thirds of the root canals of each specimen and saved in TIF format. The FD and lacunarity of the SEM images were calculated. Two-way ANOVA and Bonferroni tests were used for statistical analysis (p=0.05).

Results: The FD of dentin surfaces with or without a smear layer did not differ significantly (p>0.05). While magnification was an important factor in the FD of smear-free surfaces (p<0.01), it did not present any significant difference in the presence of a smear layer (p>0.05). Lacunarity showed a significant decrease in the images without a smear layer (p<0.0001). Although it demonstrated a slight increase with magnification, this increase was not significant (p>0.05).

Conclusions: Lacunarity was a differentiating factor in determining the presence or absence of the smear layer regardless of the magnification of the SEM images. FD was affected by magnification and could not discriminate the presence or absence of the smear layer. Lacunarity analysis may be a practical tool for evaluating SEM images of dentinal surfaces.

Keywords: Scanning electron microscopy, smear layer, fractal dimension, lacunarity

Öz

Amaç: Farklı büyütmelerdeki taramalı elektron mikroskop (SEM) görüntülerini kullanarak smear tabakası varlığı veya yokluğunda dentinin fraktal boyutunu (FD) ve lakünaritesini araştırmaktır.

Gereç ve Yöntemler: Çekilmiş mandibular premolar dişler iki gruba ayrıldı (n=5). Kronların uzaklaştırılmasından sonra, kanallar genişletildi. Birinci grupta smear tabakası olduğu gibi bırakılırken, ikinci grupta %5'lik EDTA ve %2,5'lik NaOCl irigasyonu ile uzaklaştırıldı. Kökler uzunlamasına ikiye bölünerek bir yarısı SEM için hazırlandı. Her örneğin kök orta üçlüsünden x500 ve x1000 büyütmede dört adet görüntü elde edilerek TIF formatında kaydedildi. SEM görüntüleri kullanılarak FD ve lakünarite hesaplandı. İstatistiksel analiz için, iki yönlü ANOVA ve Bonferroni testleri kullanıldı (p=0,05).

Bulgular: Smear tabakası olan veya olmayan dentin yüzeylerinin FD'si istatistiksel olarak farklı değildi (p>0,05). Smear tabakası olmayan yüzeylerin FD'sinde büyütme önemli bir faktör iken (p<0,01), smear tabakası varlığında herhangi bir farklılığa rastlanmadı (p>0,05). Smear tabakası olmayan görüntülerde, lakünarite anlamlı bir azalma gösterdi (p<0,0001). Lakünarite, büyütme ile hafif bir artış gösterse de, bu artış anlamlı değildi (p>0,05).

Address for Correspondence/Yazışma Adresi: B. Güniz Baksı Prof., Ege University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology, İzmir, Turkey Phone: +90 532 250 46 38 E-mail: bgunb@yahoo.com ORCID ID: orcid.org/0000-0001-5720-2947 Received/Geliş Tarihi: 24.01.2022 Accepted/Kabul Tarihi: 05.04.2022 Sonuç: Lakünarite, SEM görüntülerinin büyütmesinden bağımsız olarak, smear tabakasının varlığı veya yokluğunu saptamada ayırt edici bir faktördü. FD büyütmeden etkilenmekteydi ancak smear tabakası varlığı veya yokluğunu ayırt edemiyordu. Lakünarite analizi, dentin yüzeylerinin SEM görüntülerini değerlendirmek için kullanışlı bir gereç olabilir.

Anahtar Kelimeler: Taramalı elektron mikroskobu, smear tabakası, fraktal boyut, lakünarite

Introduction

Digital images are widely used to represent data in all fields of science. In order to extract subtle information from digital images, which are indiscernible to the naked eye, various image processing and texture analyses techniques are required (1-3). These techniques are used both in medical and dental radiology to supplement diagnosis of numerous pathologies (1-3). Other than the radiographic images, the use of microscopic images in medical and dental diagnostics has also its fundamental aim in the discernment of potential abnormalities. Recently, fractal and self-similarity properties have attracted substantial attention to represent the texture and physical properties of two-dimensional digital images (4,5).

Fractal analysis is a method for quantitative evaluation of complex geometric structures that exhibit patterns throughout the image. The fractal dimension (FD), which is calculated with a computer algorithm describes the complexity of the structure and is represented by a single number (6,7). FD is described as a measure of irregularity. However, the fact that fractals with identical dimensions can have greatly different appearances, the term lacunarity has been introduced to describe the characteristics of fractals of the same dimension with different textures (8). Lacunarity is considered as a scale dependent measure of heterogenity of texture (9). In dentistry, FD calculation has been performed on radiographs for the assessment of diagnosis of many systemic pathologies, periapical lesions and evaluation of osseointegration (4,10).

The quantification of surface topography of dentine is frequently done using SEM. Characterization of dentine surface using SEM can provide an understanding of the relationship between the surface topography and the microstructure, the mechanical properties as well as the surface generation process (e.g., coating & cutting process, wear). By quantifying the surface topography, it may be possible to obtain information for the development of new materials, the understanding of material's properties and for quality assurance. Quantification is also necessary for comparison of surface qualities. Even though SEM has been prevalently used for the interpretation of dentine surfaces due to its convenience, the qualitative nature of visual topography has created a need for more quantitative methods. For this purpose, some authors have recommended use of fractal analysis and lacunarity evaluation (11).

Therefore, the aim of this study was to investigate the FD and lacunarity of dentine in presence or absence of smear layer using scanning electron microscopy (SEM) images at x500 and x1000 magnifications.

Materials and Methods

Archives of SEM images obtained from the middle third of the root canal acquired from previous studies were examined and digital photographs of root canal dentine with smear layer present (n=40) or absent (n=40) at x500 and x1000 magnifications were used for the study. The selected images were saved in TIF format (Figures 1, 2). The FD and lacunarity were calculated on four SEM images of each tooth.

Fractal Dimension Calculation

A public domain Image J software and FracLac plug-in was used for all image processing and analysis using a differential box-counting method (NIH Image software (Image J version 1.34s software, National Institutes of Health, Bethesda, MD) (http://rsbweb.nih.gov/ij/). Rectangular regions of interests were created (ROIs) and selected comprising the whole SEM image. Identical ROI sizes were used for images with and without smear layer.

The box-counting method described by White and Rudolph (12) was used for the calculation of FD. Overall aim of the method was to remove large-scale differences in brightness of the images. For this purpose, Gaussian filter with a diameter of 35 pixels was used to duplicate and blur ROIs. This procedure leaves only large variations in density by removing all fine-and medium-scale structures. The blurred area was subtracted from the original image, and 128 were added to each pixel location. This step produces an image with a mean gray value of 128. The result is an image in which individual variations in the image reflect particular types of features with different brightness (i.e., trabeculae and marrow spaces). The image was then made binary and inverted (Figures 3, 4). After eroding and dilating once, it was skeletonized to reveal features that can be seen and measured and FD was calculated using the abovementioned software (Figures 3, 4). FD for each image was calculated by obtaining the mean of the two ROIs (13).

Lacunarity Calculation

Same ROIs were used for lacunarity calculations. According to the Plotnick et al. (9) lacunarity can be defined in terms of local means and variance measured for different neighborhood sizes for each pixel in an image. Images with high lacunarity values indicate wider range of sizes of structures (9). Lacunarity was calculated using FracLac plugin that compares digital images for many morphometrics including lacunarity (13).

Statistical Analysis

The FDs of ROIs from the images with and without smear layer were compared using two-way ANOVA and Bonferroni tests (p=0.05).

Results

Table 1 shows the mean and standard deviation (SD) values of FD and lacunarity for the groups with and without smear layer. Samples with smear layer and with x500 magnification had a mean FD of 1.79 (\pm SD, 0.02) while samples with smear layer at x1000 magnification showed 1.78 mean FD (\pm SD, 0.03). On the other hand samples without smear layer had a mean FD of 1.80 (\pm SD, 0.02) and 1.78 (\pm SD, 0.02) consecutively at x500 and x1000 magnifications (Table 1).



Figure 1. Smear-free root canal dentine at x500 (A) and x1000 (B) magnifications. Sample image showing the region of interest used for FD analysis



Figure 2. Root canal dentine with smear layer at x500 (A) and x1000 (B) magnifications. Sample image showing the region of interest used for FD analysis

Mean lacunarity measurements for samples with smear layer were 0.66 (\pm 0.13) and 0.71 (\pm 0.24) simultaneously at x500 and x1000 magnifications. While samples without smear layer and with x500 magnification had a mean lacunarity of 0.50 (\pm 0.15), samples without smear layer at x1000 magnification showed mean lacunarity of 0.53 (\pm 0.11).

The FDs of dentin surfaces with or without smear layer were not significantly different (p>0.05). While magnification was an important factor in FD of smear-free surfaces (p<0.01), it did not present any significant difference in presence of smear layer (p>0.05). However, the lacunarity properties of dentin presented different results. Lacunarity showed a significant decrease in images without smear layer (p<0.0001). On the other hand, it demonstrated a slight increase with magnification; however, this increase was not significant (p>0.05).

Discussion

FD uses a statistical surface examination to delineate surface microarchitecture and gives the numerical value of the complexity as demonstrated on an image (13). It is a precise, intact and effectively accessible method. Even though, fractal geometry has contributed to the description of complexity (14) computation of FD alone cannot always provide unequivocal descriptions. In order to provide unique description, further concepts such as lacunarity have

Table 1. FD and lacunarity values \pm SD of dentin images with (smear +) or without (smear -) smear layer at x500 and x1000 magnifications

	FD ± SD	Lacunarity ± SD
Smear (+) x500	1,794±0.02	0.662±0.13
Smear (-) x500	1,803±0.02	0.503±0.15
Smear (+) x1000	1,777±0.03	0.709±0.24
Smear (-) x1000	1,778±0.02	0.526±0.11

FD: Fractal dimension, SD: Standard deviations



Figure 3. Binarized (1-2), inverted (3-4), eroded (5-6), dilated (7-8) and skeletonized (9-10) sample



Figure 4. Binarized (1-2), inverted (3-4), eroded (5-6), dilated (7-8) and skeletonized (9-10) sample images of the region of interest of root canal dentine with smear layer at x500 (A) and x1000 (B) magnifications

been recommended to discriminate complex structures demonstrating similar FDs, while looking totally different. One of the individual depictions of lacunarity is scaledependent measure of heterogeneity however; it can be also characterized as the distribution of voids in the organized series (14). It has been advocated that lacunarity can be used to disentangle patterns in every kind of images including computer graphics (11,15).

SEM is the most frequently used technique for the delineation and characterization of surface topography giving details regarding the surface features. However, the major shortcoming with SEM is that it solely shows the qualitative characteristics of the surface texture. It is very difficult to match or classify SEM images of surface topographies with regard to visual inspection since it is usually performed subjectively and superficially. Accordingly, many studies have included lacunarity analysis along with FD to elucidate information from electron microscopy images (11,16). Nevertheless, this is the first study using both FD and lacunarity to compare the changes in dentine heterogeneity in presence and absence of smear layer as measured on SEM images. Once SEM images of dentine surfaces treated with different materials have been quantified using FD and/or lacunarity analyses, they can be compared and categorized in an objective and standard manner. Simultaneous use of FD and lacunarity determines surface topography changes with respect to changes in treatment materials, methods and/ or parameters.

The FD values found in the presented study indicate that FD is (moderately) reduced after EDTA and NAOCI treatment. However, in terms of overall complexity, dentine with and without smear layer showed rather similar FD values. In other words, FD was not able to discriminate presence or absence of smear layer in SEM images and was affected by magnification of the images.

The so-called box-counting method gives the estimate of the box numbers that are needed to cover the grid of particular size at various scales. Box-counting, is the most frequently used method for the calculation of FD (17). The box-counting method pretends that the image under observation has only white pixels (1's) and black pixels (0's), which are the foreground and background respectively and thus a binary image. Calculating FD can quantitatively compare inherent roughness and derangement of different images. However, it was already proved that FD is not an individual and adequate measure, i.e., two images that look quite different may generate the same FD due to similarities in roughness (9,18-20). Furthermore, it was demonstrated that FD is a poor descriptor to quantify and compare SEM images of surface topographies and FD values depend on the microscopic magnification and on the algorithm used to compute these values (21). Complexity and surface characteristics are profoundly scale dependent. Surfaces with same topography may appear coarse and very fine depending on the magnification of the image (22). Due to the abovementioned factors, FD analysis of SEM images of dentine could not discriminate the presence and absence of smear layer in the present study.

The automated image analysis particularly deals with particle shape characterization. Geometrical information that is not effected by scale, rotation and translation was defined as the shape. Several descriptors such as circularity and shape factor have been used to characterize the shape of objects independent of their size both of which requires the boundary perimeters. However, even though the shapes look very much alike to the naked eye basically the same the divergence in shape descriptor values could be very large due to the difference in perimeter, which is majorly dependent on scale of observation. Therefore, difference in FD could be expected for the same image at different magnifications. It was previously proved that shape descriptors of fractal objects involving boundary lengths are scale variant (14).

While the FD is a poor descriptor of surface complexity, lacunarity has been claimed to be a feasible technique for the analysis of SEM images for surface texture analysis (11). The results obtained in this study also substantiated

the previous results that lacunarity is in fact sensitive to the treatment applied to dentine surfaces. Lacunarity of the SEM images of dentine decreased significantly with the removal of the smear layer and this decrease was not affected by the magnification of the images. It has been described that lacunarity is the measure of the discrepancy of an object or fractal from translational uniformity (9). Objects with high and low lacunarity values were characterized as coarse and fine texture respectively (11). The results found in this study verify this definition too, since dentine with smear layer demonstrates rough and bold surface pattern while clean dentine surface free of smear will show more delicate and organized surface characteristics. Ling et al. (11) have reported similar results that lacunarity analysis is a powerful tool that can be used to characterize surface characteristics and contours of SEM images. Furthermore, its use is not limited to this task. It was previously proved that lacunarity could also be used to assess osteoporosis, to differentiate benign from malignant tumors, to analyze the behavior of prostate and breast cancer and microvascular morphology (2).

Conclusion

Finally, this study employs the box-counting FD and lacunarity from SEM images with and without smear layer at two different magnifications. Results show that lacunarity can serve as a potential tool in determining the presence or absence of smear layer regardless of the magnification of SEM images. FD was affected by magnification and was not able to discriminate presence or absence of smear layer in SEM images. Lacunarity analysis may be a practical tool to evaluate SEM images of dentinal surfaces.

Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.G.B., B.H.Ş., Design: B.G.B., B.H.Ş., Data Collection or Processing: B.G.B., Analysis or Interpretation: B.G.B., B.H.Ş., Literature Search: B.G.B., Writing: B.G.B., B.H.Ş.

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

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

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Clinical Profiles of Centenarian Patients Presenting to the Emergency Department with an Acute Disease

Acil Servise Akut Bir Hastalıkla Başvuran Asırlık Hastaların Klinik Profilleri

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Abstract

Objective: With the aging of society, the number of centenarians, i.e., individuals aged 100 years and over, is increasing. This study aimed to develop strategies to prevent mortality and morbidity by determining the clinical profiles of patients aged 100 and over who applied to the emergency department (ED).

Materials and Methods: The study was a retrospective analysis of patients aged 100 years and older that presented to the ED of tertiary hospital with an acute disease between 2012 and 2021. An analysis was performed using the patient files and computer database containing information on demographic characteristics, presentation complaints, clinical findings, emergency severity index (ESI) triage categories, final diagnoses and outcomes in the ED, length of hospital stay, and discharge characteristics.

Results: Of the 222 patients that presented to the ED, 98.6% were women, 78.8% lived in their own homes, and 71.6% were transferred to the hospital by ambulance. At the time of presentation, 72.1% of the patients were in the ESI 3 triage category. Overall, 35.1% of the patients were hospitalized, and the median length of stay in the ED was 240 minutes. Mortality was the in-hospital outcome in 10.4% of the patients. The ESI triage categories, number of consultations, and length of hospital stay were found to be directly related to mortality (p<0.001).

Conclusion: Defining patient profile and reasons for mortality and morbidity in centenarian patients, can be used to both prevent possible adverse events and effectively plan healthcare services in this population.

Keywords: Centenarian patients, emergency severity index, comorbidity, mortality, hospitalization

Öz

Amaç: Toplumun yaşlanması ile birlikte asırlık yani 100 yaş ve üzeri bireylerin sayısı artmaktadır. Bu çalışma, acil servise başvuran 100 yaş ve üzeri hastaların klinik profillerini belirleyerek mortalite ve morbiditeyi önleyebilecek stratejiler geliştirmeyi amaçlamıştır.

