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CONTENTS

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Original Article / Özgün Araştırma

Treatment Results of Dentigerous Cysts Managed by Marsupialisation, Enucleation or Enucleation with Platelet Rich Plasma-a Retrospective Study

Marsupiyalizasyon, Enükleasyon veya Enükleasyon ve Plateletten Zengin Plazma Uygulaması ile Tedavi Edilen Dentijeröz Kistlerin Tedavi Sonuçları: Retrospektif Bir Çalışma

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Keywords
Jaw cysts, dentigerous cysts, impacted tooth, enucleation, marsupialisation, platelet rich plasma

Anahtar Kelimeler
Çene kistleri, dentijeröz kistler, gömülü diş, enükleasyon, marsupiyalizasyon, plateletten zengin plazma

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Abstract

Objective: This study aimed to evaluate the effectiveness of dentigerous cyst treatment with enucleation, marsupialisation or enucleation with platelet rich plasma (PRP) application.

Materials and Methods: The study included 27 cases with dentigerous cysts. Demographic data, treatment modalities, treatment results, size of the bony defect before and after treatment and complications were analysed retrospectively. Patients were separated into three treatment arms. Group 1 comprised 11 patients treated with enucleation, group 2 comprised seven patients treated with enucleation and PRP and group 3 comprised nine patients treated with marsupialisation. Reductions in the size of the radiolucent area at the sixth month follow-up were compared in terms of the vertical and horizontal shrinkage of the lesion and monthly reductions in lesional area.

Results: The treatment results of the three groups were similar. Only the group to which PRP was applied revealed significant vertical shrinkage ($p=0.045$). Preoperative lesional area and age appeared to be factors significantly affecting bone regeneration ($p<0.05$).

Conclusion: Enucleation and marsupialisation are successful treatment modalities for dentigerous cysts. PRP application enhanced bone gain. Marsupialisation enhanced bone integrity and promoted the eruption of permanent teeth.

Öz

Amaç: Bu çalışmanın amacı marsupiyalizasyon, enükleasyon, enükleasyon ve plateletten zengin plazma (PRP) uygulaması ile tedavi edilen dentijeröz kistlerin tedavi sonuçlarının karşılaştırmalı değerlendirilmesidir.

Gereç ve Yöntemler: Dentijeröz kiste sahip 27 olgu çalışmaya dahil edildi. Demografik veriler, tedavi şekilleri ve sonuçları, tedavi öncesi ve sonrası kemik defektinin boyutsal ölçümleri ve komplikasyonlar retrospektif olarak analiz edildi. Hastalar 3 farklı tedavi grubuna ayrılarak incelendi. Grup 1 enükleasyonla tedavi edilen 11 hasta içerirken, grup 2 enükleasyon ve kist kavitesine PRP uygulanan 7 hastadan oluşmaktaydı. Üçüncü grup marsupiyalizasyon ile tedavi edilen 9 hasta içermekteydi. Altıncı ayda radyolusent alanda vertikal küçülme, horizontal küçülme ve lezyon alanında aylık küçülme olmak üzere üç parametre şeklinde incelendi.

Bulgular: Üç grubun tedavi sonuçları benzer çıkarken, PRP uygulanan grup istatistiksel açıdan anlamlı olarak daha hızlı vertikal küçülme sergiledi (p=0,045). Preoperatif lezyon alanı ve yaşın istatistiksel olarak anlamlı bir sonuçla kemik iyileşmesinde etkili bir faktör olduğu görüldü (p<0,05).
Sonuç: PRP kullanımı hem enükleasyon hem marsupiyalizasyon çene kistlerinin tedavisinde başarılı sonuçlar verirken kemik iyileşmesini hızlandırmaktadır. Kemik devamlılığının korunması, kiste eşlik eden daimi dişin sürdürülmesi marsupiyalizasyonla daha kolay sağlanmaktadır.

Introduction

Odontogenic cystic lesions grow slowly by resorbing the jaw bones. As the cysts enlarge, vital structures may have been damaged, facial asymmetry and displacement of teeth may have developed (1). Dentigerous cysts are the second most common type of odontogenic cysts. It develops around the crown of unerupted tooth caused by expansion of follicle epithelium of unerupted tooth with a well-defined, radiolucent area attached to tooth in cement-enamel junction. Treatment of a dentigerous cyst is dependent on the size of lesion, involved permanent teeth and proximity to anatomic structures (2).

Decompression, marsupialization and enucleation are main treatment modalities for jaw cysts. Enucleation is defined as a complete removal of cystic lining with intention of primary healing. Enucleation of cystic lesions can bring forth risks such as nerve injury or fracture of jaw. To avoid these complications, marsupialization or decompression is recommended to reduce the size of cyst, hence making it safe to enucleate the lesion (3,4). Marsupialization is a technique where a window is created in cystic wall and sutured to the oral mucosa. Decompression is based on a creation of a window between the cyst and oral cavity by fixing a kind of device. Marsupialization and decompression aim to release intraluminal pressure in cyst cavity and gradual decrease in lesion volume (5). Stents, acrylic plates or obturators are used for cyst shrinkage (6).

There are few studies that evaluate bone gain using panoramic views following cyst treatments (4,6-9). Lesion type, age and preoperative lesion area are investigated if these factors affect the bone gain while performing decompression (4,6,9,10). Researchers also focused on bone regeneration after cystectomy whom analysis were revealed with panaromic radiographs (11,12). Dentigerous cysts, unicystic ameloblastomas, keratocysts were predominantly evaluated to consider lesion shrinkage amount following decompression (4,6,13).

This retrospective study was conducted on cases with dentigerous cysts. The aim of this retrospective study was to compare the effectiveness of only enucleation, enucleation with platelet rich plasma (PRP) application and marsupialization in terms of reduction in cyst dimensions, analyzing the panoramic radiographs acquired pre and post-operatively. Furthermore, the study examined whether lesion shrinkage was related with age and initial lesion size.

Materials and Methods

A retrospective study was performed involving 27 patients with diagnosis of dentigerous cyst in jaw bones who presented to researcher's private practice from February 2017 to May 2019. Inclusion criterias were having histologically confirmed diagnosis of dentigerous cyst of longer diameter greater than 20 mm with minimum six months follow-up. Moderate to heavy smokers and medically unfitted patients were excluded.

Histopathological examinations were made after clinical and radiological examination. Histopathological examination revealed lesion with stratified non-keratinized epithelial lining present with a fibrous connective tissue wall mildly infiltrated with inflammatory cells (Figure 1).

Clinical, histopathological, radiological and demographic datas were collected. All ortopantomographies (OPGs) were obtained using the same machine (Promax, Planmeca, Helsinki, Finland).

Different from formula described by Gao et al. (6) the size of cystic lesion was calculated as area of elips before and after treatment on OPG. A standard lesion area was defined as

$$Lesion\ area = \pi \frac{\text{maximal vertical length (mm)}}{2} \frac{\text{maximal horizontal length (mm)}}{2}$$

The relative shrinkage size after treatment was measured as the lesion area before treatment minus the lesion area after six months treatment. The relative

speed of shrinkage of a cystic lesion was calculated as follows:

$$shrinkage\ of\ lesion = \frac{\text{the lesion area before treatment} - \text{the lesion area six months after treatment}}{\text{duration of treatment (6 months)}}$$

Ratio of vertical shrinkage (%) was calculated as

$$ratio\ of\ vertical\ shrinkage = 100\ x\ \frac{\text{preoperative vertical length} - \text{post-operative vertical length after six months}}{\text{preoperative vertical length}}$$

Ratio of horizontal shrinkage (%) was calculated as

$$ratio\ of\ horizontal\ shrinkage = 100\ x\ \frac{\text{preoperative horizontal length} - \text{postoperative horizontal length after six months}}{\text{preoperative horizontal length}}$$

Three different treatment protocols have been compared with each other in terms of vertical shrinkage (%), horizontal shrinkage (%) and monthly reduction rate of cystic lesion area (relative speed of shrinkage) (mm^2/month) on OPGs. Preoperative and six months post-operative OPGs of all patients were considered to calculate lesion shrinkage as described in math formulas above. With the aid of statistics we studied whether vertical shrinkage (%), horizontal shrinkage (%) or relative speed of shrinkage (mm^2/month) had a meaningful correlation with the host factors such as the age of the patient and the initial size of the lesion or treatment modalities.

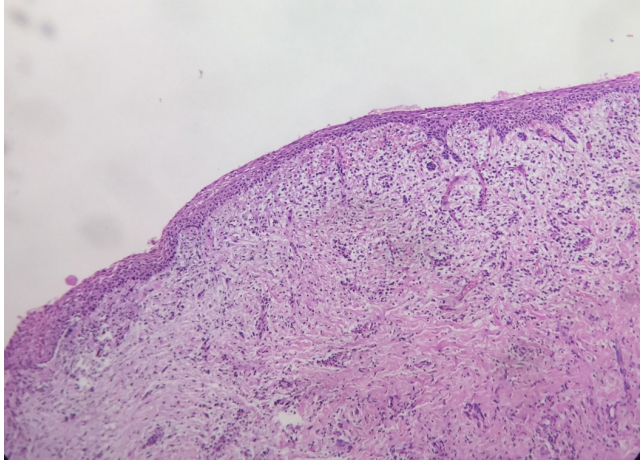

Figure 1. All patients were diagnosed histopathologically. The epitelium was stratified squamous non-keratinized and the subepitelial area showed lenfo-plasmosit infiltrate of chronic type (HEx400)

Treatment Protocol

Twenty-seven patients with dentigerous cysts were included. All patients signed informed consents before the interventions. This study follows the Declaration of Helsinki. The Ethical Committee of University of İzmir Katip Çelebi approved the study (decision no: 792, date: 02.07.2020). The patients seperated into three treatment arms. Group 1 comprised 11 patients treated with enucleation while group 2 comprised 7 patients treated with enucleation and PRP application. Group 3 comprised 9 patients treated with marsupialization.

Cysts that cause nasal passage obstruction, relatively smaller sized cysts and cysts accompanied by teeth to be extracted were treated with enucleation. Seven patients who were candidates for implant treatment after cyst treatment and close neighborhood to vital structures or roots of teeth were treated with enucleation with PRP application. Larger cysts or cysts that involved permanent teeth to be erupt or anatomic structures were treated with marsupialization. All patients have had panoramic radiographs preoperatively and at post-operative 6th month (Figure 2).

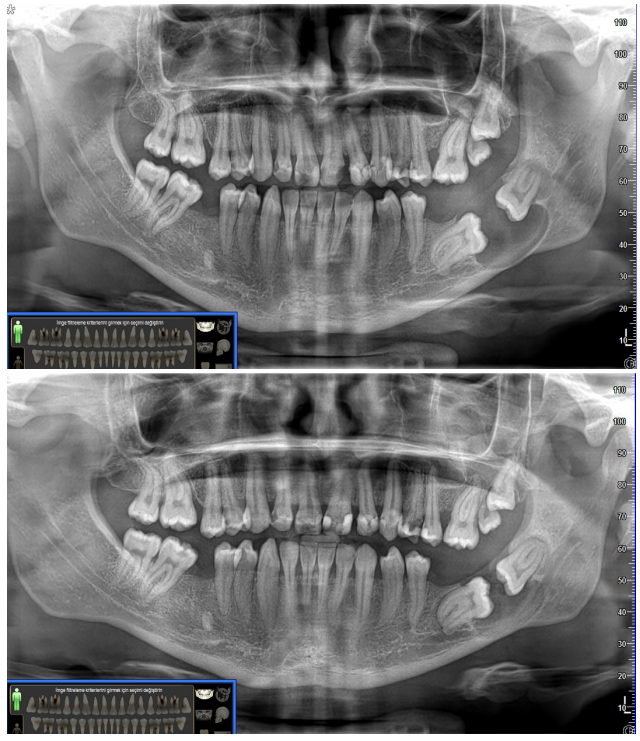

Figure 2. Dentigerous cyst related with kissing molars. A panoramic X-ray taken before marsupialization (a) a panoramic radiograph at 6th month follow up, showing shrinkage of bone cavity

All treatments were performed under local anesthesia by the same experienced surgeon. Following a careful preparation of a full thickness flap without a perforation or a rupture that may affect the bone healing period, cysts and accompanying teeth were totally removed for group 1.

PRP that derived from the centrifugation of patient's own blood was prepared for group 2. Venous blood was withdrawn from patients and collected in sterile vacuum tube coated with anticoagulant citric acid and dextrose. After first centrifugation under 800 times gravity for 5 minutes, blood was separated into plasma and red blood cells. The red blood cells were removed, and then remaining plasma underwent second centrifugation under 1,500 times gravity for 5 minutes. The bottom layer which was defined as PRP was collected and mixed with calcium chloride to obtain gel form of PRP to facilitate application to cyst cavity. After enucleation of the lesion, PRP was applied to bone cavity before suturation in group 2. Enucleated cystic lesions were examined histopathologically to confirm clinical diagnosis.

Histopathological analyses were performed with incisional biopsies of cysts at the time of marsupialization for group 3. Cystic lesion opening was sutured to obtain a marsupialization hole after biopsy procedure. Furthermore, an acrylic obturator was planned to keep the lesion uncovered and to avoid food impaction. Dental impressions were taken with silicone and casts were fabricated. Customized Hawley like acrylic plates were fabricated and applied to patients' mouth on seventh day. Until the acrylic obturator was applied to mouth, marsupialization hole was preserved with daily exchanged gauze covered with topical antibiotic pomade. After application of acrylic obturator to mouth, the patients were instructed to maintain overall proper hygiene of the oral cavity through self-irrigation of cavity every day. Cyst openings were prepared like marsupialization hole unlikely to minimal sized decompression holes. However, in our cases we have applied intraoral devices to protect food trap in the cavity and preserve the cyst opening like decompression procedure. Therefore, the marsupialization procedure which we applied to our patients has similarities to decompression procedure. The patients were scheduled for weekly follow-up to provide hygiene of the cyst cavity (Figure 3).

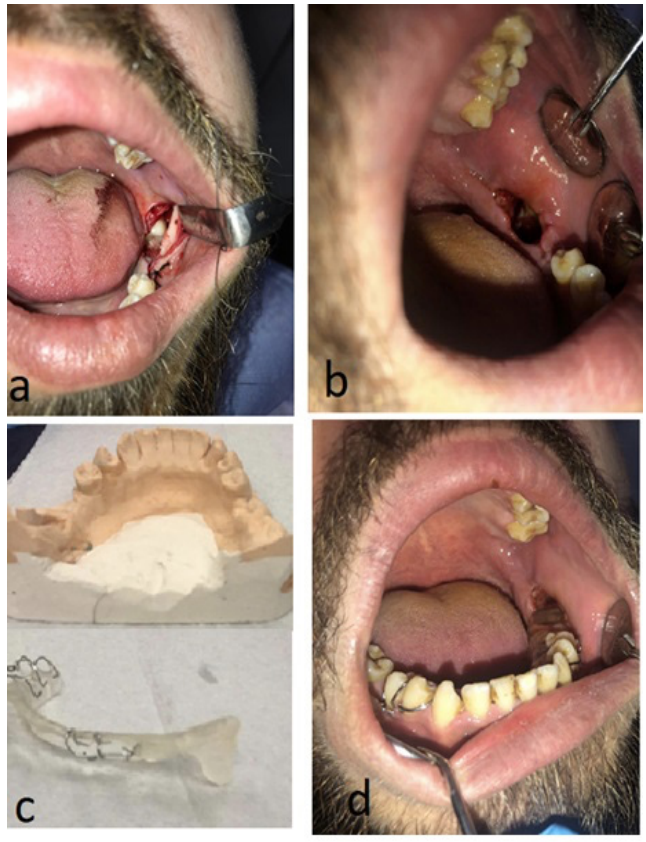

Figure 3. Patient with dentigerous cyst involving kissing molar has been treated with marsupialization. Opening of the cyst and biopsy sampling. [A hole (arrow) was created in bone nearby the window hence mobile soft tissues have been sutured to this hole]. (a) Marsupialization hole was ready a week later, (b) customized Hawley like acrylic obturator, (c) acrylic plate in mouth of patient avoiding trapping of food in the cyst cavity