Gereç ve Yöntemler: Çalışma 2012-2021 yılları arasında üçüncü basamak bir hastanenin acil servisine akut hastalık şikayeti ile başvuran 100 yaş ve üstü hastaların retrospektif analizini içermektedir. Çalışmada hastaların demografik özellikleri, başvuru şikayetleri, klinik bulguları, emergency severity index (ESI) triyaj kategorileri, acil servisteki kesin tanıları ve son durumları, hastanede kalış süreleri ve taburculuk gibi bilgiler hasta dosyaları ve bilgisayar veri tabanı kullanılarak analiz edildi.

Bulgular: Acil servise başvuran 222 hastanın %98,6'sı kadındı, %78,8'i kendi evinde yaşıyordu ve %71,6'sı ambulansla hastaneye sevk edilmişti. Başvuru anında hastaların %72,1'i ESI 3 triyaj kategorisindeydi. Genel olarak, hastaların %35,1'i hastaneye kaldırıldı ve acil serviste medyan kalış süresi 240 dakikaydı. Hastaların %10,4'ü mortalite ile sonuçlandı. ESI triyaj kategorileri, konsültasyon sayısı ve hastanede kalış süresinin mortalite ile doğrudan ilişkili olduğu bulundu (p<0,001).

Sonuç: Asırlık hastalarda mortalite ve morbidite nedenlerini ve hasta profilini belirlemek, bu popülasyonda hem olası yan etkileri önlemek hem de sağlık hizmetlerini etkin bir şekilde planlamak için kullanılabilir.

Anahtar Kelimeler: Asırlık hastalar, emergency severity index, komorbidite, mortalite, hastaneye yatış

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Introduction

The aging of the world population has led to the emergence of patient groups with different physiological and biological structures. With the continuation of aging, the number of centenarians, i.e., individuals aged 100 years and over is increasing day by day. The number of people aged 100 years and over differs from one country to another and was reported as 5,859 in Türkiye in 2021, constituting 0.1% of the entire elderly population in the country (1). Emergency departments (ED), located at the crossroads in terms of medical care, have become indispensable areas for these individuals with their easy access and provision of healthcare services 24 hours a day, seven days a week (2,3). However, there are also a series of difficulties concerning this patient population, such as the use of multiple drugs, presence of comorbid diseases in medical history, requirement of more resources in their diagnosis and treatment, and their higher need for social services, which have resulted in the necessity of restructuring the health system (4). The priority to prevent adverse events that may arise in the ED related to this patient group is to determine their specific needs, organize healthcare services accordingly, and making healthcare professionals competent in this regard, thereby improving the quality of care provided (5,6). This study aimed to develop strategies that can prevent mortality and morbidity in centenarians by determining the clinical profiles of patients aged 100 years and over that presented to the ED of a tertiary hospital.

Materials and Methods

This retrospective study was conducted with the retrospective analysis of patients aged 100 years and older, who presented to the ED of a tertiary hospital between January 2012 and December 2021. During the 10-year study period, a total of 206,309 patients aged 65 years and over presented to the ED, and 222 of these patients met the inclusion criteria. Approval for the study was obtained from the Recep Tayyip Erdogan University Faculty of Medicine Non-invasive Clinical Research Ethics Committee (decision number: 2023/08, date: 05.01.2023).

Study Design

The computer-based hospital information management system, in which patient records are kept, and ED patient files were used to collect data on the patients aged 100 years and older included in the study. Patients with missing data were excluded from the study. The patients' modes of transportation, presentation complaints, emergency severity index (ESI) triage categories, demographic data, medical history, last diagnosis, length of ED stay, ED outcome, consultation and epicrisis notes, ward admitted for treatment and follow-up, length of hospital stay, inhospital outcome, and the Charlson comorbidity index (CCI) were reached.

Study Data

The patients' modes of transportation of were categorized as by ambulance or by private vehicle, and where they lived was categorized as their own home, hospital, or nursing home. The triage categories of the patients at the time of presentation were classified between 1 and 5 according to ESI. CCI was calculated according to the comorbid conditions present in the medical history of the patients at the time of presentation and classified as 0, 1-2, 3-4, and \geq 5 points. The length of stay in the ED was recorded in minutes, and in the case of admission to any inpatient ward, the length of hospital stay was noted in days. Outcomes were evaluated as discharge with recovery or mortality.

Statistical Analysis

All statistical analyses were performed using Jamovi v.1.6 statistics software [The Jamovi Project (2021) Computer Software, version 1.6. Sydney, Australia]. Categorical data were expressed as frequency (n) and percentage. Normally distributed continuous variable data were defined as mean and standard deviation, and non-normally distributed continuous variable data as median and interquartile range (IQR). The normality of data distribution was determined using the Shapiro-Wilk test. In the comparison of continuous variables, the t-test was used in case of a normal distribution, and the Mann-Whitney U test otherwise. The chi-square test was conducted to compare categorical variables between groups.

Results

Demographic Characteristics

During the 10 year study period, there were a total of 298 ED presentations belonging to 222 patients aged 100 years or older. Of the 206,309 patients aged 65 years and over that presented to the ED during this period, 0.11% constituted those of aged 100 years and over. Our dataset included 219 women and three managed from 100 to 113 (median 102, IQR 101-104) years. Of the patients included in the study, 175 (78.8%) lived in their own homes, and 159 (71.6%) were transported to the hospital by ambulance.

When the medical history of the patients was examined, 135 (60.8%) had cardiovascular system diseases (CVSs) and 100 (45%) had central nervous system diseases (CNSs). The demographic characteristics of the centenarian patients are given in Table 1.

Presentation Characteristics and Emergency Management

At the time of presentation, the most common ESI triage category was 3 (n=160, 72.1%). Figure 1 shows the distribution of the triage category by years. In general, the most common reason for presentation to the ED was shortness of breath (n=55, 24.8%), followed by trauma (n=26, 11.7%).

As a result of their evaluation in the ED, 28 (12.6%) patients were diagnosed with pneumonia, 21 (9.5%) with heart failure,

and 14 (6.3%) with musculoskeletal injuries. According to the pathological condition detected, 91 (41%) patients were referred to one clinic and two patients (0.9%) were referred to four different clinics for consultation. Twelve of the 78 (35.1%) patients that required inpatient medical care were directly admitted from the ED to the intensive care unit (ICU) for follow-up and treatment (Table 2).

Table 1. Demographic characteristics of centenarian patients presenting to the emergency department				
Gender, female 219 (98.6%)				
Age, median (IQR) (years)	102 (101-104)			
Living arrangement				
Own home	175 (78.8%)			
Nursing home	1 (0.5%)			
Other	46 (20.7%)			
Mode of transportation to ED				
Private vehicle	63 (28.4%)			
Ambulance	159 (71.6%)			
Recurrent presentation to ED within 72 hours	10 (4.5%)			
Recurrent presentation within the same c	alendar year			
1	39 (17.6%)			
2	15 (6.8%)			
3	5 (2.3%)			
4	6 (2.7%)			
5	2 (0.9%)			
6	3 (1.4%)			
7	0			
8	1 (0.5%)			
9	1 (0.5%)			
Number of annual presentations				
2012	13 (5.6%)			
2013	18 (8.1%)			
2014	20 (9%)			
2015	25 (11.3%)			
2016	23 (10.4%)			
2017	20 (9%)			
2018	31 (14%)			
2019	30 (13.5%)			
2020	21 (9.5%)			
2021	20 (9%)			
IQR: Interquartile range, ED: Emergency depart	ment			



Figure 1. ESI triage distribution of the centenarian patients by years

ESI: Emergency severity index

Table 2. Presentation complaints and emergency department outcomes and diagnoses of the centenarian patients				
Presentation complaint				
Shortness of breath	55 (24.8%)			
Trauma	26 (11.7%)			
Chest pain	16 (7.2%)			
Weakness	14 (6.3%)			
Muscle pain	14 (6.3%)			
Abdominal pain	12 (5.4%)			
Altered state of consciousness	12 (5.4%)			
Headache	10 (4.5%)			
Fever	10 (4.5%)			
Loss of strength	7 (3.2%)			
Bloody stool	7 (3.2%)			
Fainting	6 (2.7%)			
Dizziness	6 (2.7%)			
Other	37 (17.5%)			
ED outcome				
Discharge	142 (64%)			
Inpatient ward admission	62 (27.9%)			
Admission to ICU	12 (5.4%)			
Referral to another hospital	4 (1.8%)			
Mortality	2 (0.9%)			
Last diagnosis in ED				
Pneumonia	28 (12.6%)			
Heart failure	21 (9.5%)			
Soft tissue trauma	14 (6.3%)			
Anemia	12 (5.4%)			
Cerebrovascular disease	11 (5%)			
Urinary tract infection	11 (5%)			

Table 2. Continued	
Last diagnosis in ED	
Acute bronchitis	8 (3.6%)
Acute renal failure	8 (3.6%)
Stabil angina pectoris	8 (3.6%)
Hypertensive urgency	8 (3.6%)
Gastrointestinal system bleeding	7 (3.2%)
Myalgia	7 (3.2%)
Femoral fracture	7 (3.2%)
Cerebral concussion	7 (3.2%)
Pulmonary embolism	6 (2.7%)
Other	59 (26.3%)
In-hospital outcome	
Discharge	59 (26.6%)
Mortality	23 (10.4%)
Length of stay in ED, median (IQR) (min)	240 (120-413)
Patients staying in ED for more than 480 min	43 (19.4%)
Length of stay of patients hospitalized for follow-up and treatment (day)	2.7±7.60 (min:1 - max: 90)
IQR: Interquartile range, ED: Emergency depar Minimum-maximum	tment, min-max:

The median length of stay in the ED during the follow-up and diagnosis stage was 240 (IQR, 120-413) minutes. According to the Republic of Türkiye Ministry of Health Communique on the Principles of Implementation of Emergency Services in Inpatient Healthcare Facilities (7), patients must be discharged home within eight hours after presentation or hospitalized if inpatient treatment is required or transferred to another hospital if there is no vacant patient room. Accordingly, 43 (19.4%) patients were followed up in the ED for more than 480 minutes.

Among the patients hospitalized to receive healthcare services, the mean length of hospital stay was 2.7±7.60 (minimum: 1 - maximum: 90) days. Twenty-three (10.4%) of the inpatients died, and their median length of hospital stay was 11.5 (IQR: 2.75-20.8) days.

Mortality

When factors affecting mortality were evaluated, a statistically significant relationship was found between mortality and the ESI triage category, number of consultations requested, and length of hospital stay (days) in those receiving inpatient medical care services (p<0.001). The mortality statistics of the centenarian patients are given in Table 3.

Table 3. Patients' statistics	by mortalit	у	
	No mortality (n=199)	Mortality (n=23)	p-value
CCI-Orginal			
0	29	4	
1	24	7	
2	40	4	
3	41	3	
4	40	3	0.555*
5	11	1	
6	10	1	
7	2	0	
8	2	0	
CCI-Age			
5	18	3	_
6	26	7	_
7	34	3	
8	46	4	
9	37	4	0.513*
10	22	1	
11	8	0	
12	6	1	
13	2	0	
CCI-2011			
0	37	6	_
1	9	3	_
2	62	7	_
3	30	2	0.503*
4	29	4	
5	15	1	
6	14	0	
7	3	0	
ESI			
1	2	2	-
2	24	18	-
3	157	3	0.001*
4	3	0	
5	13	0	
Length of stay in the ED (minute)	240 (IQR 120-413)	198 (IQR 129-416)	0.959^

Table 3. Continued					
	No mortality (n=199)	Mortality (n=23)	p-value		
Number of consultations					
0	95	0			
1	75	16			
2	22	6	0.001*		
3	5	1			
4	2	0			
Length of hospitalization (day)	0 (IQR 0-1.5)	4 (IQR 2-11)	0.001^		
Re-application rate within	a calendar y	rear			
0	128	22			
1	38	1			
2	15	0			
3	5	0			
4	6	0	0.212*		
5	2	0	0.313		
6	3	0			
7	0	0			
8	1	0			
9	1	0			

*Chi-square test; ^Mann-Whitney U test, CCI: Charlson comorbidity index, ESI: Emergency severity index, IQR: Interquartile range, ED: Emergency department

Discussion

The growing number of centenarian patients presenting to the ED has brought about the necessity of developing emergency service delivery strategies for this special patient group. This study was conducted to describe the impact of centenarian patients on the ED and their specific requirements.

We determined that there was a direct correlation between the centenarians' ESI triage category at the time of presentation to the ED and the mortality rates among the inpatients. Although ESI is effective in predicting ICU admission and short-term mortality in patients aged 65 years and over presenting to the ED, its utility in predicting longterm mortality has not been determined (7). In this study, 72.1% of the centenarian patients were included in triage category 3, especially in this group of patients, showing that this patient population requires the use of more resources in the presence of emergency medical conditions added to their existing comorbidities. In patients that required followup and treatment after admission, the increase in the number of hospitalization days was associated with mortality rates. This can be considered as an indication that both in the ED and other inpatient clinics, patient-centered multidisciplinary alternative care strategies should be developed and used in the management of unstable centenarians presenting to the ED with an acutely developing condition.

In this study, 71.6% of the patients were transported from their home to the hospital by ambulance. This rate is similar to previous international studies (8). However, the living spaces of centenarian patients may change due to social and sociocultural differences.

The reasons for presentation to the ED were determined as shortness of breath triggered by CVS and CNS comorbidities, followed by injuries. The presentation complaints and comorbidities of the patients were in line with their diagnoses leading to their discharge or hospitalization. Pneumonia is an important medical condition, especially in centenarians. This condition has a different clinical manifestation in centenarians than in younger patients, further contributing to negative outcomes and placing an additional financial burden on acute healthcare services (9.10). Trauma-related injuries, on the other hand, were consistent with the literature as the second most common reason for presentation to the ED in these patients (11). After trauma, 3.2% of the patients were discharged with surgical treatment due to femoral fracture, and no adverse events or mortality was encountered in this group. However, the data of our study differ from the literature (12).

Increasing age is associated with chronic comorbid diseases Although some studies in the literature have determined that centenarian patients have lower rates of chronic disease (10,13), more than half (60.9%) of the patients in our study had CVSs and 45% had chronic comorbidities affecting their cognitive functions. The association of existing comorbidities with acute events results in the need for more complex medical care involving more than one discipline, as well as increasing the possibility of mortality. Similarly, in the current study, a statistically significant correlation was found between the increased number of consultations requested and mortality, once again revealing the necessity of multidisciplinary care strategies.

We observed that 10.3% of the patients included in the study died while receiving treatment in the hospital. The presentation complaints and last diagnoses in the ED were parallel to each other, with neurological and renal system pathologies being the most common causes of mortality. In studies conducted with centenarian patients, the causes of mortality vary (14,15). However, we did not evaluate the main causes of mortality, which can be considered as one of the limitations of our study. The identification of the leading causes of death in patients aged 100 years and older can provide a more accurate estimate of the future healthcare needs of this population.

In terms of gender, it is anticipated that the increase in the number of men is higher than that of women in the centennial population across the world (16). However, when we evaluated the centennial patients that presented to the ED according to gender, we observed that the number of women was higher. This can be attributed to physiological differences between men and women, and it is an issue that should not be overlooked in healthcare service provided.

The main limitation of our study concerns its single-center and retrospective design. The data were not collected specifically for the study; we obtained them from the existing routine hospital records.

Conclusion

The increased number of hospital presentations of centenarians increases the average life expectancy. Thus, this patient population has become important users of emergency services. It is important to establish multidisciplinary health strategies by identifying data on when and how centenarians benefit from healthcare services. However, the lack of studies on the care and management of these patients and the lack of education of healthcare service providers emerge as problems in this process. A multidisciplinary approach should be adopted in the development of care strategies for this patient group. In addition, in vulnerable centenarians, improving prehospital services to reduce the need for hospitalization should be part of the programs to be developed in order to minimize adverse events associated with hospital stay, such as delirium and functional impairment. Therefore, the data obtained from this study, defining patient profile and reasons for mortality and morbidity in centenarian patients. can be used to both prevent possible adverse events and effectively plan healthcare services in this population.

Ethics

Ethics Committee Approval: Approval for the study was obtained from the Recep Tayyip Erdogan University Faculty of Medicine Non-invasive Clinical Research Ethics Committee (decision number: 2023/08, date: 05.01.2023).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.B., Concept: Ö.B., Design: M.M.Y., G.E., Data Collection or Processing: G.E., Analysis or Interpretation: M.M.Y., Literature Search: İ.A., Writing: Ö.B., İ.A.

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

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

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Metformin Alleviates Doxorubicin-induced Liver Injury in Rats via Reducing Oxidative Stress, inflammation and Excessive Cell Death

Metformin, Sıçanlarda Oksidatif Stresi, Enflamasyonu ve Aşırı Hücre Ölümünü Azaltarak Doksorubisin Kaynaklı Karaciğer Hasarını Azaltır

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Abstract

Objective: The liver is one of the most important internal organs in the human body and has high regenerative properties. It performs the functions of protein synthesis, intake, storage, and distribution of nutrients and vitamins from the blood. In this study, we investigated the curative effect of metformin (Met) on doxorubicin (DOX)-induced liver damage.

Materials and Methods: A total of 32 Wistar-albino rats were divided into four groups: control, Met, DOX, and DOX + Met groups. The DOX and DOX + Met groups received four doses of DOX. Met was gavaged daily for 15 days in the DOX + Met and Met groups. Structural liver injury was evaluated with hematoxylin-eosin, picro-sirius, TUNEL, and nuclear factor kB (NF-kB) antibody staining. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total antioxidative status (TAS), total oxidative status (TOS), and hydroxyproline levels were measured as biochemical parameters.

Results: The DOX group was found to have a significant structural liver injury characterized by hyperchromatic nuclei in hepatocytes, widespread sinusoidal dilatations, and granular and vacuolar degeneration. Increased NF-kB staining and the apoptotic index were also detected in the DOX group. Biochemical tests revealed an increase in ALT, AST, and TOS levels and a decrease in TAS levels in the DOX group. Met administration provided a significant improvement in the structural changes caused by DOX. In addition, the DOX + Met group had lower NF-kB staining, apoptotic index, ALT, and TOS levels and a higher TAS level compared to the DOX group.

Conclusion: Our findings indicate that Met alleviates DOX-induced structural liver injury by reducing oxidative stress, inflammation, and excessive cell death.

Keywords: Doxorubicin, metformin, liver injury, apoptosis, oxidative stress

Öz

Amaç: Karaciğer, yenilenme özelliği yüksek olan insan vücudundaki en önemli iç organlardan biridir. Protein sentezi, besin maddelerinin ve vitaminlerin kandan alınması, depolanması ve dağıtılması işlevlerini yerine getirmektedir. Bu araştırmada, doksorubisin (DOX) ile oluşturulan karaciğer hasarı üzerindeki metforminin (Met) iyileştirici etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Toplam 32 Wistar-albino sıçan dört gruba ayrıldı: kontrol, Met, DOX ve DOX + Met grupları. DOX ve DOX + Met gruplarına dört doz DOX verildi. DOX + Met ve Met gruplarında 15 gün süreyle günde Met verildi. Yapısal karaciğer hasarı, hematoksilen-eozin, pikro-sirius, TUNEL ve nükleer faktör kB (NF-kB) antikor boyaması ile değerlendirildi. Biyokimyasal değerlendirmeler için alanın aminotransferaz (ALT), aspartat aminotransferaz (AST), total antioksidan durum (TAS), total oksidatif durum (TOS) ve hidroksiprolin düzeyleri ölçüldü.

Bulgular: DOX grubunda, hepatositlerde hiperkromatik çekirdekler, yaygın sinüzoidal dilatasyonlar, granüler ve vakuoler dejenerasyon ile karakterize önemli yapısal karaciğer hasarı olduğu bulundu. DOX grubunda artmış NF-kB boyama ve apoptotik indeks de tespit edildi. Biyokimyasal testler DOX grubunda ALT, AST ve TOS düzeylerinde artış ve TAS düzeyinde azalma gösterdi. Met uygulaması,

Address for Correspondence/Yazışma Adresi: Cevat Gençer PhD, Aydın Adnan Menderes University Faculty of Medicine, Deparment of Histology and Embryology, Aydın, Turkey Phone: +90 505 710 98 26 E-mail: cevat.gencer@adu.edu.tr ORCID ID: orcid.org/0000-0001-8204-4581 Received/Geliş Tarihi: 03.02.2023 Accepted/Kabul Tarihi: 24.05.2023 DOX'un neden olduğu yapısal değişikliklerde belirgin bir iyileşme sağladığı gözlemlenmiştir. Ayrıca DOX + Met grubunda DOX grubuna göre NF-kB boyama, apoptotik indeks, ALT ve TOS düzeyleri daha düşük ve TAS düzeyi daha yüksekti.

Sonuç: Bulgularımız, Met'in oksidatif stresi, enflamasyonu ve aşırı hücre ölümünü azaltarak DOX kaynaklı yapısal karaciğer hasarının etkisini hafiflettiğini göstermektedir.

Anahtar Kelimeler: Doksorubisin, metformin, karaciğer hasarı, apoptoz, oksidatif stres

Introduction

Doxorubicin, a broad-spectrum anticancer agent, has a limited clinical usage due to its cardiotoxic and hepatotoxic effects (1). Doxorubicin toxicity is largely caused by an overabundance of reactive oxygen species (ROS) or a reduction in antioxidant defenses, resulting in an imbalance in oxidative status. Increased ROS induces excessive calcium retention in the mitochondria, increasing mitochondrial permeability and resulting in mitochondrial enlargement and adenosine triphosphate depletion, finally leading to cell death (2).

Numerous drugs with antioxidant and anti-inflammatory effects have been utilized to treat doxorubicin-induced liver damage. Metformin is a biguanide derived from a perennial plant (Galega officinalis) extensively used to treat type 2 diabetes (3). In addition to its effects on glucose metabolism, it has anti-oxidative, anti-inflammatory, and anti-apoptotic effects. Thus, metformin inhibits lipid peroxidation and has protective effects against toxic agents in several tissues (4).

In the current study, we aimed to investigate the possible protective effects of metformin on doxorubicin-induced liver damage in a rat model.

Materials and Methods

Animals

Thirty-two male Wistar albino rats, 8-week-old, weighting between 250-300g, were obtained from a private company. All animals were housed in transparent cages with a relative humidity of 40-60% and an ideal temperature (22 °C) during the study period. They were exposed to 12 hours of light/darkness. Throughout the study, rats were fed with a regular rat diet, and clean tap water ad libitum. The study protocol was approved by the Aydin Adnan Menderes University Animal Experiments Local Ethics Committee (Ref. No: 64583101/2018/112, date: 23.10.2018).

Study Protocol

The study was planned on 4 groups: control, metformin (Met), doxorubicin (DOX) and DOX + Met (DOX + Met). For the group, Met (200 mg/kg) was given every day for 15 days by gavage. The study protocol for each group and timeline was given in Table 1.

The rats were sacrificed with exsanguination under general anesthesia [xylazine (5 mg/kg) and ketamine (100 mg/kg)] at the end of the study protocol. Rat liver tissues were

preserved in 10% neutral formalin and blood samples were collected into biochemistry tubes for further analyses.

Routine Histopathological Evaluation

Routine tissue follow-up procedure was performed after the fixation material was removed. Liver tissues were embedded in paraffin blocks and sections with 5 μ m thickness were prepared with a microtome. Hematoxylin-eosin, Masson's trichrome, and picro-sirius staining were performed for routine histopathological evaluation.

Light microscopic assessment of liver sections was performed with an optical microscope (Olympus BX50, Tokyo, Japan). Structural liver injury was graded with hepatic injury score calculated via evaluation of 4 different areas selected from the liver tissues of four groups by two experienced histologists (5).

Light microscopic observations were converted into quantitative data using the hepatic damage score created by Jacevic et al. (6).

Immunohistochemical Staining

Immunohistochemical staining was performed on 5 µm thick sections mounted on polylysine coated slides. Immunohistochemical staining with nuclear factor kB (NF-kB) primary antibody (Santa Cruz Biotechnology sc-8008, Texas, USA) was performed to reveal inflammatory activity in the liver specimens. Sections were then visualized with chromogen 3,3'-diaminobenzidine solution and Mayer's hematoxylin was used for nuclear background staining.

TUNEL Staining

Extent of cell death in the liver specimens were determined using TUNEL method. Sections were stained according to the kit protocol (Millipore, 2470976, USA). TUNEL positive cells were counted at 400x magnification. Apoptotic index scoring was used to analyze the data. Hundred cells in five distinct locations were counted, and proportion of apoptotic cells were identified to calculate apoptotic index (7).

Biochemical Analyses

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were defined as biochemical markers of liver injury. Measurements were performed using commercially available kits in the biochemistry laboratory. As a marker of collagen turnover and fibrotic activity, hydroxyproline levels were studied in hydrolyzed liver tissue samples using a colorimetric assay (Elabscience, E-BC-K061, Wuhan, China).

Assessment of Oxidative Status

In order to assess oxidative status in liver specimens, total oxidative status (TOS) and total antioxidative status (TAS) were measured. TOS and TAS in liver tissues were measured using commercial kits (Rel Assay Diagnostics).

Statistical Analysis

Normally distributed parameters were presented as mean \pm standard deviation, the remaining parameters were given as median (minimum-maximum). One-way ANOVA and Kruskal-Wallis H analysis were used to compare the normally distributed and abnormally distributed variables, respectively. The SPSS 21 program (SPSS Inc., Chicago, Illinois, USA) was used in the application of this protocol. A p value <0.05 was set statistically significance level.

Results

Body and Liver Weights

Initial body weights of the studied rats were similar between the four groups (p=0.981). On the other hand, there was a statistically significant difference regarding final body weights (p<0.001). DOX group had lower final body weight compared to control and Met groups. DOX + Met group had lower body weight than the control group (Table 2). Liver weights were also found to be different between four groups (p=0.013). DOX group had lower liver weight compared to control and Met groups (Table 2).

Histopathological Evaluation of Structural Liver Injury

Light microscopy revealed typical histological findings in the control and Met groups (Figure 1a, b). On the other hand, evaluation of liver specimens from DOX group demonstrated significant structural damage characterized by mononuclear cell infiltrations, hyperchromatic nuclei in hepatocytes, sinusoidal dilatations and granular and

Table 1. De	Table 1. Detailed timeline of the study protocol					
Days	Control group (IP + G)	DOX (IP)	DOX (IP) + Met (G)	Met (G)		
1	Dw 2 mL/kg (IP) +	5 mg/kg dox (IP)	5 mg/kg dox (IP) + 200 mg/kg Met (G)	200 mg/kg (G)		
2	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
3	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
4	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
5	Dw 2 mL/kg (IP) + Dw 1 mL (G)	5 mg/kg dox (IP)	5 mg/kg dox (IP) + 200 mg/kg Met (G)	200 mg/kg (G)		
6	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
7	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
8	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
9	Dw 2 mL/kg (IP) + Dw 1 mL (G)	5 mg/kg dox (IP)	5 mg/kg dox (IP) + 200 mg/kg Met (G)	200 mg/kg (G)		
10	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
11	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
12	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
13	Dw 2 mL/kg (IP) + Dw 1 mL (G)	5 mg/kg dox (IP)	5 mg/kg dox (IP) + 200 mg/kg Met(G)	200 mg/kg (G)		
15	Dw 1 mL (G)		200 mg/kg Met (G)	200 mg/kg (G)		
16	Sacrification	Sacrification	Sacrification	Sacrification		
DOX: Doxorubicin, Met: Metformin, Dw: Distilled water, G: Gavage, IP: Intraperitoneally						

vacuolar degeneration (Figure 1c). Met administration significantly attenuated structural changes induced by DOX (Figure 1d). Hepatic injury score was significantly higher in the DOX group compared to control and Met groups. Met administration significantly reduced hepatic injury score in DOX treated animals.

Assessment of Inflammation and Apoptosis

Immunohistochemical staining for NF-kB was used to assess inflammatory activity in liver specimens. In the DOX group, there was intense NF-kB positivity around the central vein, compared to control and Met groups. In the DOX + Met group, a significant decrease in NF-kB staining intensity was observed compared to DOX group (Figure 2).

Apoptotic index was significantly higher in the DOX group compared to control and Met groups. Met administration significantly reduced apoptotic index in the DOX administered animals (Figure 3, Table 2).

Biochemical Evaluation of Liver Injury

Serum ALT values were substantially higher in the DOX group compared to control and Met groups. Although not statistically significant, ALT values were slightly lower in the DOX + Met group than those in the DOX group. The DOX group had considerably higher AST values than control, Met, and DOX + Met groups. When DOX + Met group was compared to the control group, no significant difference was detected regarding AST values (Table 2).

1.01

Assessment of Oxidative Status

TOS was significantly higher in the DOX group compared to control, Met and DOX + Met groups. There were no significant difference between control, Met and DOX + Met groups regarding TOS. On the other hand, TAS was significantly lower in the DOX group compared to control, Met and DOX + Met groups. There were no significant difference between control, Met and DOX + Met groups regarding TAS (Table 2).

Assessment of Liver Fibrosis

In the Picro-sirius-stained sections, small fibrotic areas around the central vein were observed in the DOX group (Figure 1g). There was no statistically significant difference between the groups regarding hydroxyproline levels, although the DOX group values were tended to be higher (Table 2).

Discussion

In the present study, we investigated the possible effects of oral Met administration on DOX induced liver injury for the first time in the literature. Our findings revealed that Met alleviates structural and biochemical markers of liver damage in a rat model of DOX-induced hepatotoxicity. We also demonstrated that these beneficial effects were possibly related to a reduction in oxidative stress, inflammatory activity and excessive cell death in the liver tissue.

and liver tissue oxidative stress and tibrosis markers						
	Control group	Met	DOX	DOX + Met	p-value	
Body and liver weight of rats						
Initial weight (g)	295.75±13.13	296.50±11.53	295.38±12.85	297.63±8.72	0.981	
Final weight (g)	364.88±33.65	350.88±36.37	270.63±31.38ª,b	308.13±36.54ª	<0.001*	
Liver Weight (g)	12.64±1.23	12.63±1.20	9.09±2.63 ^{a,b}	11.07±2.64	0.013*	
Hepatic Injury Score and A	poptosis Index of liver	specimens				
Hepatic Injury Score	0 (0-1)	1 (0-1)	7 (4-8) ^{a,b,d}	3 (2-4) ^{a,b,c}	<0.001*	
Apoptosis Index	2.6 (1.8-3.2)	4.6 (4.2-8.0)ª	40.2 (37.8-47.6) ^{a,b,d}	14.8 (13.4-18.2) ^{a,b,c}	<0.001*	
Serum ALT, AST levels						
ALT	44.00±16.88	47.14±8.21	63.75±16.07ª,b	52.57±6.99	0.033*	
AST	110.00±17.46	119.14±17.69	195.62±49.23 ^{a,b,d}	117.57±25.61°	<0.001*	
Liver tissue oxidative stress	s and fibrosis markers					
TOS	1.11±0.45	0.66±0.52	1.76±0.25 ^{a,b,d}	1.17±0.33°	<0.001*	
TAS	2.05±1.00	1.94±0.17	1.45±0.41 ^{a,b,d}	1.95±0.17°	<0.001*	
Hydroxyproline	0.94±0.40	0.92±0.32	1.06±0.42	0.86±0.17	0.747	
and one we control had one we had sold one we DOX dado one we DOX . Not ALT, Alapine eminetranefarance AST, Apportate eminetranefarance						

Table 2. Body and liver weight of rats. Hepatic Injury Score and Apoptosis Index of liver specimens. Serum ALT, AST levels

^ap<0.05 vs control, ^bp<0.05 vs Met, ^cp<0.05 vs DOX, ^dp<0.05 vs DOX + Met, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TAS: Total antioxidative status, TOS: Total oxidative status, Met: Metformin



Figure 1. Liver sections stained with hematoxylin-eosin. Control group (a, x200), Met group (b, x200), DOX group (c, x200) and DOX + Met group (d, x200). Liver sections stained with Picrosirius red. Control group (e, x200), Met group (f, x200), DOX group (g, x200) and DOX + Met group (h, x200).