Statistical Analysis

Data were recorded using Excel (Microsoft, Redmond, WA) and analysed using SPSS 13.0 (SPSS, Inc, Chicago, IL). Data showed normal distribution according to Kolmogorov-Smirnov and Shapiro-Wilk therefore parametric tests were appropriate for our study. One-Way ANOVA and Pearson correlation analysis were performed. The results were considered significant at a p-value lower than 0.05.

Results

Twenty-three male and 4 female patients with histopathologically confirmed dentigerous cysts whose ages range between 6 and 71 years (mean: 33.6±12.1) were included.

All groups demonstrated significant healing changes over the 6 months of follow-up. The Paired t-test showed a significant difference in terms of vertical length, horizontal length and area of the cyst cavity comparing measurements of before and after treatment on OPG (Table 1). The mean relative speeds of shrinkage (mm^2/month) of group 1, group 2 and group 3 were 28.8 mm^2/month, 50.9 mm^2/month, 44.9 mm^2/month, respectively. There was no significant difference among the 3 treatment modalities. Velocity of horizontal shrinkage of group 1, group 2 and group 3 were 32%, 50%, 43%, respectively. There was no significant difference among the 3 treatment modalities regarding to ratio of horizontal shrinkage. Ratio of vertical shrinkage of group 1, group 2 and group 3 were 37%, 57%, 54%, respectively. There was a significant difference between group 1 and group 2 (Table 2).

Some of the host factors seemed to contribute to process of bone healing. Ratio of vertical shrinkage was significantly associated with the age of the patient (Table 3). When analyzing the preoperative lesion area, there was a statistically significant correlation with monthly reduction rate (Table 4).

All patients showed good bone healing without any wound dehiscence, although we face with some complications. Three temporary paresthesias were noted in enucleation group that continued for three weeks to three months. Thirteen of cases were originated by third molar teeth. Eight of cases were related with maxillary canine teeth, and remaining cysts of 6 cases were caused by mandibular premolars.

Table 1. Significant difference between preoperative and six months post-operative lesion dimesions in three treatment groups were noted

	Preoperative Vertical length (mm) (mean ± SD)	Vertical length at six months (mm) (mean ± SD)	p	Preoperative horizontal length (mm) (mean ± SD)	Horizontal length at six months (mm) (mean ± SD)	p	Preoperative lesion area (mm^2) (mean ± SD)	Lesion area at six months (mm^2) (mean ± SD)	p
Group 1	19.8±2.33	12.6±2.08	0.000	22.2±1.47	15.3	0.000	356.7±55.59	171.4±40.56	0.000
Group 2	16.9±2.13	7±1.16	0.001	32.3±5.16	16.6	0.000	473.9±128.84	158.4±47.58	0.011
Group 3	23.3±3.80	11.3±3.18	0.000	27.8±7.40	16.3	0.002	562±193.43	213.6±100.89	0.007

Group 1 cases treated with enucleation. Group 2 cases treated with enucleation and platelet rich plasma application. Group 3 cases treated with marsupialization. SD: Standard deviation

Table 2. Different treatment groups were compared in terms of monthly reduction in cyst area, ratio of vertical shrinkage and ratio of horizontal shrinkage of cyst dimensions

	Group 1		Group 2		Group 3		
	n	Mean	n	Mean	n	Mean	p
Montly reduction in cyst area (mm^2/month)	11	28.8	7	50.9	9	44.9	0.172
Ratio of vertical shrinkage (%)	11	37%a	7	57b%	9	54ab%	0.045
Ratio of horizontal shrinkage (%)	11	32%	7	50%	9	43%	0.215

Group 1 cases treated with enucleation. Group 2 cases treated with enucleation and platelet rich plasma application. Group 3 cases treated with marsupialization. a, b, ab The difference was significant between a and b

Table 3. Relation between age and shrinkage of cyst cavity in three different parameters

		Montly reduction in cyst area (mm^2/month)	Ratio of vertical shrinkage (%)	Ratio of horizontal shrinkage (%)
Age	r	0.192	-0.422	-0.336
	p	0.337	0.028	0.086

Group 1 cases treated with enucleation. Group 2 cases treated with enucleation and PRP application. Group 3 cases treated with marsupialization

Table 4. Preoperative lesion area had a significant correlation with cyst shrinkage		
		Montly reduction in cyst area (mm²/month)
Preoperatif lesion area (mm²)	Pearson correlation	0.779**
	Significant (2-tailed)	0.000
	n	27
**There was a 78% correlation ratio between monthly shrinkage and preoperative lesion area		

Sixteen patients revealed complete healing in bone defect during follow up period while the others are ongoing shrinking phase. Also, 5 children with dentigerous cyst related with permanent teeth treated with marsupialization were evaluated in that study. The permanent teeth have given a good response to marsupialization and come towards dental arch.

Discussion

This study evaluated the healing in dentigerous cysts of jaws to substantiate our understanding of bone healing after enucleation or marsupialization with analysis of OPGs and discussed the indications, treatment results and host factors that affect the bone healing such as age and preoperative cyst size. The patients with odontogenic keratocysts accompanied with or without Gorlin Goltz syndrome, glandular odontogenic cysts, pseudocyts and inflammatory cysts were not included to study. Treatment modalities and growth patterns of pseudocysts are completely different from traditional jaw cysts. Developmental jaw cysts such as keratocysts or glandular odontogenic cysts have an aggressive nature that could affect study results and cause bias of results. Inflammatory jaw cysts such as radicular cyst have an infectious origin that may trigger re-infection during healing period after cyst treatment or apical resections. Therefore, inflammatory cysts could not show a standard healing process due to risk of re-infection. As a result, dentigerous cysts were chosen for this study to understand affective factors on bone healing, since they have been expected to show a standardized healing process.

Enucleation of lesion is an aggressive and fast technique however; it has the risk of compromising important structures nearby. In such cases marsupialization is an alternative that allow the shrinkage of lesion before removal. In both methods, bone gain has occurred. With regard to bone gain after enucleation, different authors have given bone regeneration ratios in cyst cavities at various time periods. Ihan Hren and Miljavec (11) reported 7%, 27% and 46 % bone gain in large bone defects after 2, 6 and 12 months, respectively. Chacko et al. (7) reported 25.8%, 57% and 81% average reduction in size of cysts at post-operative 6 months, 9 months and 12 months, respectively. Chiapasco et al. (14) evaluated both reduction in size and increase in bone density by computed analysis of panoramic radiographs after enucleation of 27 jaw cysts. Mean reduction of cyst cavities were 12%, 43.5% and 81.3% after 6, 12 and 24 months, respectively. In our study, mean vertical reduction in size was 37% for patients treated with enucleation while mean horizontal reduction in size was 32% at six months follow up. Bone gain at six months follow-up in our patient population was found slightly higher than that of Ihan Hren and Miljavec's (11) and Chiapasco et al.'s (14) studies. Inconsistency between the results caused by difference between measurement techniques as we evaluated the bony healing with different math formulas. Furthermore, we included patients with only dentigerous cysts; previous studies evaluated inflammatory cysts, keratocysts and even tumoral lesion defects. Lesion type was reported as an affective factor on bone healing (9). Ihan Hren and Miljavec (11) noted that radical approaches to aggressive lesions resulted with removal of cyst walls with part of the surrounding bone wall and lack of bone wall around the defect after operation. However, cystic cavities in our study were surrounded by bone except the most conservative hole that was prepared by surgeon.

The patients who were treated with PRP application after enucleation showed better reduction in cyst cavity in which vertical shrinkage was significantly different from group 1 and 2. Although, conflicting results were reported about efficacy of PRP on bone regeneration, the use of PRP was reported to have enhanced bone regeneration of

jaw bone defects such as cleft defects, cyst cavities or segmental defects (15-18). Special attention should be paid to preservation of periosteum which has a critical importance for spontaneous ossification (19). Stabilization of blood clot is another important factor for spontaneous bone regeneration (20). In our study, all cysts were surrounded by bone except the smallest window as possible that was prepared for enucleation during surgery in group 1 and group 2.

Studies investigating the bone gain with marsupialization or decompression have been published for several times. Articles reporting affective factors on bone gain in jaw cysts were identified via literature search. Various factors such as lesion type, initial lesion area, age of patient and defect configuration have been investigated that have probability to influence effectiveness of treatment (4,6,9,10,21,22). In our study, we have mainly compared different treatment modalities regarding bone gain. Only PRP applied enucleation group showed significantly better healing in term of vertical shrinkage of lesion while patient groups who were treated with only enucleation or marsupialization showed similar bone gain. Also monthly reduction rates of three groups have not showed statistically significant difference. There is limited data of that compares results of different treatment modalities, enucleation and marsupialization on bone gain.

Preoperative lesion area is statistically significant factor in bone healing according to our study. Anavi et al. (10) investigated relationship between lesion shrinkage and preoperative lesion size in a population of 57 cases with dentigerous cyst, keratocyst, radicular cyst and glandular odontogenic cyst who were treated with decompression and resulted that larger lesions showed faster shrinkage. Other studies have also supported that initial lesion area is a significantly affective factor on bone healing period (6,9,10,22). Age was a significant factor on the vertical shrinkage while correlation values were not statistically significant regarding horizontal shrinkage and monthly reduction rate of cyst lesion in our study. Age was reported as an affective factor on bone healing following jaw cyst treatment in some studies (4,6,10). In contrary, age was reported not to have a significant effect on bone healing by some researchers (9,21,22). Lesion type has been reported as an ineffective factor on bone gain by several researchers. Keratocyst, radicular cyst, dentigerous cyst, unicystic ameloblastoma showed similar speed of bone regeneration during marsupialization (4,6,10). In contrary, some studies claimed that the lesion type affect the bone healing (9,22). In our study, we included the cases of dentigerous cysts to evaluate the effect of treatment modalities on bone gain. Dentigerous cyst is defined as a developmental jaw cyst associated with a crown of unerupted or impacted tooth. Mandibular third molars, maxillary canines, mandibular premolars are involved teeth by dentigerous cysts (2). In our study, 14 impacted mandibular third molar (one case with kissing molar), 8 maxillary canines, 6 mandibular premolars with dentigerous cysts (total 28 teeth in 27 cases) were included.

Many researchers have attempted grafting cyst cavities with autogenous bone or bone substitutes. Bicsak et al. (23) supported ß-tricalcium phosphate application to cyst cavities since it is claimed to facilitate the bone healing. Autologous grafting after enucleation accelerated defect ossification. Missing of cortical borders or defects with periosteal covering are leading factors for using additional grafts (20). In our study even large cyctic lesions that are included to study have intact cortical walls, even though the cyst walls thin out by cyst pressure. Therefore additional bone grafts have not been preferred. Instead of grafts, PRP that could be easily obtained from patient's blood without extra charge was used in group 2. Nagaveni et al. (16) supported PRP application to cyst cavities since it has been claimed to have significant effect on bone healing of 10 patients with jaw cyst. Subramaniam et al. (24) have used PRP in traumatic bone cyst cavity. PRP related studies were predominantly run-on animal models and have been used with graft materials in cyst cavities of human beings (24-28). Pappalardo and Guarnieri (27) reported histological analysis of cyst cavities augmented with PRP and bovine xenograft and concluded that this combination served promoted bone regeneration. Although, some researchers have shown no benefit of PRP on bone formation (15,26), some reports have confirmed the effectiveness of PRP in enhancing bone regeneration. (29,30). PRP was observed to accelerate bone healing in term of vertical shrinkage in cyst cavity according to our study.

Rahpeyma and Khajehahmadi (31) have reported indications and results of marsupialization in jaw

cysts. Large cysts, cysts with permanent tooth to be erupted were good candidates to marsupialization. Ugurlu et al. (32) performed decompression for 34 patients with dentigerous cysts related with permanent teeth and reported successful results. In our study, the patients with the similar indications were treated with marsupialization, as a result neurovascular bundle had been protected, tooth loss had been reduced, and permanent teeth come closer to dental arch. Although, permanent teeth that are located in cyst cavity come to a better position with marsupialization, further orthodontic treatment may be required (32). Customized thermoplastic resin stents (6), serum sets, dentures, acrylic devices (31), syringe needle cover (13), decompression stent (33) were reported as marsupialization equipment. In our study, we have used Hawley like acrylic plate which offer stable and comfortable equipment in the mouth of the patient. Requirement of patient compliance to device in the mouth is a drawback of the marsupialization technique.

OPGs have been used to follow the healing of jaw cysts and measure the efficacy of treatments by many researchers (6,7,9-11,13,14). In our study, we have used OPGs to measure reduction in size of cyst cavities in three different parameters; vertical diameter, horizontal diameter and the area of cyst. We calculate the area of cyst using the formula of area of ellipsoid. Gao et al. (6) used a different way to calculate the cyst area (vertical diameter × horizontal diameter). Since cysts have ellipsoidal shape, our calculation method gives more precise results. Although, OPG is widely used tool in that area, it has some drawbacks as it is a kind of two dimentional imaging. There are limited studies which used computed tomographies or cone beam computed tomographies and calculated the volume of lesion in three dimensions (21).

Conclusion

The present study showed that enucleation and marsupialization have given similar bone regeneration speed while PRP application has accelerated the bone healing. Although, retrospective nature of this study, standardization of the procedures in terms of treatment protocols, imaging, measurements, follow up periods that was applied to the patients included makes our results valuable. This study is one of the rare studies that compare marsupialization and enucleation. However, controlled prospective studies on larger population are needed to understand effect of treatment methods on bone regeneration better.

Ethics

Ethics Committee Approval: The Ethical Committee of University of İzmir Katip Çelebi approved the study (decision no: 792, date: 02.07.2020).

Informed Consent: All patients signed informed consents before the interventions.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.D., Design: E.D., Supervision: E.D., Fundings: E.D., Materials: E.D., Ö.G., Data Collection or Processing: E.D., Ö.G., Analysis or Interpretation: E.D., Ö.G., Literature Search: E.D., Writing: E.D., Critical Review: E.D., Ö.G.