Figure 2. Liver sections immunostained for NF- κ B. Control and Met groups demonstrate light staining for NF- κ B (a and b, x200). DOX group demonstrates intense staining for NF- κ B (c, x200). DOX + Met group demonstrates less intense staining for NF- κ B compared to the DOX group (d, x200)



Figure 3. Fluorescent microscopic images of liver sections stained with TUNEL assay to detect cell death

Histopathological evaluation of liver tissues revealed significant structural changes in the DOX group characterized by mononuclear cell infiltrations, hyperchromatic nuclei, sinusoidal dilatations, granular and vacuolar degeneration in hepatocytes. Similar structural alterations were also observed in sections stained with Masson's trichrome. Many previous studies also reported similar structural changes in the histopathological assessment of liver tissues following DOX administration (8-10).

We observed a decrease in sinusoidal dilatations, mononuclear cell infiltrations, and a substantial decrease in the number of pyknotic cells in liver tissue samples taken from DOX + Met group rats compared to the DOX group alone. Numerous researchers have reported similar beneficial effects with Met administration in different hepatotoxicity models (11-13). Met exerts these effects by activating AMPactivated protein kinase in the injured cells, suppressing "cyclin d1" expression, halting mitotic cell division in the G1 phase, and therefore halting the increase in the number of damaged cells (14).

In the DOX group, we observed NF-kB positivity in and around the central vein reflecting an increase in the inflammatory activity. Met administration reduced NF-kB expression in the DOX treated animals. In line with these findings, Rizk et al. (15) reported that 200 mg/kg Met substantially lowered NF-kB, DOX-2, and Caspase 3 expression in rats treated with methotrexate for seven days. Nguyen et al. (16) also demonstrated that Met inhibited ROS generation generated by lithocholic acid (LCA) in HCT116 CRC cells and thereby inhibited NF-kB activity, abolishing LCA-mediated IL-8 overexpression.

A number of *in vivo* and *in vitro* studies have demonstrated that DOX promotes cell death in various tissues by increasing oxidative stress (17-19). We detected a significant increase in TOS and apoptotic index in the liver tissue following DOX administration. Met significantly reduced TOS and apoptotic index in DOX treated animals. Similar to our results, Li et al. (20) reported a decrease in the number of apoptotic cells after Met administration in a cisplatin-induced kidney injury model.

Significant increase in serum AST and ALT levels were detected following DOX administration. Met treatment led to a significant decrease in AST but not in ALT levels. In a similar study, Rizk et al. (15) used Met in a methotrexate-induced liver injury model. When they compared AST and ALT levels before and after treatment, they found that Met -treated rats had substantially lower AST and ALT values (15). Tripathi et al. (21) likewise observed a significant decrease in AST and ALT levels in the Met treatment group in their study. Discrepancy in our findings regarding ALT level may be possibly due to the small number of animals in our groups or due to diffuse toxic effects of DOX in different tissues.

Conclusion

To conclude, our findings have revealed that Met administration attenuates DOX induced structural liver injury via reducing oxidative stress, inflammation and excessive cell death. These data suggest that Met may be useful for prevention of DOX induced hepatotoxicity. Exact mechanisms underlying these beneficial effects of Met on DOX induced liver injury and its potential for clinical application need to be elucidated in further studies.

Ethics

Ethics Committee Approval: The study protocol was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Ref. No: 64583101/2018/112, date: 23.10.2018).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: This research was supported by Aydın Adnan Menderes University Scientific Research Projects Coordination Unit (project number: 19005).

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Role of Hybrid Treatment Technique in Radiotherapy Planning for Early Stage Left Breast Cancer

Erken Evre Sol Meme Kanseri Radyoterapi Planlamasında Hibrit Tedavi Tekniğinin Rolü

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Abstract

Objective: The application of effective techniques has an important place in the control of the disease in the breast, which is an anatomically complex region. The aim of this study is an early stage left breast irradiation, in addition to Intensity-Modulated Radiotherapy (IMRT), volumetric-modulated arc therapy (VMAT) plans, it is to compare the data obtained by the HYBRID planning technique obtained with 5 different loadings and also to determine the appropriate weight ratios in HYBRID plans in terms of critical organ doses.

Materials and Methods: IMRT and VMAT plans were prepared for all patients included in the study. HYBRID plans with different beam weight rates were prepared to determine the optimal dose distribution hybrid beam weight ratio. Dose distributions, heterogeneity, and conformity indices of all plans were compared among themselves. In addition, dosimetric comparison was made in terms of critical organ doses.

Results: In the study, it was seen that the HYBRID3 plan was statistically significant in terms of the heart dose. This result shows that in terms of cardiac dose, the HIBRID 3 plan provides the desired low dose in terms of cardiac diseases. Considering the D_{mean} (s1 Gy) value of the contralateral breast (CB) for the plans created in this study, it was found that all HYBRID loading plans were statistically significant compared to the IMRT plans. HYBRID plans have the lowest CB D_{mex} .

Conclusion: If one is unable to obtain appropriate results with VMAT techniques, then HYBRID plans formed by the combination of IMRT and VMAT plans reduce organs at risk doses, in the mean time ensuring that the dose wraps around the planned target volume in a targeted manner.

Keywords: Breast cancer, Intensity-Modulated Radiotherapy, volumetric arc therapy, HYBRID plans, critical organ doses

Öz

Amaç: Meme, anatomik olarak kompleks bir bölgede olduğundan etkili tekniklerin uygulanması hastalığın kontrolünde önemli bir yer tutar. Bu çalışmanın amacı; erken evre sol meme ışınlaması için Yoğunluk Ayarlı Radyoterapi (IMRT), hacimsel yoğunluk ayarlı ark tedavisi (VMAT) planlarına ilave olarak 5 farklı yüklemeyle elde edilen HİBRİT planlama tekniği ile elde edilen verileri kıyaslamak ve ayrıca kritik organ dozları açısından HİBRİT planlarda uygun ağırlık oranlarını tespit etmektir.

Gereç ve Yöntemler: Çalışmaya dahil edilen tüm hastalar için IMRT ve VMAT planları hazırlandı. Optimal doz dağılımlı hibrit yükleme oranının tayini için farklı yükleme oranları ile HİBRİT planları hazırlandı. Tüm planların doz dağılımları, hetrojenite ve konformite endeksleri kendi aralarında karşılaştırıldı. Ayrıca kritik organ dozları açısından dozimetrik karşılaştırma yapıldı.

Bulgular: Çalışmada HİBRİT 3 planlarının kalp dozu açısından istatistiksel olarak anlamlı olduğu görüldü. Bu sonuç kardiyak dozda HİBRİT 3 planın kalp hastalıkları açısından istenilen düşük dozu sağladığını göstermektedir. Bu çalışmada oluşturulan planlar için karşı memenin D_{mean} (≤1 Gy) değeri dikkate alındığında, tüm HİBRİT yükleme planlarının IMRT planlarına göre istatistiksel olarak anlamlı olduğu görülmüştür. HİBRİT planlar en düşük kontra lateral meme D_{meak} değerine sahiptir.

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) **Sonuç:** VMAT teknikleri ile uygun sonuçlar elde edilemiyorsa, IMRT ve VMAT planlarının birleşiminden oluşan HİBRİT planlar, kritik organ dozlarını azaltırken, dozun PTV'yi istenen şekilde sarmasını sağlar.

Anahtar Kelimeler: Meme kanseri, Yoğunluk Ayarlı Radyoterapi, volümetrik ayarlı ark terapi, HİBRİT planları, kritik organ dozları

Introduction

Adjuvant radiotherapy following breast-conserving surgery is effective in reducing the risk of loco-regional recurrence and distant metastasis in patients with earlystage breast cancer (1). With advances in radiation therapy (RT) techniques, 5-year survival of breast cancer patients has increased to 89%, 10-year survival to 83%, and 15-year overall survival to 78% (2). This improvement in survival suggests the need to reduce complications associated with RT.

Since the breast is in an anatomically complex region, the application of effective techniques has an important place in the control of the disease. To prevent complications in the late period, several clinical studies have recommended preservation of organs at risk (OAR) such as ipsilateral lung (IL), contralateral lung (CL), heart and contralateral breast (CB) at different dose volume levels (3). They also mentions that the literal dose homogeneity and the reduction of skin dose help to minimize radiation-induced toxicities such as fibrosis, erythema and wet-desquamation (4).

Since each patient is different, the right choice of the individual treatment technique becomes important. With the right treatment technique, it provides the desired adequate dose coverage to the planned target volume (PTV), while providing low critical organ doses. Compared to Intensity-Modulated Radiotherapy (IMRT)/volumetric-modulated arc therapy (VMAT) plans, Three dimensional (3D) conformal radiotherapy (CRT) plans tend to result in lower target coverage, more heterogeneous dose coverage, and greater volumes of doses up to 20 Gy (5).

It has been proven that IMRT and VMAT techniques provide more appropriate and homogeneous dose distribution when it is desired to be treated by defining it as the only PTV that includes the chest wall and regional nodes after modified radical mastectomy (6).

In studies on the subject, although the IMRT technique provides the desired dose homogeneity throughout the breast volume, it has been determined as a disadvantage that it is more sensitive to set-up and movement uncertainties. In addition, studies report that IMRT increases dose homogeneity and reduces acute skin toxicity and CB dose. The IMRT technique causes the doses of critical organs and normal tissues close to the target volume to decrease compared to conventional treatment, resulting in a decrease in acute and late toxicity rates (7).

VMAT, another technique that has started to be used outside of IMRT, is an approach applied using single or multiple arc irradiation. In this technique, depending on the device structure, the multi leaf collimator (MLC) position, gantry rotation speed and dose rate are modulated to treat the field to be irradiated (8). Advanced treatment techniques such as IMRT and VMAT improve PTV coverage, quality index and homogeneity, while reducing the dose received by critical organs, especially the heart. Despite this, studies say that while high doses of critical organs decrease, these organs that receive low doses cause an increase in irradiated volumes, and accordingly, the risks of secondary cancer cases may increase (9).

The primary aim of this study is to dosimetrically compare IMRT, VMAT and HYBRID (IMRT/VMAT) planning techniques for whole breast irradiation in terms of PTV coverage, Heterogeneity index (HI-), Confirmity index (CI) and OAR. In addition, it is to investigate the clinical usability in practice compared to IMRT and VMAT plans by determining which loading is more appropriate in the hybrid plans obtained by trying different loadings.

Materials and Methods

Patient Selection

Images of 20 randomly selected Caucasian origine, female patients were included in this study with left breast carcinoma (Ca) aged 40-69 years (median mean: 48) who had previously received primary breast radiotherapy for ductal Ca. In the patient images used in the study, patient personal information is hidden for privacy.

During the simulation, the breast board and arms were positioned up, with the chest wall parallel to the gantry, with an under-head support pillow suitable for the neck structure. After the immobilization of the patients in the supine position with suitable angled wedge supports under the legs for patient comfort, the patient is given fixed breathing training in advance, adhering to the clinical protocol with the Toshiba Aquillion 64 CT (computed tomography) simulator (by adding at least 5 cm to the upper and lower limits of the irradiation volume). While breathing steadily, 3 mm thick sections were taken.

The patient's information was submitted to the ethics committee. The study was approved by Aydın Adnan Menderes University Faculty of Medicine, Non-interventional Clinical Research Ethics Committee (approval number: 10, protocol no: 2022/07) and was carried out in accordance with the Declaration of Helsinki.

Volume definition: "Breast Atlas for Radiation Therapy Oncology Group Radiotherapy" is considered as a reference for contouring in all patient plans. After the PTV and critical organs adjacent to the PTV are determined, a 3 mm automatic margin is applied over the PTV in accordance with our clinical protocol. For organs risk evaluation, the treatment dose (total: 50 Gy) was defined as the isodose covering 95% of the PTV, using the criteria in Table 1.

IMRT plans: Treatment planning system (TPS) (Elekta, Business Area-Software-Systems, UK)are in the Monaco (version 5.10), using 6 MV Elekta Agility Linear Accelerator (Elekta LIMITED, UK) device (leaf-thickness 0.5 cm), 6 MV photon energy parameters, IMRTplans have 7 areas with an angle of approximately 310°, 330°, 342°, 30°, 80°, 105°, 132° (±5) dynamic IMRT treatment technique and using Monte-Carlo Algorithm. The collimator angle is defined as 2° to prevent leaf leakage.

VMAT plans: It was calculated using the Monte Carlo Algorithm with the double-arc treatment technique [approximately angles CW 286° starting-angle, 232° arcangle as reference (±5) 15° intervals], starting from the CW direction, using the same concentricity in VMAT plans, on the same IMRT contours, with the same central axes. The collimator angle was defined as 2° to prevent leaf leakage.

HYBRID plans: HYBRID plans; of previously calculated IMRT and VMAT plans (HYBRID 1: 20% IMRT - 80% VMAT, HYBRID 2: 80% IMRT - 20% VMAT, HYBRID 3: 40% IMRT - 60% VMAT, HYBRID 4: 60% IMRT-40% VMAT and considering planning algorithm structure, in terms of set up practicality, HYBRID 5: 52% IMRT - 48% VMAT) is the combination of loadings at different rates, in accordance with the device parameters in the TPS, MLC in the IMRT treatment technique modulates irradiation at fixed gantry angles,while according to the doses defined in the VMAT treatment technique (PTV, OAR) MLCs irradiate modulated depending on the gantry speed and dose rate during irradiation.

The CI defines the degree to which the predicted isodose volume matches the shape and size of the target volume. The ideal value of a correct plan in CI was expected to be close to "1". As OAR, lung dose, mean CL dose, maximum and minimum CB doses and mean heart dose ratio were

Table 1. Dose constra organs at risk used fo	ints for planning target volumes and r treatment plans
PTV (total dose 50 Gy)	95% REF Iso dose D ₉₅ %≥47.5 Gy
Lung dose	Nodal irradiation (-) V_{20} (%10-20 Nodal irradiation (+) V_{20} (%25
Contralateral lung dose	V _{5Gy} ≤10%
Contralateral breast dose	D _{mean} ≤1 Gy
Heart dose	V _{56y} ≤40% V _{256y} ≤10%
Mean heart dose (LAD)	D _{mean} 3-5 Gy
Normal tissue	Minimum dose

considered Table 1 values were used as evaluation criteria for OARs (10). For the HI-, the value in the algorithm of the Monoco Planning System was applied.

Statistical Analysis

The obtained data were analyzed using the Statistical Package for the Social Sciences (SPSS Statistics 25.0; SPSS Inc. Chicago, IL, USA) program. The distribution of the data was evaluated with the Kolmogorov-Smirnov test. While evaluating the study data, paired sample t-test was used to evaluate the descriptive statistical methods [mean, standard deviation (SD), frequency] as well as pairwise comparisons of normally (Gaussian) distributed parameters. Values at the significance level were accepted at p<0.05 levels. As a result of the analysis, p<0.05 and below values were accepted as significant. According to the parameters examined, the data in this study are the values obtained by taking the averages of all patients (20 patients), and the SD were obtained by using these averages, and the results were evaluated accordingly.

Results

Considering the statistical results, then the following points gain importance.

HYBRID plans were obtained with different loading rates of IMRT and VMAT plans, while PTV reference isodoza (95%) was given 50 Gy. Statistical differences of HYBRID plans compared to IMRT and VMAT plans, HI-, conformity index (CI) values and the statistical results of critical organ doses (OAR: lung dose, CL dose, CB and heart dose) are shown in Table 2 collectively.

When a total dose of 50 Gy to PTV is given, we look at the differences between the IMRT and VMAT plans according to the reference 95% isodose volume (V_{gs}) and the HYBRID plans according to IMRT and VMAT appears to be statistically significant. When the HI-, HYBRID 1 (p<0.010), HYBRID 2 (p<0.037) and HYBRID 5 (p<0.028) loading plans were eamined, it is found statistically significant compared to IMRT when VMAT plans and HYBRID 5 (p<0.012) plans were compared. This shows that the HI is high in IMRT plans and low in VMAT plans.

In terms of CI, it is seen that all HYBRID plans compared to VMAT plans (p<0.003, p<0.013, p<0.002, p<0.004, p<0.005) and VMAT plans are statistically compared to IMRT plans (p<0.018).

There was no statistical significance in any of the loadings in terms of counter-lung dose. However, when the IMRT plan with 20% loading (HYBRID 1) is compared with the VMAT plans, it shows a value close to statistical significance (p<0.095) when other results are taken into account.