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

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Original Article / Özgün Araştırma

Evaluating Well-being Among Dental Students Using the Warwick-Edinburgh Mental Well-being Scale and the Relationship Between Mental Well-being and Socio-demographic Findings

Warwick-Edinburgh Mental İyi Oluş Ölçeği ile Diş Hekimliği Öğrencilerinde İyi Oluşun Değerlendirilmesi ve Mental İyi Oluş ile Sosyo-demografik Bulgular Arasındaki İlişki

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Keywords
Dental education, dental students, mental well-being, questionnaire, Warwick-Edinburgh Mental Well-Being Scale

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Abstract

Objective: The present study aimed to measure mental well-being among dental students using the Warwick-Edinburgh Mental Well-being Scale (WEMWBS) and to investigate factors affecting well-being.

Materials and Methods: Başkent University Faculty of Dentistry students were invited to participate in the study in 2019. The participants were asked to complete the WEMWBS and a questionnaire describing their demographic details, social background and lifestyle habits.

Results: A total of 268 dental students (68% females) participated in the survey, with a response rate of 85%. The overall WEMWBS score of the study population was 48.18±9.6. Statistically significant differences in WEMWBS scores were detected between first- and fourth-year ($p<0.001$) students and between fourth- and fifth-year students ($p<0.001$). Male students ($p<0.001$) whose first choice was to attend the school of dentistry ($p=0.024$) and who pursued hobbies ($p=0.001$) or regular sports activities ($p=0.001$) had significantly higher WEMWBS scores than those who did not. Dental students' smoking status ($p=0.630$), living accommodation ($p=0.71$) or parents' education level did not affect WEMWBS scores significantly.

Conclusion: The mental well-being of dental students was influenced by their year of study, gender, first choice of school and pursuit of hobbies or regular sports activities. Factors that may affect the mental well-being of students should be carefully considered, and strategies should be implemented to monitor and promote well-being during the students' academic career.

Öz

Amaç: Bu çalışmada, Warwick-Edinburgh Mental İyi Oluş Ölçeği (WEMİOÖ) kullanılarak diş hekimliği öğrencilerinin mental iyi oluşunu ölçmek ve iyi oluşu etkileyen faktörleri incelemek amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmaya Başkent Üniversitesi Diş Hekimliği Fakültesi'nde öğrenim gören öğrenciler dahil edilmiştir. Katılımcılara WEMİOÖ ile demografik detaylar, sosyal geçmiş ve yaşam tarzı alışkanlıklarını içeren bir anket uygulanmıştır.

Bulgular: Çalışmaya 268 (%68 kadın) diş hekimliği öğrencisi katılmıştır. Çalışma popülasyonunun genel WEMİOÖ skoru 48,18±9,6 olarak bulunmuştur. Birinci ve dördüncü sınıf öğrencileri (p<0,001) ile dördüncü ve beşinci sınıf öğrencilerinin WEMİOÖ skorları (p<0,001) arasında istatistiksel olarak anlamlı farklılıklar tespit edilmemiştir. Erkek öğrencilerin (p<0,001), ilk tercihleri diş hekimliği fakültesine gitmek olan (p=0,024) ve hobileri (p=0,001) veya düzenli spor alışkanlıkları olan (p=0,001) öğrencilerin WEMİOÖ skorları daha yüksek bulunmuştur. WEMİOÖ skorları açısından diş hekimliği öğrencilerinin sigara içme (p=0,630), barınma (p=0,71) veya ebeveynlerin öğrenim durumları açısından anlamlı bir farklılık bulunmamıştır.
Sonuç: Mevcut sonuçlar, diş hekimliği öğrencilerinin mental sağlıklarının fakültedeki öğrenim yılı, ilk tercihlerinin diş hekimliği fakültesi olması, cinsiyet, hobi veya düzenli spor alışkanlıklarının varlığından etkilendiğini göstermiştir. Öğrencilerin mental sağlıklarını etkileyebilecek faktörlerin iyi değerlendirilmesi ve akademik kariyerleri boyunca refahlarını izlemek ve geliştirmek için stratejilerin mevcut olması önemlidir.

Introduction

The significance of recent studies focused on positive mental health and its influence on our lives has elevated greatly. Positive mental health has been characterized as a condition that enables people to comprehend their capabilities including overcoming the everyday life pressure, being productive and participating in the society (1). In policy and academic literature, the term positive mental health is in accordance with the term mental well-being, in which both have impacts on psychological functioning in terms of hedonic and eudaemonic aspects. Personal understanding of happiness and life satisfaction constitute the core of hedonism, whereas psychological functioning and self-realization widely refer to eudaimonism (2).

Previous studies have shown a strong link between mental and physical health (3,4). In addition, it was reported that individuals having elevated levels of mental well-being had higher creativity levels, improved immune systems, better relationships with other people; in addition to being more productive at work and living longer (5).

In order to determine the well-beings, either mental or psychological, there were widely accepted and commonly used appliances. Among those, Scale of Psychological Well-Being (6), the Satisfaction with Life Scale (7), PANAS Scale (8) and Short Depression-happiness Scale (9) evaluated the mental well-being by addressing to either hedonic or eudaemonic perspectives, but not both. Therefore, a new scale, which assessed the entire concept including hedonic and eudaemonic perspectives, was needed to be proposed. For this reason, researchers in the United Kingdom (UK) introduced Warwick-Edinburgh Mental Well-Being Scale (WEMWBS) (10).

WEMWBS has been frequently used in studies as a convenient tool to score mental well-being (11,12). This scale has been translated into numerous languages such as Norwegian, Swedish, Italian, Dutch, German, French and Spanish and has been validated (13-15). Validity and reliability studies of the Turkish version were performed in 2015 and it was concluded that the Turkish WEMWBS had a single factor structure and could be constituted as a valid and reliable research instrument in order to evaluate mental well-being (16).

Stressful occupations and work environments are both known to have negative influences on health, especially the mental health. It has been shown that university students suffer from psychological problems due to educational stress that are usually faced while trying to satisfy ascending demands from the university (17). This is particularly the case for students who study medical care fields since they have to cope with increased hours of work, tiring workload, and challenging theoretical education (18). In addition, dental students are required to obtain training in practical education which includes laboratory requirements in the preclinical years and clinical requirements in the clinical parts of their training. All of the aforementioned factors combine to cause a significant level of stress in dental students (19). Therefore, dental education is considered to be one of the most stressful health fields (20) and this led researchers to focus on examining the stress sources and psychological well-being among dental students in the last decades. In order to investigate the sources and levels of stress that these students experience, multiple researches have been performed in different dental schools globally. Surveys with the Dental Environment Stress (DES) questionnaire have been

used in great amount of studies, which were also conducted with dental students and demonstrated that they have been suffering increased levels of stress during the ongoing training (19,21). Although there are several studies in the literature that evaluate the mental well-being of dental students (22-24), few studies used WEMWBS (25,26). Therefore, this study is conducted to measure the mental well-being of dental students by WEMWBS and investigate the factors that affect their well-beings.

Materials and Methods

This cross-sectional study was carried out by first to fifth year dental students of Başkent University. The study protocol was approved by Institutional Review Board and Ethics Committee of Başkent University (decision no: 19/97, date: 18.09.2019). This study was registered under the Clinical Trials protocol registration system (NCT04100928).

The survey was scheduled in a way that the assessment stage of the students would be independent. The participants were chosen among the students who attended a lecture together. By the end of the lecture, a researcher has informed the students about the aim of this voluntary survey and has also addressed the confidentiality issues, in which all identities would remain anonymous. The questionnaires were handed to the volunteered students. The questionnaire consisted of demographical details (age, gender, year of study), social background (parents' education levels, first choice for admission, living accommodation) and lifestyle habits (smoking status, presence of regular sports activities and hobbies). In addition, WEMWBS was applied to the participants of this study.

In a broad population of UK WEMWBS has been validated, where it was also introduced initially (10). In 2015, it has been translated into Turkish and validation process has been accomplished. The internal consistency coefficient and combined reliability coefficient of the scale were determined as 0.92 (16). Total of 14 features that address hedonic, as well as eudaemonic perspectives of mental health were found in this self-administered scale. These perspectives included positive influences such as optimism, happiness, enjoyment, relaxation; accomplishment of interpersonal contacts and positive actions such as energy, clear mind. Participants were asked to mark the boxes that describe 5-point Likert scale (none of the time, rarely, some of the time, often, all of the time) based on their situations of past 2 weeks. Giving a score of 1 to 5, the minimum of 14 and maximum of 70 points could be acquired from the Likert scale. The features were scored in positive manner and all of them had equal weights. The overall grade was calculated by adding all scores. Thus, higher score represented elevated level of mental well-being and vice versa. The demographical, socio-economical and smoking information of participants were also obtained.

Statistical Analysis

Data analysis was performed using SPSS for Windows, version 21 (SPSS Inc., Chicago, IL, United States). The data were analyzed for normality of distribution with Shapiro-Wilk test. Mann-Whitney U test was used for two group comparisons (gender, sport habit, presence of a hobby and whether the dentistry was their first choice). The Kruskal-Wallis test was used to compare more than two independent groups (overall WEMWBS scores in the five years of the dental program, living accommodation, education level of the parents, smoking status). When the p-value of the Kruskal-Wallis test was statistically significant, Bonferroni adjusted Mann-Whitney U test was used for multiple post-hoc comparisons. Cronbach's alpha coefficients were computed to determine the reliability of the WEMWBS.

Results

Table 1 provides the detailed demographics of study population. A total of 268 dental students (68% female) participated, with a response rate of 85%; among them 183 (68%) were female, while 85 (32%) were male students. The age range was 18 to 25 years with mean age of 21.11±1.77 years. The majority of the students (89.1%) reported that dentistry was their first career choice. 48% of participants were living with their parents and 186 (69.4%) students were non-smokers.

The overall WEMWBS scores and WEMWBS scores according to the year of study are summarized in Table 2. Based on the reliability analysis, the internal consistency (Cronbach's alpha) was 0.90 for the WEMWBS. The overall WEMWBS score of the study

Table 1. Demographics of study population and response rate by year of study

Year of study	n	Gender		Response rate by year (%)	Mean age
		Female	Male		
1	69	41	28	66.3	19.45
2	63	44	19	94	20.48
3	56	46	10	93.3	21.37
4	35	22	13	100	22.35
5	45	30	15	95.7	23.27
Total	268	183	85	85	21.11

N: Number

population was 48.18±9.6. When the WEMWBS scores were analyzed by the year of the study, statistically significant difference was detected. The WEMWBS scores were highest in first year students (51.1±7.21) and lowest in 4th year students (42.47±10.33). The difference was statistically significant between year 1 and year 4 ($p<0.001$); along with year 4 and year 5 ($p<0.001$).

Table 3 illustrates the role of demographical details, social background and lifestyle habits on overall WEMWBS scores. Statistically significant differences were observed when overall WEMWBS scores were compared by gender. Female dental students (46.4±9.4) exhibited lower WEMWBS scores compared to their male (52.12) counterparts ($p<0.001$). Dental students who reported that dentistry was their first career choice had higher WEMWBS scores ($p=0.024$). This trend could also be observed in case of sports activities and presence of hobbies. Significantly higher WEMWBS scores were acquired among students who had hobbies (WEMWBS scores: 50.11±9.8 versus 46.1±8.9, $p<0.001$) or regular sports activities (WEMWBS scores: 49.9±9.4 versus 46.6±9.6, $p=0.01$). WEMWBS scores did not differ regarding to dental students' smoking status ($p=0.630$), living accommodation ($p=0.710$), or educational levels of the parents ($p=0.522$ and 0.669 for mothers and fathers, respectively).

Discussion

The aim of this study was to assess the mental well-being of dental students using WEMWBS. In addition, factors with potential influences on dental student's well-being were investigated in the present study. Our research included 268 dental students and the overall WEMWBS score of this population was 48.18±9.6. In this study, we obtained a response rate of 85% from the students enrolled. Moreover, Cronbach's alpha coefficients for WEMWBS demonstrated very high reliability. Thus, we acknowledge that our findings clearly represent the ongoing conditions of dental students of Başkent University Faculty of Dentistry.

There are many studies in the literature evaluating mental well-being with different scales (10,22,23,27,28). Among these, WEMWBS was firstly developed by Tennant et al. (10) in 2007 and it was validated on a student and representative population sample. The median score was found to be 50 in the student sample and 51 in the population sample. Similarly, WEMWBS was used to assess mental well-being in Northern Ireland by analyzing the responses from 3,355 people of the general population, aged 16 years and over. Parallel with the results of Tennant et al. (10), the overall median score for the WEMWBS in this sample was 50 (29). In a recent research mental well-being in Denmark was measured and cross-cultural comparison was made through four European settings. The results of this study manifested that mean scores were 52.2±8.7 in Denmark. When a comparison was made through four European settings, the highest overall mental well-being scores were reported to be that of Catalonia (58.1), which was then followed by Denmark, Iceland and England (49.8) (12). A cross-sectional survey that was performed on doctors, nurses, physiotherapists, pharmacists and dentists to measure their mental well-being has demonstrated that the mean score of this population was 48.1±9.4, which was comparable with the present study (30).

Table 2. Respondents' overall WEMWBS scores and WEMWBS scores by year of study

Variable	1st year		2nd year		3rd year		4th year		5th year		Overall		Difference between years
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
1st item	3.75±0.85	4.00	3.2±1.11	3.00	3.39±0.90	3.50	3.25±1.18	3.00	3.55±0.84	4.00	3.45±0.99	4.00	0.028 (1>2)
2nd item	3.86±0.78	4.00	3.5±0.87	3.00	3.71±0.86	4.00	3.36±1.11	4.00	3.84±0.73	4.00	3.67±0.89	4.00	NS
3rd item	2.85±1.12	3.00	2.46±1.17	2.00	2.72±1.04	3.00	2.05±0.96	2.00	2.62±1.11	2.00	2.59±1.11	3.00	0.011(1-3>4)
4th item	3.58±0.98	4.00	3.55±1.07	4.00	3.17±0.97	3.00	2.72±1.05	3.00	3.66±1.00	4.00	3.39±1.05	4.00	0.001(1-2-5>4)
5th item	3.26±1.07	3	2.87±1.30	3.00	2.69±1.18	3.00	2.27±1.03	2.00	3.33±1.12	3.00	2.93±1.20	3.00	0.001 (1-5>4)
6th item	3.39±0.75	3.00	3.12±1.12	3.00	3.25±0.74	3.00	2.83±1.08	3.00	3.55±1.07	4.00	3.25±0.97	3.00	NS
7th item	3.8±0.89	4.00	3.52±1.13	4.00	3.67±0.87	4.00	3.38±1.04	3.5	3.75±1.04	4.00	3.65±1.00	4.00	NS
8th item	3.76±0.86	4.00	3.44±1.21	4.00	3.48±1.04	4.00	3.41±1.33	4.00	3.75±1.09	4.00	3.58±1.10	4.00	NS
9th item	3.47±0.95	4.00	3.41±0.96	4.00	2.98±1.08	3.00	2.77±1.73	3.00	3.55±1.13	4.00	3.27±1.07	3.00	0.001 (1-5>4)
10th item	3.94±0.78	4.00	3.73±0.97	4.00	3.82±0.85	4.00	3.48±1.12	4.00	3.80±0.99	4.00	3.78±0.93	4.00	NS
11th item	4.29±0.59	4.00	4.15±0.78	4.00	4.14±0.77	4.00	3.75±0.90	4.00	4.20±0.81	4.00	4.14±0.77	4.00	NS
12th item	4.01±0.87	4.00	3.84±0.86	4.00	3.98±0.67	4.00	3.63±1.12	4.00	3.93±0.91	4.00	3.90±0.88	4.00	NS
13th item	3.77±0.89	4.00	3.61±1.03	4.00	3.35±1.15	3.00	3.11±1.14	3.00	3.75±1.28	4.00	3.55±1.10	4.00	0.014 (1>4)
14th item	3.36±1.020	3.00	2.74±1.28	3.00	3.05±1.03	3.00	2.55±1.25	2.00	3.33±1.24	3.00	3.04±1.19	3.00	0.02 (1>4)
Overall WEMWBS scores	51.11±7.21	51.00	47.19±10.17	48.00	47.41±8.56	48.00	42.4±10.33	43.5	50.66±10.70	52.00	48.18±9.64	49.00	0.001 (1-5>4)