In the comparison made in terms of lung dose in the irradiated field, all other loadings were found to be statistically significant except for the HYBRID 4 loading

Table 2. Device a	Statistical summary of CI, HI- nd Monaco (version 5.10) Pla	·, OAR and PTV (V ₉ Inning System	5) value	s obtained from	IMRT, VMAT an	d hybrid diff	erents loadin	g plans using Ele	ekta Agility Line	ar Accelerator
			c	PTV (V ₉₅)	Heterogeneity Index (-)	Conformity Index	Lung dose	Contralateral lung dose	Contralateral breast dose	Mean heart dose (LAD)
		Correlation		0.355	0.449	0.251	0.635	0.597	0.836	0.450
Pair 1	HYBRID 1 & IMRT	Significant	20	0.315	0.193	0.484	0.049	0.068	0.003	0.192
		d		0.317	0.054	0.079	0.020	0.237	0.027	0.219
		Correlation		-0.559	0.456	0.969	0.981	0.988	0.990	0.792
Pair 2	HYBRID 2 & IMRT	Significant	20	0.093	0.185	0.000	0.000	0.000	0.000	0.006
		d		0.322	0.087	0.498	0.027	0.236	0.007	0.958
		Correlation		-0.011	0.000	0.467	0.580	0.814	0.894	0.707
Pair 3	HYBRID 3 & IMRT	Significant	20	0.977	1.000	0.173	0.079	0.004	0.000	0.022
		þ		0.311	0.344	0.525	0.022	0.239	0.009	0.594
		Correlation		-0.371	0.000	0.832	0.656	0.937	0.919	0.778
Pair 4	HYBRID 4 & IMRT	Significant	20	0.291	0.999	0.003	0.040	0.000	0.000	0.008
		ď		0.314	0.344	0.362	0.398	0.237	0.009	0.676
		Correlation		-0.241	0.445	0.704	0.889	0.893	0.935	0.777
Pair 5	HYBRID 5 & IMRT	Significant	20	0.503	0.198	0.023	0.001	0.000	0.000	0.008
		d		0.316	0.012	0.990	0.022	0.209	0.007	0.601
		Correlation		0.941	0.659	0.957	0.968	0.936	0.698	0.861
Pair 6	HYBRID 1 & VMAT	Significant	20	0.000	0.038	0.000	0.000	0.000	0.025	0.001
		d		0.002	0.010	0.003	0.033	0.095	0.813	0.385
		Correlation		-0.034	0.559	0.256	0.574	0.518	0.754	0.400
Pair 7	HYBRID 2 & VMAT	Significant	20	0.926	0.093	0.475	0.083	0.125	0.012	0.252
		d		0.216	0.037	0.013	0.023	0.150	0.130	0.111
		Correlation		0.671	0.305	0.878	0.394	0.826	0.856	0.515
Pair 8	HYBRID 3 & VMAT	Significant	20	0.034	0.392	0.001	0.260	0.003	0.002	0.128
		d		0.014	0.344	0.002	0.741	0.107	0.697	0.038
		Correlation		0.266	0.304	0.614	0.561	0.671	0.622	0.434
Pair 9	HYBRID 4 & VMAT	Significant	20	0.458	0.392	0.059	0.092	0.034	0.055	0.210
		d		0.066	0.344	0.004	0.020	0.132	0.795	0.054
		Correlation		0.270	0.054	0.703	0.790	0.729	0.833	0.494
Pair 10	HYBRID 5 & VMAT	Significant	20	0.450	0.881	0.023	0.007	0.017	0.003	0.147
		þ		0.107	0.028	0.005	0.023	0.146	0.720	0.051
		Correlation		0.554	0.272	0.168	0.421	0.389	0.681	0.122
Pair 11	VMAT & IMRT	Significant	20	0.097	0.447	0.643	0.226	0.266	0.030	0.738
		d		0.332	1.000	0.018	0.023	0.168	0.070	0.206
1: HYBRID	1: 20% IMRT - 80% VMAT, HYB	RID 2: 80% IMRT - 20	AMV % (T, HYBRID 3: 40%	5 IMRT - 60% VM/	AT, HYBRID 4: 6	0% IMRT - 40	% VMAT, HYBRID	5: 52% IMRT - 48	3% VMAT. 2: HI-,
Cl: Critica	l organ dose (OAR) values obtair	ied by giving 50 Gy to	the 95%	reference dose	covered PTV. HI-:	Heterogeneity I	ndex, Cl: Confo	rmity Index, N: Nu	mber of samples,	IMRT: Intensity-
modulated test, statis	I radiotherapy, VMAI: Volumetric tical methods paired sample t-te:	-modulated arc therap st. P<0.05 and less w	oy. Vn: I ere cons	ne percentage vol dered significant	ume (V) of an org	an receiving (n) dose. Data di	stributions were c	alculated by Kolm	logorov-Smirnov

plans IMRT (p<0.398) and 40% IMRT - 60% VMAT loading plan was according to VMAT (p<0.741).

Considering the contralateral-breast dose, it was seen that all HYBRID loading plans statistically significant compared to IMRT plans.

Considering the heart dose, the 40% IMRT - 60% VMAT loading plans were statistically significant compared to the VMAT plan (p<0.038). In addition, HYBRID 4 (p<0.054) and HYBRID 5 (p<0.051) loading plans showed a value close to significance, even though they were outside the significance acceptance limits compared to the VMAT plan.

Discussion

The two main goals in breast radiotherapy are to deliver the desired homogeneous dose to the PTV, while preserving as much normal tissue as possible and reducing patient toxicity. It is aimed to provide disease control with these criteria. While using radiotherapy techniques, targeted PTV coverage leads to low dose exposure of adjacent OARs such as the IL, contralateral chest, and lung. It is known that this low dose effect leads to an increase in the rate of radiation-induced secondary malignancies. Studies also show that free breathing and chest/chest wall motion are in the range of 3 mm or less (11). Also, Jeulink et al. (12) concluded in their dosimetric study that the free breathing mode was sufficient for left breast irradiation.

IMRT and VMAT techniques are not always sufficient depending on the patient's structure, and HYBRID treatment techniques are needed for both homogeneous irradiation of PTV and lowering of OAR doses. Studies reporting planning comparisons have shown that in post-mastectomy breast cases, VMAT is preferred over IMRT and 3 DCRT to OARs to achieve lower dose and better PTV coverage with CI and HI.

In addition, in the study of Chen et al. (13), it is mentioned that the HYBRID - VMAT technique is advantageous in terms of appropriate PTV dose, heart dose and MU. On the other hand, Pignol et al. (14) reported about the decrease in target dose homogeneity during treatment leading to significantly increase in acute skin toxicity dose homogeneity in the irradiated PTV volume provides superiority in tumor control and reduces the possibility of radiation-induced toxicity.

This study showed the comparison of PTV in terms of target coverage and homogeneity, and thus the desired target coverage can be achieved by considering HYBRID 1 and HYBRID 3 values provided that the mean HYBRID loading rate is ± 10 (30% IMRT - 70% VMAT). In the study, the HI-shows that it index is high in IMRT plans and low in VMAT plans.

In terms of statistical significance the CI shows that all HYBRID plans are more suitable than VMAT plans.

The risk of radiation-induced pneumonia is an important complication of radiotherapy in breast cancer patients after radiotherapy. Willner et al. (15) reported that the incidence of radiation-induced pneumonitis increased by 10% for every 10% increase in V_{106v} . Yorke et al. (16) suggested the

use of $V_{_{5Gy}}\text{and}~V_{_{10Gy}}$ in the affected lung may be the cause of radiation-induced lung injury.

The study of Lai et al. (5) shows that the use of VMAT covers smaller volumes in the lung at higher doses (V_{20Gy}) smaller volumes, while low doses (V_{5Gy}) cover larger lung volumes.

In the study, however, there was no statistical significance in any plan in terms of CL dose. Considering the values, it is seen that the HYBRID 1 loading plan gives a better result than the others, although it is not statistically significant (p<0.095) compared to the VMAT plan.

From the lung dose adjacent to the treatment area point of view, all plans except HYBRIT 3-HYBRID 4 provide the desired low lung dose.

Doses vary for different patient anatomies, although common dose limits are used. According to the study of Taylor et al. (17), right breast doses are lower than left breast in terms of changes in cardiac doses. Darby et al. (18) reported a linear relationship between ischemic heart disease and D_{mean} for the heart. However, Hu et al. (19) used different techniques and observed that the heart dose was significantly reduced in the so called target segmented plans of IMRT that received 9 fields of the heart dose was used as the critical organ. In addition, in the same study, it was stated that VMAT plans significantly reduced the irradiated dose volume in the IL and the D_{max} dose was lower in the CB.

In the study, in terms of heart dose, HYBRID loading plan with 40% IMRT - 60% VMAT rates (p(0.038) is found statistically significant.

Breast cancer radiotherapy also has an effect on the CB. Popescu et al. (20) used the Rapid Arc[®] technique and reported that the D_{mean} of the CB was below 3.2 Gy,which could significantly reduce the risk of secondary carcinogenesis induced by RT, especially for the young female patient. In this study, consideration of the $D_{mean}(sl Gy)$ value in the CB, it is determined that the HYBRID planes had the lowest CB D_{max}

Conclusion

There is no standard radiotherapy treatment planning technique for breast cancer after radical mastectomy yet, and there are several options using different technologies. It is seen both in our study and in the literature that patientbased HYBRID methods provide more protection especially in terms of critical organ doses.

The literature indicates that the VMAT technique provides dose appropriateness and homogeneity while providing an adequate prescription dose for the target of RT. It also significantly reduces the risk of complications of the IL and CB with lower dose radiation exposure for breast cancer patients. In cases where we cannot obtain appropriate results with VMAT techniques, HYBRID plans reduce the OAR doses, while the dose envelops the PTV in a targeted manner. In the study, it was observed that HYBRID plans,
especially HYBRID 2, HYBRID 3 and HYBRID 4 loading plans had lower OAR values than IMRT and VMAT. In addition, HYBRID 3 and HYBRID 4 loading plans were statistically significant for PTV.

The results show that it is possible to obtain the desired dose includinge the desired critical organ doses and PTV, with patient-specific HYBRID plans obtained with different loadings in line with clinical needs.

Ethics

Ethics Committee Approval: The study was approved by Aydın Adnan Menderes University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (approval number: 10, protocol no: 2022/07) and was carried out in accordance with the Declaration of Helsinki.

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ö., N.Özb., Concept: N.Ö., N.Özb., Design: N.Ö., N.Özb., Data Collection or Processing: N.Ö., N.Özb., Analysis or Interpretation: N.Ö., N.Özb., Literature Search: N.Ö., N.Özb., Writing: N.Ö., N.Özb.

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

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

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Comparison of Machine Learning Methods to Predict Incomplete Atypical Femoral Fracture After Bisphosphonate Use in Postmenopausal Women

Menopoz Sonrası Kadınlarda Bifosfonat Kullanımı Sonrası Tamamlanmamış Atipik Femur Kırıklarının Tahmini için Makine Öğrenmesi Modellerinin Karşılaştırılması

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Abstract

Objective: Long-term use of bisphosphonates (BP) for treating osteoporosis may cause incomplete atypical femoral fracture. In this study, we compared the classification and risk estimation of incomplete atypical femoral fractures, which is an alternative approach to clinical risk assessment.

Materials and Methods: A data set was randomly selected from women using postmenopausal BP. We identified a class imbalance problem in the population and created a balanced structure using the density-based synthetic minority over-sampling technique. We compared machine learning algorithms and conducted a case study.

Results: We solved the class imbalance problem with the density-based synthetic minority over-sampling technique and found that the random forest and adaboost methods achieved the highest performance in the classification step.

Conclusion: It is recommended to apply resampling methods in cases where there is an unbalanced class problem such as incomplete atypical femoral fracture. Ensemble methods perform better than traditional methods in this study.

Keywords: Classification, unbalanced data, disease diagnosis, orthopedics, incomplete atypical fractures

Öz

Amaç: Bifosfonatların (BP) osteoporoz tedavisinde uzun süreli kullanımı tam olmayan atipik femur kırığına neden olabilir. Bu çalışmada, tamamlanmamış atipik femur kırıklarının sınıflandırılması ve risk tahmini için gelişmiş makine öğrenimi modellerinin performansını karşılaştırmayı amaçlanmaktadır.

Gereç ve Yöntemler: Veri seti, menopoz sonrası BP kullanan kadınların rastgele bir alt kümesini içerir. Popülasyonda bir sınıf dengesizliği sorunu belirledik ve yoğunluğa dayalı sentetik azınlık aşırı örnekleme tekniği kullanarak dengeli bir yapı oluşturduk. Makine öğrenmesi algoritmalarını karşılaştırdık ve bir olgu çalışması gerçekleştirdik.

Bulgular: Bu çalışmada, geleneksel lojistik regresyon yaklaşımını birkaç gelişmiş topluluk öğrenme yöntemiyle karşılaştırılmıştır ve rastgele orman ve Adaboost yöntemlerinin en iyi tahmin performansını elde ettiği sonucuna varılmıştır.

Sonuç: Bu çalışmada, atipik femur kırığını tahmin etmek için tekrarlanabilir bir makine öğrenimi iş akışı gösterilmiştir. Gelişmiş tahmine dayalı modeller, geleneksel modellerle karşılaştırılmış ve bunların geleneksel modellerden daha iyi performans gösterdiğini gösterilmiştir.

Anahtar Kelimeler: Sınıflandırma, dengesiz veri, hastalık tanısı, ortopedi, tamamlanmamış atipik kırıklar

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Introduction

Osteoporosis is an important orthopedic disease that occurs with low bone density and deterioration of bone structure (1-3). Early diagnosis of osteoporosis and appropriate and effective treatment such as bisphosphonates (BP) is very important to prevent potential fractures (4). However, longterm use of BP in the treatment of osteoporosis can result in incomplete atypical femoral fractur (iAFF) (5). These fractures are life-threatening (6). Therefore, determining the risk of fracture can be a great difficulty (7). More recently, machine learning (ML) methods have been increasingly utilized as they provide robust and versatile means of risk prediction for various medical domains. Although ML is relatively new to the field of orthopedics, it is essential for researchers in this field to fully understand ML (8,9).

In this study, we present a comparison of ML algorithms to risk predict iAFFs in the post-menstrual period. In our previous study, we had some difficulties in identifying risk factors due to numerical imbalances between groups (10). From this point of view, it is aimed to develop ML in data with class imbalance between groups in investigating risk factors in diseases with low prevalence. Using existing ML algorithms without considering data preprocessing to balance data sets makes it very difficult to develop an effective model. To prevent this, oversampling methods are applied before the training step in the application. Here, we first applied DBSMOTE to the training dataset with class imbalance problem because it is easy to extract information from small datasets and is needed in the real world. Next, we made a comparison between traditional learning methods and advanced ML methods in terms of evaluation criteria such as accuracy, sensitivity, specificity and kappa. To test the interpretability of the model that was selected after comparisons, we conducted a validation study for two patients.

Materials and Methods

Data

Study Population

This study was carried out in Menteşe district of Muğla province, where a population. The national health registry showed that 2,746 postmenopausal women in this region were using BP. Inclusion criteria were defined as age >50, female gender, diagnosis of osteoporosis and duration of BP use (BITIME). The true prevalence of iAFF is unknown, as some patients with iAFF are asymptomatic and do not seek treatment. We assumed 10% prevalence of iAFF and used 5% α significance and ±5% precision levels. As a result, the study continued with 132 patients and iAFF was detected in 14 of these 132 patients. Imbalanced ratio was 8.42 showing a moderate imbalance.

The data were obtained during the research project 17/064 supported by the Scientific Research Projects unit of Muğla Sıtkı Koçman University and ethics committee approval was obtained between 08.2016-08.2017 (approval number: 2016/55, date: 17.06.2016). Informed consent was obtained from all participants included in this study. Table 1 shows the descriptive statistics of the iAFF data set.

Imaging Studies

In order not to miss the early insufficiency fracture, whole body bone scintigraphy and anteroposterior and lateral radiographs were taken from all subjects. The presence of increased involvement in the lateral cortex of the femur by bone scintigraphy, focal changes in the lateral cortex of the femur (radiolucent line, focal-generalized cortical thickening, lines, cavities) were accepted as an iAFF. A consensus diagnosis of an iAFF was made with both radiographic and scintigraphic images (a team consisting of 2 orthopedists, 1 radiology and 1 nuclear medicine specialist). Although the ASBMR case definition does not include a bone scan or MRI, many authors suggest that an advanced imaging modality can be used for definitive diagnosis if there is a high suspicion of an iAFF (6).

Laboratory Tests

All measurements of the patients are given in Table 1. Bone mineral density (BMD) measurements of the femoral neck and anteroposterior lumbar spine were made using a dualenergy X-ray absorptiometry machine. According to World Health Organization criteria, normal BMD was defined as less than 1 standard deviation (SD) of young adult peak BMD (T-score), osteopenia was defined as a value between 1.0 and 2.5 SD. Young adult peak BMD and osteoporosis were defined as a value greater than or equal to 2.5 SD of young adult peak BMD.

Statistical Analysis

ML is defined as a multidisciplinary area that uses statistics, mathematics and computer science. It focuses on building models that learn from data and increase accuracy over time. ML can be thought of as deciding which treatment is most effective for a patient with certain characteristics, providing more accurate answers to clinical questions such as the expected risks/benefits in the short and long term for a specific disease (11,12). They can be performed using approaches such as traditional and advanced learning (13,14). In this study, we used logistic regression, decision tree, random forest, adaptive boosting, extreme gradient boosting methods

Logistic Regression (LR): LR is a traditional statistical learning method that uses a logistic link function to model a bilateral orthopedic outcome based on patient-level risk factors (15).

Decision Tree (DT): DT is a tree-based algorithm consisting of a series of decision tests that work with the divide and conquer method. Thanks to the tree structure, it makes it easier for experts to interpret the model and to detect highrisk patients (16). **Random Forest (RF):** RF is an ensemble method (bagging) created from decision trees. By combining more than one decision trees in the RF, a decision forest is created and the final estimation of the patient risk is made by combining the estimation results obtained from each decision tree (17).

Adaptive Boosting (ADABOOST): ADABOOST is an ensemble method (boosting) proposed by Freund and Schapire (18). ADABOOST initially starts with an even distribution for each sample and finds the weakest classifier based on classification performance. Then, it updates the weights, focusing on weakly classified samples. By combining weak classifiers as a result of a certain iteration, a strong classifier is created for disease classification (17). **Extreme Gradient Boosting (XGBOOST):** Gradient boosting is an ensemble method (boosting) that creates a prediction model for classification problems. XGBOOST builds and generalizes the model iteratively as other incremental methods. One of the most important features that distinguishes this method from others is its extra randomization parameter can be used to reduce the correlation between trees (19).

Density-Based Synthetic Minority Over-sampling Technique (**DBSMOTE**): DBSMOTE, Bunkhumpornpat et al. (20). It is based on the oversample randomly shaped set developed

Table 1. Descriptive statistics	s for iAFF dataset raw and DBSMOTE		
		RAW data	DBSMOTE
Feature abbreviations	Feature descriptions	Mean ± SD/n	Mean ± SD/n
Characteristic			
Age (years)	Patient's age	72.79±7.35	73.67±7.76
Height (cm)	Patient height	149.02±5.23	149.70±0.04
Weight (kg)	Patient's weight	63.82±12.11	64.91±10.25
BMI (kg/m²)	Body mass index	28.71±5.14	28.93±4.21
Medication			
BITIME	Bisphosphonate usage time	7.71±3.40	8.74±3.92
Steroid	Steorid usage history		
Present		19	13
Absent		113	85
PPI	Proton pump inhibitor		
Present		57	42
Absent		75	56
DM	Diabetes mellitus		
Present		25	18
Absent		107	81
Thyroid	Thyroid status		
Normal		109	74
Hypothyroidism		12	14
Hyperthyroidism		11	8
Test result			
DVIT (ng/mL)	Vitamin D level	30.24±12.81	30.21±12.89
PTH (pg/mL)	Parathyroid hormone level	58.12±27.39	68.18±28.78
ALP (U/L)	Alkaline phosphatase	65.05±20.38	66.11±26.58
HIPTS	Hip T-score	-1.78±0.77	-1.80±0.68
Vertebrats	Vertebra T-score	-2.49±1.13	-2.48±0.89
BMI: Body mass index SD: Stan	dard deviation DM: Diabates mellitus PTH: Pa	arathyroid bormone AI P. Alka	line phosphatase

by DBSCAN. The purpose of the DBSMOTE algorithm is to try to solve the class imbalance problem by adaptively generating synthetic new examples from the minority class through linear interpolation between existing minority class instances. DBSMOTE aims to reduce the bias in SMOTE and can adaptively change the decision boundary to focus on hard-to-learn samples (21,22).

Evaluation Criteria Methods Advocated in the Paper

Sensitivity (TP/((TP + FN))) is the ratio of predicted positive class values (TP) to all positive class values (TP+FN) (23,24). Specificity (TP/((TP + FN))) is the ratio of correctly predicted negative class values (TN) to all negative class values (23,24). Precision [TP/((TP+FP))] is the ratio of correctly predicted positive class value (TP) to all positively predicted class values (23,24). Balanced accuracy and kappa values were also considered.

Proposed ML Workflow

The steps of the proposed ML workflow are given in Figure 1.

The applications were conducted in R, an open-source software. During the study, caret, caretEnsemble, smotefamily, ggplot2, gridExtra and lime packages were used. Data set is divided into two parts as 25% test and 75% training. In this study DBSMOTE was also applied. Here, we install a 5-fold cross-validation approach to avoid overfitting. In the classification with raw and post-DBSMOTE data set, random search tunelength =5 was made to adjust the hyper parameters of the models. To increase the interpretability of the mode we use the lime package for interpretation purposes.

Results

Class Imbalance Reduces the Performance of ML Methods to Effectively Detect iAFFs after BP Use

The class imbalance problem directly affects the

performance criteria in the application of ML methods. Table 2 shows the results per model in the presence of class imbalance.

LR (balanced accuracy =0.45, kappa =-0.07, sensitivity =0, specificity =0.90, F1=NA, Precision =0) was observed to have the lowest performance results among the other methods. XGBOOST (balanced accuracy =0.90, kappa =35, sensitivity =0.50, specificity =0.98, F1=40, Precision=0.33) compared to other methods although it is good in balanced accuracy and kappa, it is still not sufficient for other criteria.

DBSMOTE Helps ML Methods to Effectively Detect iAFFs after BP Use

In this step, we applied DBSMOTE and observed a significant improvement in the performance of models shown in Table 2.

RF (balanced accuracy =0.88, kappa =0.79, sensitivity =1.00, specificity =0.77, F1=0.91, precision =0.84) method was found to provide higher accuracy than other methods. Classification success order after RF is followed by LR (balanced accuracy =0.73, kappa =0.47, sensitivity =0.88, specificity =0.58, F1=0.77, Precision =0.69), ADABOOST (balanced accuracy =0.88, kappa =0.78, sensitivity =0.96, specificity =0.80, F1=0.92, Precision=0.87), DT (balanced accuracy =0.80, kappa =0.65, sensitivity =0.90, specificity =0.70, F1=0.85, precision =0.81) and XGBOOST (balanced accuracy =0.84, kappa =0.70, sensitivity =0.93, specificity =0.75, F1=0.88, precision =0.84) methods.

The very low classification success of the diagnosis of iAFF made with raw data was interpreted as the result of imbalance. When these results are compared, it shows that LR method is relatively inadequate compared to RF, ADABOOST and XGBOOST methods in the presence of class imbalance. Another point to note here is that the XGBOOST method performs significantly higher than other methods in the presence of class imbalance. When the results after DBSMOTE are examined, it is observed that the RF and ADABOOST methods over performs others.



Figure 1. Proposed ML workflow for risk prediction of femoral fracture ML: Machine learning

ML Methods Help Effectively Interpret the Risk Factors for iAFFs after BP Use

Fracture risk assessment can guide clinicians and individuals in understanding the risk of having a fracture and speed up the decision-making process to reduce these risks. Figure 2 shows the order of the most important risk factors based on RF method.

As Figure 3 we also conducted a study of RF risk assessment on two test cases where the labels were not provided. In this case study, the characteristics that contribute to the classification of each patient and the decision rules for these characteristics were determined. After the RF model was trained, randomly selected patients #7 and #119 were classified for iAFFs. Here the y-axis gives the decision rules, and the X-axis gives the weights in this decision (Figure 3).



Figure 2. Feature importances based on RF RF: Random forest

Here, patient 7 was assigned class 0-label with probability 0.96 and patient 1 was assigned 1 labeled class with probability 0.86. When the result of the 7th patient is examined, the fact that BITIME <=6, PTH <=41.5 and weight <=54.6 contribute to the patient entering the no-risk class. 10.2< BITIME and HIPTS <=-2.3 contributed to the classification of patient #119 as involvement, while BMI <=24.6 does not support the presence of involvement.

The findings obtained after this case study were approved by the orthopedic and traumatology specialist, and it was concluded that the 7th and 119th patients were in the classes we predicted.

Conclusion

Today, an orthopedist needs accurate predictions of the outcome of their patients' disease, and therefore highperformance methods are vital to support treatment decisions. The percentage of people in the geatric age group in the general population is increasing and the use of BP group drugs for the prevention of osteoporotic fractures in this age group is becoming widespread (4). In the investigation of risk factors for iAFF in patients using BP, numerical group imbalances emerge between iAFF and non-iAFF groups. It is a common problem in studies to determine the risk factors associated with such diseases with low prevalence values. Early detection of iAFF significantly changes patient mortality and morbidity. In this respect, it is important to reveal the risk factors and true prevalence. As an important decision support tool, machine learning methods are used to potentially transform large medical data sets int

Table 2. Performance	result	after	classification	with	the	raw	data	set	and	performance	results	after	classification	with
DBSMOTE. Darker color codes show better performance														

		••			
Metric (raw data)	LR	RF	DT	ADABOOST	XGBOOST
Balanced accuracy	0.45	0.50	0.48	0.48	0.90
Карра	-0.07	-0.04	-0.04	-0.04	0.35
Sensitivity	0	0	0	0	0.50
Specificity	0.90	0.90	0.96	0.96	0.98
F1	NA	NA	NA	NA	0.40
Precision	0	0	0	0	0.33
Metric (DBSMOTE)					
Balanced accuracy	0.73	0.88	0.80	0.88	0.84
Карра	0.47	0.79	0.65	0.78	0.70
Sensitivity	0.88	1.00	0.90	0.96	0.93
Specificity	0.58	0.77	0.70	0.80	0.75
F1	0.77	0.91	0.85	0.92	0.88
Precision	0.69	0.84	0.81	0.87	0.84
LR: Logistic regression. RF: Random forest.	DT: Decision tree				

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Figure 3. Model agnostics of the classification procedure for the 7th and 119th patient in the iAFF test data set

Into a meaningful and efficient structure (9,13-14,25). However, ML methods are affected by the class imbalance problem and this problem has a big impact on classification performance. LR, DT, RF, ADABOOST and XGBOOST methods were used in this study. We observed that the LR method was highly affected by the class imbalance problem and that the DT, RF, ADABOOST and XGBOOST methods also achieved low classification success. Therefore, the data set is balanced with DBSMOTE to eliminate the class imbalance problem. The balanced data set was integrated into LR, DT, RF, ADABOOST and XGBOOST methods. The results showed that the RF and ADABOOST methods was the best performing method among these algorithms. Then, risk factors were determined by conducting a case study using RF method. When evaluating the risk of iAFF, we considered risk factors. We have reported varying degrees of severity of the RF method to help better understand key risk factors. Besides, we found that risk factors such as the duration of BP use, PTH level, age, DVIT level and body mass index contribute significantly to the high fracture risk.

Our study has some limitations. At the data level, the study was conducted in a small number of cohorts, and the data set used was obtained from patients in a particular region. In addition, this study provides cost effective determination of the risk factors of the disease without the need for X-ray and scintigraphy in the field of orthopedics.

Ethics

Ethics Committee Approval: The data were obtained during the research project 17/064 supported by the Scientific Research Projects unit of Muğla Sıtkı Koçman University and ethics committee approval was obtained between 08.2016-08.2017.

Informed Consent: Informed consent was obtained from all participants included in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.T., U.C., E.D., Design: S.T., U.C., E.D., Data Collection or Processing: T.D.C., U.C., E.D., Analysis or

Interpretation: S.T., T.D.C., U.C., E.D., Literature Search: S.T., U.C., E.D., Writing: S.T., T.D.C., U.C., E.D.

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

Financial Disclosure: The data were obtained during the research project 17/064 supported by the Scientific Research Projects unit of Muğla Sıtkı Koçman University.

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The Importance of Serum Procalcitonin and C-reactive Protein Levels in Patients with Lymphoma

Lenfomalı Hastalarda Serum Prokalsitonin ve C-reaktif Protein Düzeylerinin Önemi

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Abstract

Objective: Increased inflammatory cells in the tumor microenvironment are important in the formation and progression of lymphomas. C-reactive protein (CRP) and procalcitonin (PCT) are biomarkers that can be used to detect infection and inflammation. We investigated the clinical significance of CRP and PCT levels in lymphoma cases.

Materials and Methods: We evaluated 82 Hodgkin and 120 diffuse large B-cell lymphoma (DLBCL) cases. Pre- and post-treatment PCT and CRP values were compared. The relationship between pre-treatment CRP and PCT levels and response to chemotherapy treatment, disease stage, performance score, extranodal involvement, presence of bulky mass, and bone marrow involvement was determined.

Results: In the Hodgkin lymphoma group, the CRP level was increased in 40 (48.8%) patients and the PCT level was increased in 16 (19.5%) patients. The CRP level was high in 36 (30%) cases and the PCT level was high in 34 (18.3%) cases in the DLBCL group. CRP and PCT levels were significantly lower after chemotherapy treatment in all of our cases compared to pretreatment (p<0.001, p<0.001, respectively).

Conclusion: The decrease in CRP and PCT levels after chemotherapy treatment in our Hodgkin and DLBCL cases compared with pretreatment supports the role of inflammation in the pathogenesis. In addition, these parameters may contribute to the determination of prognosis.

Keywords: Procalcitonin, C-reactive protein, Hodgkin lymphoma, diffuse large B-cell lymphoma, prognosis

Öz

Amaç: Fazla enflamatuvar hücrenin bulunduğu tümör mikro çevresi, lenfomaların oluşumunda ve ilerlemesinde önemlidir. C-reaktif protein (CRP) ve prokalsitonin (PCT), enfeksiyon ve enflamasyonu tespit etmek için kullanılabilen biyobelirteçlerdir. Biz lenfoma olgularında CRP ve PCT düzeylerinin klinik önemini araştırdık.

Gereç ve Yöntemler: Seksen iki Hodgkin ve 120 diffüz büyük B-hücreli lenfoma olgusunu değerlendirdik. Tedavi öncesi ve sonrası PCT ve CRP değerleri karşılaştırıldı. Tedavi öncesi CRP ve PCT düzeyleri ile kemoterapi tedavisine yanıt, hastalık evresi, performans skoru, ekstranodal tutulum, hacimli kitle varlığı ve kemik iliği tutulumu arasındaki ilişki belirlendi.

Bulgular: Hodgkin lenfoma grubunda 40 (%48,8) hastada CRP düzeyi, 16 (%19,5) hastada PCT düzeyi yüksek idi. Diffüz büyük B-hücreli lenfoma grubunda CRP düzeyi 36 (%30) olguda, PCT düzeyi 34 (%18,3) olguda yüksekti. CRP ve PCT düzeyleri kemoterapi tedavisi sonrası tüm olgularımızda tedavi öncesine göre anlamlı olarak düşüktü (sırasıyla p<0,001, p<0,001).

Sonuç: Hogkin ve diffüz büyük B-hücreli lenfoma olgularımızda kemoterapi tedavisi sonrası CRP ve PCT düzeylerinin tedavi öncesine göre azalması inflamasyonun patogenezdeki rolünü desteklemektedir. Ayrıca bu parametreler prognozun belirlenmesinde de katkı sağlayabilir.

Anahtar Kelimeler: Prokalsitonin, C-reaktif protein, Hodgkin lenfoma, diffüz büyük B-hücreli lenfoma, prognoz

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Introduction

Chronic inflammation causes genetic instability and oncogenic mutations, creates the appropriate microenvironment for tumor development (1,2). Inflammation plays an important role in the pathogenesis of breast, lung, and liver cancer, multiple myeloma, Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) (3-5). In classic HL, Hodgkin/reed Steinberg (HRS) cells constitute approximately 1% of the tumor tissue (6). Lymphocytes, eosinophils, neutrophils, and plasma cells form an inflammatory microenvironment around HRS cells (7). These inflammatory cells contribute to the survival and proliferation of HRS cells through the cytokines they secrete and also allow them to escape from the body's immune system (6,7). The JAK-STAT pathway activated by interleukin (IL) 2, IL-6, IL-10 secreted from inflammatory cells contributes to the pathogenesis of the disease in diffuse large B-cell lymphoma (DLBCL) (8).