Kruskal-Wallis test, according to the Bonferroni Correction $p<0.005$ was considered statistically significant
WEMWBS: Warwick-Edinburgh Mental Well-being Scale, NS: Non-significant, SD: Standard deviation

Table 3. Role of demographical details, social background and lifestyle habits on students' overall WEMWBS scores

Variables	N (%)	Overall WEMWBS score		p-value
		Mean ± SD	Median	
Gender				
Male	85 (32)	52.12±9.03	49	<0.001
Female	183 (68)	46.42±9.43	47	
Living accommodation				
With parents	129 (48)	47.92±9.56	49	0.710
At dorm	51 (19)	47.62±8.57	48	
At home (alone)	60 (22)	48.45±10.66	51	
At home with room mate	28 (11)	50.08±10.88	48	
Dentistry first preference?				
Yes	239 (89)	48.7±9.31	49	0.024
No	29 (11)	43.8±11.36	43	
Smoking status				
Never smoked	186 (69.5)	47.9±9.07	48	0.630
<1 pack/day	52 (29.5)	49.6±10.38	51	
1 pack/day	28 (10)	48.1±11.10	47	
>1 pack/day	2 (1)	51± 5.21	51	
Education level of the mother				
Primary school	8 (3)	52.28±7.69	53	0.522
Secondary school	9 (3)	42.12±11.98	44	
High school	55 (21)	48.54±9.44	48	
University	147 (55)	47.9±10.18	49	
Master/PhD	49 (18)	48.77±7.97	48	
Education level of the father				
Primary school	6 (2)	49±10.67	47	0.669
Secondary school	10 (4)	42.33±12.05	45	
High school	32 (12)	48.78±9.51	48	
University	142 (53)	48.08±9.78	49	
Master/PhD	78 (29)	48.6±9.29	49	
Presence of regular sport activity				
Yes	125 (47)	49.9±9.40	51	0.001
No	143 (53)	46.6±9.63	47	
Having a hobby?				
Yes	139 (52)	50.11±9.85	51	<0.001
No	129 (48)	46.1±8.99	47	

Data are given as n (%). Mann-Whitney U test was used for two group comparisons (gender, sport habit, presence of a hobby and whether the dentistry was their first choice). The Kruskal-Wallis test was used to compare more than two independent groups (living accommodation, education level of the parents, smoking status). N: Number, WEMWBS: Warwick-Edinburgh Mental Well-being Scale, SD: Standard deviation

These scores were lower than the scores obtained in general population surveys in the UK, Scotland, Northern Ireland, and Denmark (10,12,29) and were in agreement with the previous studies showing worse psychological well-beings in medical students and higher levels of anxiety in dental students compared to general populations (31-33). Regarding the comparison of well-being scores with previous studies, it should be kept in mind that there were some minor varieties between the studies. Obtaining lower values could be explained by the work environments of the health care professions and also dental education programs which are known to be highly demanding and stressful.

Previous studies did not determined a standardized cut-off scores for the WEMWBS and it was reported that the average population mean was around 51 based on the study results which were conducted on different countries (10,12,29). Present finding might seem to be lower than the previously reported average population mean. This difference may arise from the fact that subjective wellbeing varies across cultures (34). Therefore, we should be extremely cautious when using cut-off points for the classification and only nation-specific cut-off points should be employed.

Few studies exist in the literature that assessed the mental well-being of dental students with WEMWBS (25,26). Lewis and Cardwell (25) compared the prevalence of mental well-being among veterinary medicine, medicine, dentistry and pharmacy students. They found that the mean score of the dental students was 45.41. The overall WEMWBS score of the current study population is in line with the findings of Lewis and Cardwell (25). Other study which was conducted by Knipe et al. (26) was performed on medical, dentistry and veterinary students. The prevalence of mental health appeared to be higher in the first few years of college and decreased as students remained in education among medical and veterinary science students, although this pattern was not evident in dental students. Similarly, in this study, it was found that dental students exhibited a worse mental well-being in their last years.

When mean WEMWBS scores of each grade were compared, it was determined that the fourth-year students had the lowest (42.47±10.33), whereas the first-year students had the highest (51.11±7.21) WEMWBS scores. The differences between year 1 and 4, along with year 4 and 5 were statistically significant. The fourth year of dental education is highly demanding because in addition to their theoretical trainings, dental students also need to obtain clinical training, which includes enrolling patients, taking care of them, executing permanent dental treatments, as well as pursuing clinical requirements and taking tests (19). Low WEMWBS scores in fourth-year students may be attributed to the accumulation of all aforementioned factors. Five-year students presented WEMWBS scores which were closed to the first-year scores and this score was significantly higher compared to fourth-year student's values. This variation in WEMWBS scores could be explained by the fact that the students were accustomed to both theoretical and clinical requirements. In addition, they were used to perform dental treatments after the first year of their clinical trainings; this might have reduced their anxiety about the career and increase their self-confidence which results in promoting their well-beings. However, this finding is in contrast with that of previous studies which reported higher level of stress in senior students than their younger peers. They suggested that the coursework becomes more difficult with each passing year. This difference could be explained by the factors specific to the participant population used in the studies or by the difference of the curricula between the universities (21,35-37).

While investigating the factors that may affect well-being, the present study found that male students' well-being scores were significantly higher than that of females. This finding was in accordance with previous studies (21,38,39) and suggests that male students' overall well-being status was better than females. On the other hand, first choice for admission was previously reported as an important demographic variable and the studies clarify that the students, who could not achieve their initial career preferences, but ended up in dental school had lower well-beings (21,38,40,41). In accordance with previous studies, our research has also proved that students who reported that dentistry was their first career choice had significantly higher WEMWBS scores. Students who have hobbies or regular sports activities yielded significantly higher WEMWBS scores. Presence of a hobby or regular sports activity promote good human relationships and values. Therefore, having hobbies

or regular sports activities may well assist reducing the stress and promoting a sense of well-being of dental students as well (38). There were no significant differences in living accommodations, smoking status and parents' education levels in terms of the overall WEMWBS scores. There were contradictory findings in the literature about the effects of these factors on well-being. Number of studies reported a significant association of well-being with some of these factors (39,42) whereas the others did not (38). This difference could be explained by the varieties between the studies such as different social, economic and cultural characteristics of the study populations.

Dental students' mental well-being was explored in a limited number of studies by analyzing both feelings and functional perspectives of mental well-being. Thus, current study contributes to the literature in this field. However, the results of this study should be interpreted with caution since the cross-sectional design of our study, number of the participants and involving of students from private institution present limitations.

Conclusion

Present study adds significant contribution to the relevant literature by assessing dental students' well-beings using WEMWBS, which includes both hedonic and eudaemonic dimensions. The mental well-beings of dental students were influenced by gender, first choice for admission, year of study, having hobbies or regular sports activities. Taken these results together, the factors that might affect the mental well-beings of the students should be considered and strategies should be in place to monitor and promote their well-beings during their academic careers. However, the results of the present study may not represent students' well-being in other dental schools. It would be of interest to compare well-beings of the present dental students to other faculty students and general populations. In addition, further research with prospective design involving a cohort of students over five years academic periods is recommended to monitor the mental well-beings of dental students' more objectively.