C-reactive protein (CRP) and procalcitonin (PCT) are acutephase reactants (9,10). CRP increases in cases of bacterial and viral infections, collagen tissue diseases, burns and cancer. IL-6 secreted by HRS cells also stimulates CRP synthesis in HL cases. PCT used to distinguish bacterial infections from non-bacterial infections (10). PCT level increases extensive metastatic solid cancers, thyroid cancers, and neuroendocrine tumors (11).

Although there are many different factors that play a role in the pathogenesis of HL and DLBCL, inflammation is important in the pathogenesis. Inflammatory biomarkers are important in both the diagnosis and prognosis of HL (12). In DLBCL cases, CRP contributes to the determination of prognosis both alone and in combination with the international prognostic index score.

Inflammation is a factor that negatively affects cancer development and prognosis, and CRP and PCT are inflammatory biomarkers. Therefore, we investigated CRP and PCT levels and their clinical significance in HL and DLBCL.

Materials and Methods

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Atatürk University (decision no: 33, date: 25.11.2021). The requirement for informed consent was waived because the data used in the study were anonymous and the study was designed as a retrospective file review. We analyzed the files of 339 patients with HL and DLBCL. Two hundred-two cases who were given adriamycin, bleomycin, vinblastine, dacarbazine treatment for HL and rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone treatment for DLBCL treatment were included in the study. The lymphoma diagnosis and subgroup determination of the cases were made according to the World Health Organization 2016 criteria. Patients with fever, signs of active infection, and a history of inflammatory disease were not included in this study.

Age, gender, Ann Arbor stage, extranodal involvement, bone marrow infiltration. Eastern Cooperative Oncology Group (ECOG) performance score, presence of bulky mass, lactate dehydrogenase (LDH), beta 2 (β 2) microglobulin, CRP, and PCT values at the time of admission were recorded. CRP and PCT values of the cases were also recorded after the first course of chemotherapy (15-30 days), and these values were accepted as post-treatment. Bone marrow biopsy was not performed in HL cases with bone marrow involvement in positron emission tomography/computerized tomography (PET-CT) and were considered stage 4. Bone marrow involvement was evaluated by bone marrow biopsy in all other cases. The response of the cases to chemotherapy was evaluated by PET-CT. Deauville criteria were used for PET-CT response evaluation of patients with HL. Response to treatment was classified as complete remission, partial remission, stable disease, and progressive disease.

Statistical Analysis

Statistical analyzes were performed using the SPSS (Statistical Package for the Social Sciences) 20 program. The distribution normality of the data was analyzed with the Kolmogorov-Smirnov test. Comparisons between the two groups were made with the independent t-test if the data were normally distributed, otherwise with the Mann-Whitney U test. One-Way analysis of variance was used for comparisons between groups of more than two normally distributed quantitative variables. Comparisons of more than two groups without normal distribution were made with Kruskal-Wallis and Dunn-Bonferroni tests. For correlation analysis. Pearson correlation analysis was used if the data were normally distributed, and Spearman Rank correlation analysis was used if the data were not normally distributed. Survival analyzes were performed with the Kaplan-Meier test. P<0.05 value was considered statistically significant.

Results

Eighty-two (40.6%) of our cases were HL and 120 (59.4%) were DLBCL. The mean age of HL cases was 40.68±15.26 years; 38 (46.3%) were female and 44 (53.7%) were male. In the DLBCL group, the mean age was 51.95±11.95; 44 (36.7%) were female and 76 (63.3%) were male. Laboratory findings and clinical features of our cases were shown in Table 1.

There was no significant correlation between LDH value and pre-treatment PCT and CRP values in the HL (r=0.09, p=0.57; r=0.08, p=0.52, respectively) and DLBCL groups (r=0.198, p=0.21; r=0.04, p=0.75; respectively). There was no significant correlation between pre-treatment CRP and β 2 microglobulin values in the HL group (r=0.238, p=0.23), but a significant correlation was found between pre-treatment PCT and β 2 microglobulin values (r=0.63, p=0.015). Pretreatment PCT and CRP values and β 2 microglobulin levels were not significantly correlated in the DLBCL group (r=0.14, p=0.51; r=0.03, p=0.89; respectively).

CRP values were high in 40 (48.8%) of the HL patients and 36 (30%) of the DLBCL patients, and PCT values in 16 (19.5%) of the HL group and 34 (18.3%) of the DLBCL group. Pre-treatment CRP and PCT values were significantly higher than post-treatment CRP and PCT values in HL and DLBCL groups (Table 2). The relationship between the response status of lymphoma cases to chemotherapy treatment and pre-treatment CRP and PCT levels was shown in Table 3. The association with pre-treatment CRP and PCT levels and ECOG score, extranodal involvement status, presence of bulky mass, bone marrow infiltration,

and Ann Arbor stages of our HL and DLBCL cases were shown in Tables 4 and 5.

The mean follow-up period of the cases was 24±11 months. Six cases in the HL group and 12 cases in the DLBCL group died due to disease-related reasons. The mean survival time of patients with high and normal CRP values in the HL group was 15±2.1 months and 19±3.1 months, respectively (p=0.04). The survival time of patients with high and normal PCT levels in the HL group was 14±1.8 months and 18±2.4 months, respectively (p=0.03). In the DLBCL group, the survival time of patients with high CRP value (12±2.1 months). In the DLBCL group, the survival time was shorter in patients with high CRP (12±2.1 months) value than in

Table 1. Laboratory findings and clinical features of HL and DLBCL cases								
	Group							
Parameters	HL	DLBCL	All patients					
Lactate dehydrogenase (135-225 U/L)	293±156.73	445.39±168.74	500.91±1303					
β2 microglobulin (0.8-2.4 mq/L)	2.49±1.33	3.34±2	2.97±1.8					
Sedimentation (0-20 mm/h)	19±6.2	14±5.1	15±4.8					
ECOG performance score	HL n (%)	DLBCL n (%)	All patients n (%)					
ECOG 1	8 (9.8%)	22 (18.3%)	30 (14.9%)					
ECOG 2	60 (73.2%)	66 (55%)	126 (62.4%)					
ECOG 3	14 (17%)	22 (18.3%)	36 (17.8%)					
ECOG 4	0 (0%)	10 (8.3%)	10 (5%)					
Ann Arbor stage	HL	DLBCL	All patients					
Stage 1	4 (4.9%)	10 (8.3%)	14 (6.9%)					
Stage 2	24 (29.3%)	48 (40%)	72 (35.6%)					
Stage 3	36 (44%)	44 (36.7%)	80 (39.6%)					
Stage 4	18 (22%)	18 (15%)	36 (17.8%)					
Bulky mass (present)	12 (14.6%)	18 (15%)	30 (14.9%)					
Extranodal involvement (present)	20 (2.4%)	18 (15%)	38 (18.8%)					
Bone marrow infiltration (present)	18 (22%)	18 (15%)	36 (17.8%)					
HL: Hodgkin lymphoma, DLBCL: Diffuse large B cell lymphor	ma. ECOG: Eastern Coo	operative Oncology Group						

Table 2. CRP and PCT values of our cases before and after treatment								
Group	Parameters	Pre-treatment	Post-treatment	p-value				
	CRP	52.51±53.1	10.47±15.44	<0.001				
HL	РСТ	3.23±3.15	0.48 ±1.95	0.002				
DLBCL	CRP	39.12±49.3	10.79±14.01	0.045				
DEDOL	РСТ	1.69±2.64	0.36 ±1.61	0.006				
	CRP	44.23±50.91	10.67±14.49	<0.001				
All patients	РСТ	2.16±2.87	0.4 ±1.7	<0.001				

HL: Hodgkin lymphoma, DLBCL: Diffuse large B cell lymphoma, CRP: C-reactive protein (normal range: 0-5 mg/L), PCT: Procalcitonin (normal range: 0-0.5 ng/mL)

Table 3. The relationship between pre-treatment CRP and PCT levels and response to chemotherapy						
Group	Chemotherapy response status	CRP (mg/L)	PCT (ng/mL)			
	CR	5.93±3.3	0.06±0.08			
	PR	47.62±27.62	0.14±0.34			
пс	Stable disease	93.34±26.36	0.32±0.35			
	Progressive disease	124.35±54.45	1.89±3.99			
p-value		<0.001	0.04			
Group	Chemotherapy response status	CRP (mg/L)	PCT (ng/mL)			
	CR	7.57±8.87	0.04±0.04			
DLDCI	PR	33.72±31.2	0.33±0.38			
DLBCL	Stable disease	98.02±46.79	0.99±0.55			
	Progressive disease	121.02±54.51	2.67±0.55			
p-value		<0.001	0.04			

HL: Hodgkin lymphoma, DLBCL: Diffuse large B cell lymphoma, CRP: C-reactive protein, PCT: Procalcitonin, CR: Complete remission, PR: Partial remission

Table 4. The relationship between pre-treatment CRP level and ECOG score, extranodal involvement, bulky mass, bone marrow infiltration and Ann Anbor stage

Parameters	CRP		
ECOG performance score	HL	DLBCL	All patients
ECOG 1	33.03±46.74	36.86±44.73	37.84±43.59
ECOG 2	46.08±48.44	39.79±50.57	42.77±49.24
ECOG 3	60.06±57.43	32.83±51.83	41.9±53.35
ECOG 4		40.17±54.35	40.17±54.35
p-value	0.71	0.98	0.97
Extranodal involvement	HL	DLBCL	All patients
Present	65.35±15.55	36.07±16.07	46.46±18.32
Absent	43.3±20	48.11±19.69	40.11±18.89
p-value	0.04	0.03	0.04
Bulky mass	HL	DLBCL	All patients
Present	48.25±10.16	44.74±11.72	43.74±10.91
Absent	35.77±8.95	23.6±15.5	27.95±10.06
p-value	0.04	0.04	0.03
Bone marrow infiltration	HL	DLBCL	All patients
Present	64.33±20.12	47.01±19.32	55.16±14.2
Absent	41.66±17.78	36.35±17.07	38.35±17.09
p-value	0.02	0.03	0.02
Ann Arbor stage	HL	DLBCL	All patients
Stage 1	24.03±15.22	8.8±10.59	18.33±10.94
Stage 2	38±13.97	33.6±9.75	33.21±12.24
Stage 3	49±16.55	42.89±16.78	43.53±18.97
Stage 4	67.59±17.9	47.01±9.32	57.3±13.36
p-value	0.01	0.04	0.03
HL: Hodgkin lymphoma, DLBCL: Diffuse large Group	B cell lymphoma, CRP: C-reactive	protein, PCT: Procalcitonin, ECOG:	Eastern Cooperative Oncology

Parameters	PCT		
ECOG performance score	HL	DLBCL	All patients
ECOG 1	0.02±0.01	0.24±0.4	0.18±0.35
ECOG 2	0.46±1.7	0.1±0.18	0.27±1.18
ECOG 3	0.04±0.02	0.07±0.07	0.06±0.06
ECOG 4		2.33±4.96	2.33±4.96
p-value	0.76	0.014	0.017
Extranodal involvement	HL	DLBCL	All patients
Present	0.97±0.03	0.94±0.61	0.99±0.64
Absent	0.03±0.01	0.15±0.04	0.03±0.04
p-value	0.04	0.02	0.04
Bulky mass	HL	DLBCL	All patients
Present	1.4±0.59	1.36±0.61	1.38±0.59
Absent	0.04±0.02	0.05±0.04	0.05±0.03
p-value	0.03	0.04	0.04
Bone marrow infiltration	HL	DLBCL	All patients
Present	1.39±0.06	1.36±0.01	1.37±0.02
Absent	0.21±0.44	0.08±0.07	0.14±0.3
p-value	0.03	0.04	0.04
Ann Arbor Stage	HL	DLBCL	All patients
Stage 1	0.03±0.009	0.01±0.01	0.02±0.01
Stage 2	0.7±0.08	0.7±0.02	0.4±0.29
Stage 3	1.21±0.44	1.36±0.07	1.14±0.3
Stage 4	2.66±0.03	2.36±0.05	2.63±0.08
p-value	0.03	0.04	0.04

Table 5. The relationship between pre-treatment PCT level and ECOG performance score, extranodal involvement, bulky mass

HL: Hodgkin lymphoma, DLBCL: Diffuse large B cell lymphoma, CRP: C-reactive protein, PCT: Procalcitonin, ECOG: Eastern Cooperative Oncology Group

patients with normal CRP (16±3.1 months) value, and in patients with high PCT level (13±1.8 months) compared to those with normal PCT (15±2.4 months) level (p=0.03, p=0.04, respectively).

Discussion

There is systemic inflammation in cancer cases and it causes an increase in the level of IL-6. It triggers an increase in CRP levels. Legouffe et al. (13) reported that the CRP value was high in 42% of the NHL cases. Wieland et al found that the CRP value was high in 54% of the HL cases (14). In our study, 48.8% of HL cases and 30% of DLBCL cases had elevated CRP values, and these rates were lower than in the literature. This may be because we included only DLBCL cases from the NHL group in our study and excluded cases with signs of infection from our study.

Inflammation affects not only the development of cancer but also its spread. Weinstein et al. (15) evaluated solid tumor

or hematologic malignancy cases and found a correlation between disease stage and CRP level. It has been reported that CRP value before treatment is associated with disease stage and IPI score in DLBCL cases (16). There were also studies reporting a relationship between disease stage and pre-treatment CRP value in HL cases (14,17). A relationship was found between CRP value and the presence of extranodal involvement in HL (18). In our study, a correlation was found between pre-treatment CRP and extranodal involvement, presence of bulky mass, bone marrow infiltration, and disease stage in HL and DLBCL.

Da Silveira da Rocha et al. (17) stated that after the first course of chemotherapy treatment in HL cases, the CRP value decreased to near normal levels. In our study, the post-treatment CRP value was significantly lower than the pre-treatment CRP value in HL and DLBCL. This result supports the role of inflammation in the pathogenesis of both HL and DLBCL. We think that the reason for the decrease in CRP value is the corticosteroid treatment of inflammatory cells in HL cases. Therefore, we think that the pre-treatment CRP value may be a biomarker to predict response to chemotherapy treatment.

It was reported high PCT levels in 30.6% of patients with hematological malignancies. In our study, PCT values were elevated in 19.5% of the HL group and 18.3% of the DLBCL group, and these rates were lower compared to the literature. This may be because cases with signs of infection were not included in our study.

Very few studies investigate the diagnostic and prognostic importance of PCT levels in solid organ cancers. Chaftari et al. (19) stated that PCT levels can be used as a biomarker to predict cancer in cases without fever. The significant decrease in the post-treatment PCT level compared to the pre-treatment PCT level in our study supports this hypothesis for HL and DLBCL cases. PCT level is elevated in patients with medullary thyroid cancer, and elevated PCT level is associated with short surveillance. Matzaraki et al. (11) stated that PCT level is high if primary tumors have liver metastases. Our study is the first in the literature to investigate the relationship between PCT level, disease severity, and treatment response in lymphoma cases. We found a significant correlation between pre-treatment PCT level and response to chemotherapy treatment, extranodal involvement, presence of bulky mass, and disease stage. This result supports that PCT level is a laboratory test that can be used to evaluate disease prognosis and response to treatment.

Kawaguchi et al. (20) detected that progression-free survival was significantly shorter in follicular lymphoma patients with a high CRP value (>5 mg/dL). In our study, we found that the survival time was shorter in patients with high CRP and PCT levels in both HL and DLBCL groups compared to patients with normal levels. This supports that increased inflammation at the time of diagnosis may be associated with worse response to treatment and worse prognosis.

Conclusion

A better understanding of the pathogenesis of lymphoma disease will provide a better determination of prognostic factors. As etiopathogenesis is better understood, the parameters used in the prognostic scoring of lymphoma are updated. PCT and CRP are inexpensive biochemical tests that can be performed in almost every hospital. In our study, we found that CRP and PCT values can be used as additional biomarkers in predicting prognosis and response to chemotherapy in HL and DLBCL cases.

Ethics

Ethics Committee Approval: Approval was granted by the Ethics Committee of Atatürk University (decision no: 33, date: 25.11.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.S., Concept: S.S., G.S., F.E., Design: S.S., G.S., F.E., Data Collection or Processing: M.N.K., Z.A., Analysis or Interpretation: S.S., M.N.K., Z.A., Literature Search: S.S., M.N.K., Z.A., F.E., Writing: S.S., G.S.

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

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

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The Investigation of *Blastocystis* Subtypes in Fecal Samples of Diabetes Mellitus Patients

Diabetes Mellitus Tanısı ile İzlenen Hastaların Gaita Örneklerinde *Blastocystis* Subtiplerinin Araştırılması

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Abstract

Objective: This study aimed to detect *Blastocystis* spp. To determine the incidence, genotypes, regulation of blood glucose level and its role in gastrointestinal symptoms.

Materials and Methods: The current study was conducted at Aydın Adnan Menderes University Medical Faculty Hospital, where stool samples of 100 patients diagnosed with type 2 diabetes mellitus (DM) and 100 healthy volunteers as a control group were examined. Stool samples were evaluated by direct microscopic examination and polymerase chain reaction (PCR). Statistical analysis of the data was performed using the SPSS 17.0 program, and a significance level of p<0.05 was determined.

Results: The presence of *Blastocystis* was investigated in a total of 200 samples. Of the cases, 81 (40.5%) were female and 119 (59.5%) were male. The mean age was 57.1±11.2. Most of the participants resided in the city center (52.5%). Eighteen samples that were *Blastocystis* positive by direct microscopy were also found to be positive by PCR. Additionally, two samples that were negative by direct microscopy in the control group were found to be positive by PCR. There was no significant difference between the groups in terms of *Blastocystis* positivity. The sensitivity and specificity of the direct microscopy method were calculated as 81.81% and 100%, respectively. Nine (45%) of the *Blastocystis* subtypes were ST3, seven (35%) were ST2, three (15%) were ST1, and one (5%) was ST6.

Conclusion: *Blastocystis* spp. is a pathogen that should be considered in patients with diabetes. Our study is one of the few studies investigating the prevalence and clinical features of *Blastocystis* spp. in patients with type 2 DM.

Keywords: *Blastocystis* spp., genotype, diabetes mellitus

Öz

Amaç: Bu çalışmanın amacı diyabetik hastalarda *Blastocystis* spp. insidansını, genotiplerini, kan glukoz düzeyinin regülasyonu ve gastrointestinal semptomlardaki rolünü belirlemektir.

Gereç ve Yöntemler: Aydın Adnan Menderes Üniversitesi Tıp Fakültesi Hastanesi'nde tip 2 diabetes mellitus (DM) tanısı almış 100 hasta ile kontrol grubu olarak 100 sağlıklı gönüllünün dışkı örnekleri incelenmiştir. Dışkı örnekleri direkt mikroskobik olarak ve polimeraz zincir reaksiyonu (PZR) ile değerlendirilmiştir. Verilerin istatistiksel analizi SPSS 17.0 programı ile yapılmış, anlamlılık değeri p<0,05 olarak belirlenmiştir.

Bulgular: Çalışma kapsamında toplam 200 örnekte *Blastocystis* varlığı araştırılmıştır. Olguların 81'ini (%40,5) kadınlar, 119'unu (%59,5) erkekler oluşturmaktadır. Yaş ortalaması 57,1±11,2 olarak saptanmıştır. Çalışmaya katılanlar büyük oranda şehir merkezinde 105 (%52,5) ikamet etmekte idi. Direkt mikroskopi ile *Blastocystis* saptanan toplam 18 örneğin tamamı PZR ile pozitif bulunmuştur. Ayrıca kontrol grubunda direkt mikroskopi ile negatif çıkan iki numune PZR ile pozitif bulundu. İstatistiksel olarak gruplar arasında

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[®]Copyright 2023 by the Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. Meandros Medical and Dental Journal published by Galenos Publishing House. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) anlamlı bir fark saptanmamıştır. Direkt mikroskopi yönteminin duyarlılığı %81,81, özgüllüğü ise %100 olarak hesaplandı. *Blastocystis* alt tiplerinin analizinde dokuzunun (%45) ST3, yedisinin (%35) ST2, üçünün (%15) ST1 ve birinin (%5) ST6 olduğu görülmüştür.

Sonuç: *Blastocystis* diyabetli hastalarda dikkate alınması gereken bir patojendir. Çalışmamız tip 2 DM hastalarında *Blastocystis* prevalansını ve klinik özelliklerini araştıran az sayıdaki çalışmadan biridir.

Anahtar Kelimeler: Blastocystis spp., genotip, diabetes mellitus

Introduction

Blastocystis spp., first described in 1911, is the most common intestinal protozoan found in human stool samples. The life cycle of *Blastocystis* has not been fully elucidated due to many intermediate forms that have not yet been identified (1). At least 30 subtypes of *Blastocystis* have been confirmed and 14 subtypes have been identified in humans. ST3 has been reported most frequently, followed by ST1 (2).

It has been reported that the high prevalence detected in developing countries is due to the risk factors for *Blastocystis* transmission, such as sanitation, contaminated food and drinking water, and contact with animals (3). In our country, the prevalence of *Blastocystis* has been reported to vary between 1% and 24% (2). Although it is often asymptomatic, it has been reported that non-specific symptoms such as diarrhea, abdominal pain, nausea, bloating, weight loss, fatigue, vomiting, constipation and excessive gas were observed in studies on the clinical findings of cases with *Blastocystis* (4).

The widespread use of molecular methods in diagnosis facilitates the detection of *Blastocystis*. Polymerase chain reaction (PCR) is one of the molecular methods accepted as the gold standard for detecting *Blastocystis* (5).

Blastocystis infections were studied in patients with colorectal cancer and acquired immunodeficiency syndrome (AIDS) (4). However, the number of studies investigating the presence of *Blastocystis* in patients with DM is relatively few. This study aimed to determine *Blastocystis* spp. incidence, genotypes and role in gastrointestinal symptoms in type 2 diabetes mellitus (DM) patients.

Materials and Methods

Stool samples were collected from 100 patients diagnosed with type 2 DM and 100 healthy volunteers as a control group in Aydın Adnan Menderes University Faculty of Medicine. Written informed consent was obtained from the volunteers before sample collection. A standardized questionnaire was designed presence of gastrointestinal symptoms. All procedures performed in this study were approved by the Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine, with decision no 2017/1090 (date: 09.02.2017).

Stool samples were examined directly microscopically with Lugol's lodine solution. DNA isolation was performed using a commercial kit (MagPure Stool DNA Kit) to determine *Blastocystis* by PCR. After DNA isolation, *Blastocystis* approximately 600 bp SSU-rDNA gene amplification performed using primers RD5 (forward) (5'-ATC TGG TTG ATC CTG CCA GT-3') and *Blastocystis* specific BhRDr (reverse) (5'-GAG CTT TTT AAC TGC AAC AAC G-3') (6).

Sequences were also submitted to phylogenetic analysis and published reference sequences from the GenBank database to confirm the subtype classification.

The samples were sequenced by a commercial Company with the Applied Biosystems 377 DNA Sequencer instrument. Sequence data were sequenced using the ClustalW software Bioedit for Windows (Tom Hall) with reference sequences downloaded from https://www.ncbi. nlm.nih.gov/genbank.

Reference sequences used for this purpose are: EU445486, KF848573, AB107967, EU445488, JF792497, EU445492, KF848534 for genbank samples ST1; AB070987, DQ232794, EU679345, JF792496 and JN003684 genbank sequences for ST2; sequences KF848561, AB070988 and JN587546 were included in the analysis for ST3.

Edited *Blastocystis* partial SSU rDNA sequences were compared with the sequences in the Genbank nucleotide database on a web-based basis using Basic Local Alignment Search Tool software.

After sequencing and editing, a genetic distance referenced tree was created by Neighbor-Joining method, 1,000 repetitive bootstrap test in Molecular Evolutionary Genetics Analysis program. The 18S ribosomal RNA gene of *Proteromonas lacertae* was used as the outgroup while plotting the tree based on genetic distance.

Statistical Analysis

The positivity of *Blastocystis* spp. was compared between diabetic patients and the control group using the chi-square test. Classified data were evaluated using the chi-square test while analytical data were assessed using the Student's t-test. Statistical data analysis was performed using the SPSS 17.0 Windows[®] program, and a significance level of p<0.05 was determined.

Results

The presence of *Blastocystis* was investigated in 200 samples, including 100 with DM and 100 controls. Of the cases, 81 (40.5%) were female, and 119 (59.5%) were male. The mean age was 57.1±11.2, with an age range of 21-86. Most of the participants resided in the city center (105,

52.5%), while 49 (24.5%) lived in districts, and 46 (23%) lived in villages.

Direct microscopic examination of stool samples revealed *Blastocystis* in nine individuals in the DM group and nine in the control group. There was no significant difference between the groups regarding *Blastocystis* positivity (p>0.05).

In the isolated genomic DNA samples, PCR detected amplification at the expected size (~600 bp) in 9 and 11 samples in the DM and control groups, respectively. All 18 *Blastocystis* samples positive by direct microscopy were also positive by PCR. Additionally, two samples that were negative by direct microscopy in the control group were found to be positive by PCR.

Identification of Blastocystis Subtypes

A total of 20 samples found positive by PCR were sent to the commercial company for sequencing, sorted with reference sequences, and evaluated by examining possible wrong readings. Partial SSU rDNA sequences of the edited *Blastocystis* were compared from the GenBank database, and it was determined that nine (45%) were ST3, seven (35%) were ST2, three (15%) were ST1, and one (5%) was ST6. Since the number of subtypes was low, no additional statistical analysis was performed (Table 1).

The samples were sequenced with the subtype reference sequences in GenBank and their evolutionary distances were determined using phylogenetic tools (Figure 1).

When *Blastocystis*-positive and negative cases were compared regarding age, gender, residence, and clinical findings, there was no statistically significant difference between the groups (p>0.05). A comparison of symptoms in *Blastocystis* positive and negative cases is shown in Table 2.

In our study, PCR found positivity in two samples that were not positive by the direct microscopy method. The total number of positives increased to 11 with PCR. As a result, when PCR was accepted as the gold standard, the sensitivity and specificity of the direct microscopy method were calculated as 81.81% and 100%, respectively.

Discussion

Intestinal parasitic infections are globally distributed and their prevalence is higher, especially in developing countries. It has been reported that they can cause serious clinical problems up to death in patient groups with impaired

Table 1. Distribution of <i>Blastocystis</i> subtype							
Group	ST3	ST2	ST1	ST6			
DM (n=9)	4	3	1	1			
Control (n=11)	5	4	2	-			
Total (n=20)	9	7	3	1			
DM: Diabetes mellitus							

immunity, such as AIDS, organ transplant recipients, diabetics and oncology patients (7,8). In our study group, DM patients, the immune system is suppressed by the effect of hyperglycemia. In this situation, infections are more frequent and severe (9).

The few studies investigating the relationship between DM and parasitic infections suggest that DM is associated with an increased incidence of intestinal protozoa such as Entamoeba histolytica and Giardia duodenalis, and helminths such as Ascaris lumbricoides. Although Blastocystis spp. has been one of the most prevalent protozoa in human stool samples, there are many uncertainties, particularly regarding its pathogenic potential. On the other hand, the number of studies examining the relationship between the incidence of DM and *Blastocystis* spp. is guite insufficient (10,11). In a study investigating the prevalence of *Blastocystis* in diabetic patients in Thailand, it was reported that the parasite was detected in 12.3% of DM patients and 9% of the control group. However, the difference detected was insignificant (12). Poorkhosravani et al. (13) reported that Blastocystis spp. prevalence is 9.1% in the diabetes group and 10.1% in the control group. Similarly, in our study, 200 cases were evaluated and *Blastocystis* spp. was found in 9% of the patients with DM and 11% of the control group. There was no significant difference between the two groups regarding the



Figure 1. Evolutionary distances of partial SSU rDNA sequences

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Table 2. Comparison of symptoms in <i>Blastocystis</i> positive and negative cases									
Blastocystis	Pozitive (n=20)		Negative (n=18	0)					
Symptom	Number	%	Number	%	Total	χ²	p-value		
Pruritus	1	5	21	11.7	22	0.817	0.700		
Diarrhea	2	10	9	5	11	0.866	0.352		
Abdominal pain	3	15	34	18.9	37	0.181	0.671		
Nausea-vomiting	1	5	17	9.4	18	0.434	1.00		
Constipation	1	5	4	2.2	5	0.570	0.413		

presence of *Blastocystis* (p>0.05). In studies investigating the prevalence of *Blastocystis* spp., it is noteworthy that the incidence of the parasite shows significant differences even in close geographies and similar study groups. In various studies, the prevalence of *Blastocystis* spp. in diabetes patients has been reported to range from 2.7% to 65% (14,15). The positivity rates obtained also differed due to the use of different diagnostic methods, different study designs, the difference in the specificity/sensitivity of the diagnostic methods used, and the differences between the experiences of the laboratory personnel involved in the study. With the widespread use of molecular techniques in recent years, it has been shown that the prevalence of the parasite is much higher than expected (16). In our study, two samples that were negative by direct microscopy in the control group were also found to be positive by PCR.

Many studies comparing different diagnostic methods in the diagnosis of *Blastocystis* spp. Eida and Eida (17) showed that, when PCR was accepted as the gold standard, the sensitivity of direct microscopy was 62.2% and the specificity was 95.7% in the diagnosis of Blastocystis. In a similar study, Mohamed et al. (18) showed that 10% of 1330 stool samples had *Blastocystis* spp. by PCR, but only 6.5% were positive by direct microscopy. In a different study in our country, which also included molecular methods, the sensitivity of the direct microscopy method was reported as 12%, and the sensitivity of real-time PCR as 100%, and the high difference between them was remarkable (19). In our study sensitivity and specificity of PCR were found to be 100%. In comparison, the sensitivity of direct microscopy was 81.81% and the specificity was 100%, and these results were similar to other studies.

Blastocystis remains surrounded by many uncertainties, especially regarding its pathogenic potential. Nowadays, the most widely accepted opinion is that the clinical manifestations caused by *Blastocystis* are related to the parasite subtype, the host immune system, parasite density, intestinal microbiota and other accompanying pathogens. The evaluation should be made as a whole, guided by these data. It has been reported that it may be associated with abdominal pain, flatulence, diarrhea or constipation, nausea and anorexia in cases in which the parasite shows pathogenic features (4). In a study investigating symptoms in *Blastocystis* cases, the most common symptom was abdominal pain (40%), followed by bloating (36%) and vomiting (20%) (20). Dogan et al. (21) reported that no significant relationship exists between the presence and subtypes of *Blastocystis* and symptoms. In our study, abdominal pain was the most common (15%) in patients with *Blastocystis* spp., followed by diarrhea (10%). In addition, pruritus, nausea-vomiting and constipation were also seen in 5% of the cases. However, most patients were asymptomatic and no significant correlation was found between parasites and clinical findings (p>0.05).

In our study investigating the prevalence of *Blastocystis* and its role in gastrointestinal symptoms in diabetic patients, the difference between diabetic patients and the control group was not significant, and this result is similar to other studies. In addition, cases with *Blastocystis* and those that were not detected were statistically compared in terms of clinical findings, and no significant correlation was found with any of them. Since the number of subtypes was low, no statistical analysis was performed. The limitations of this study are that the sample size is small, the study period coincides with the COVID-19 pandemic, and a single stool sample can be obtained from the patients. However, our study is one of the few studies investigating the prevalence and clinical features of *Blastocystis* spp. in patients with type 2 DM.

Conclusion

In conclusion, *Blastocystis* spp. is a pathogen that should be considered in patients with diabetes. More comprehensive studies are needed in both diabetic and other immunocompromised patient groups.

Ethics

Ethics Committee Approval: All procedures performed in this study were approved by the Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine, with decision no 2017/1090 (date: 09.02.2017).

Informed Consent: Written informed consent was obtained from the volunteers before sample collection.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.B.Ö., S.E., Concept: Ş.B.Ö., H.E., S.E., Design: Ş.B.Ö., E.M., H.E., Data Collection or Processing: Ş.B.Ö., E.M., İ.Y., E.T., Analysis or Interpretation: Ş.B.Ö., E.M., İ.Y., E.T., H.E., S.E., Literature Search: Ş.B.Ö., E.M., İ.Y., E.T., H.E., Writing: Ş.B.Ö., H.E., S.E.

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

Financial Disclosure: This study was supported by the Scientific Research Projects Unit of Aydın Adnan Menderes University with the project number "TPF-18037".

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