Ethics

Ethics Committee Approval: The study protocol was approved by Institutional Review Board and Ethics Committee of Başkent University (decision no: 19/97, date: 18.09.2019).

Informed Consent: This cross-sectional study was carried out by first to fifth year dental students of Başkent University.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.B.Ç., Y.S., Design: N.Ö.A., S.A., Supervision: M.B.Ç., Y.S., S.A., N.Ö.A., Fundings: M.B.Ç., Y.S., S.A., N.Ö.A., Materials: S.A., Data Collection or Processing: S.A., Analysis or Interpretation: N.Ö.A., Literature Search: Y.S., M.B.Ç., Writing: Y.S., M.B.Ç., Critical Review: M.B.Ç. Y.S., S.A., N.Ö.A.

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Original Article / Özgün Araştırma

Effect of Universal Shade of Resin Cement on the Final Color of Ceramic Restorations

Üniversal Renk Rezin Simanın Seramik Restorasyonların Final Rengi Üzerine Etkisi

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Abstract

Objective: To investigate the effect of neutral (N) and universal (Un) shade resin cement on the final color of thin ceramic restorations.

Materials and Methods: Forty specimens (8×8×0.8 mm^3) were sliced from five leucite reinforced glass-ceramic blocks. Another 40 rectangular composite specimens were prepared from a resin die material (8×8×4 mm^3; IPS Natural Die Material, A1 and A 3,5 IvoclarVivadent) simulating prepared teeth and divided into two cement colors. (N and Un) After baseline color measurements, each slice was cemented to a composite resin specimen, and measurements were obtained with a spectrophotometer on a white background in daylight by a single experienced investigator. The difference between the baseline and final color measurements was calculated in accordance with the CIEDE2000 color measurement formula. The CIEDE2000 perceptibility and acceptability thresholds were set to 0.8 and 1.8. The data were statistically analysed with Shapiro-Wilk and Mann-Whitney U test.

Results: No significant difference was observed between Δ_{00} value in the N (1.18±0.40) and Un (1.26±0.52) groups for the light background. The mean differences between the N (0.74±0.31) and Un (0.88±0.55) groups were insignificant for the dark background ($p>0.05$).

Conclusion: The Un shade cement used in this study may mask the dark tooth structure when using 0.8 mm-thickness ceramic restorations. However, the results may not be clinically satisfying. The Un shade resin cement used in this study provided an improvement in color although it was insufficient to mask the underlying dark background completely. Using Un shade resin cement is a challenge under 0.8 mm-thickness ceramic restorations of dark-colored teeth. If preferred, the ceramic thickness should be increased.

Öz

Amaç: Çalışmanın amacı, nötral (N) ve üniversal (Un) rezin simanın ince seramik restorasyonlarının final rengine etkisinin değerlendirilmesidir.

Gereç ve Yöntemler: Çalışmamız için kırk adet lösit ile güçlendirilmiş cam seramik örnek (8×8×0,8 mm) elde edildi. Dental dokuyu taklit eden bir rezin kompozit malzemesinden (8×8×4 mm; IPS Natural Die Material, A1 ve A 3,5 IvoclarVivadent) kırk adet dikdörtgen altyapı örneği hazırlandı ve 2 rezin siman grubuna ayrıldı

(N ve Un). Başlangıç renk ölçümlerinden sonra, her seramik dilim bir kompozit rezin örneğinin üzerine yapıştırıldı ve deneyimli bir araştırmacı tarafından gün ışığında beyaz zemin üzerinde bir spektrofotometre ile ölçümler alındı. Başlangıç ve sonuç ölçümleri arasındaki farklılık CIEDE2000 renk ölçüm formülüne göre hesaplandı. CIEDE2000 renk ölçümü için algılanabilirlik ve kabul edilebilirlik eşik değerleri 0,8 ve 1,8 olarak ayarlandı. Veriler, Shapiro-Wilk ve Mann-Whitney U testi ile istatistiksel olarak analiz edildi.
Bulgular: Açık arka plan için N (1,18±0,40) ve Un (1,26±0,52) gruplarda Δ_{00} değerleri arasında anlamlı bir fark yoktu. Koyu arka plan için alınan değerlerde N (0,74±0,31) ve Un (0,88±0,55) gruplar arasında anlamlı bir fark bulunmamıştır (p>0,05).
Sonuç: Bu çalışmanın sonuçlarına göre kullanılan Un renk rezin simanın, 0,8 mm kalınlığında seramik restorasyonlar altında koyu diş yapısını maskeleyebildiği, ancak sonuçların klinik olarak tatmin edici olmadığı görülmektedir. Bu çalışmada kullanılan rezin siman, alttaki koyu arka planı tamamen maskelemek için yetersiz olsa bile renkte bir iyileşme sağlamıştır. Koyu renkli dişlerde 0,8 mm kalınlığında seramik restorasyonların simantasyonunda Un renkte rezin siman tercih edilecek ise restorasyonun seramik kalınlığının artırılması gereklidir.

Introduction

Ceramic veneer restorations are an esthetic treatment option with a high clinical survival rate of up to 93.5% during long-term follow-up (1). In addition, this type of restoration, which aims to protect the dental tissue with minimal or no preparation, meets the patient's esthetic needs. Thanks to recently developed ceramic materials and adhesive systems, it has become easier to achieve superior esthetic results, especially with anterior restorations (2).

There are many causes of tooth discoloration, including trauma, intrapulpal hemorrhage, root resorption, and dental tissue after endodontic treatment (3). Ceramic restorations are one of the most common and preferred treatments to reestablish the esthetic appearance of discolored teeth. However, due to the translucent nature of ceramics, underlying dental background may reflect, which negatively impacts the esthetic outcome, especially if ultra-thin ceramic restorations are used. During the rehabilitation of a dark-colored tooth with ceramic restorations, it can be challenging to perfectly match an appropriate cement and ceramic thickness. To overcome this problem, newly developed resin cements and ceramics have been produced in order to mask dark-colored teeth (4-7).

Color measurement can be determined visually or with a device. The accuracy of color measurements may vary depending on the type of measurement. Color measuring devices, including spectrophotometers and colorimeters, provide more objective results compared to visual evaluation, and they are often preferred because they provide numerical color expression and standardization (8,9).

The International Commission on Illumination (CIE) mentioned different color determination systems. One of them is the CIELAB formula (ΔEab*) system. It was found that this formula measures the dimensions of color differences between initial and final color differences. These three measurements are related to three dimensions of the space in the coordinate axis (X, Y, Z, for L*, a*, b*) and human color perception. The L*axis measures lightness (vertical axis), where values from 0 to 100 represent the transition from black to white. The a*axis measures the quantity of the red and green values, which refers to the transition from positive to negative from red to green. The b*axis measures yellow and blue values, which represents the transition from positive to negative from yellow to blue. According to the CIELAB formula (ΔEab*), new color-difference formulas, including CIEDE2000 (ΔE_{00}), have been developed and reinforce the importance of the original hue and value concepts used by Munsell (10). The Δ_{00} color-difference formula includes various revisions for CIELAB color values (weighting functions: SL, SC, SH, rotation term: RT, parametric factors: KL, KC, KH) (11). The perceptibility and acceptability thresholds of Δ_{00} were set to 0.8 and 1.8 (12).

The purpose of this study was to investigate the effect of neutral (N) and universal (Un) shade resin cement on the final color changes of thin ceramic restorations on light and dark backgrounds using the Δ_{00} color-difference formula. The null hypothesis was that the application of N and Un shade resin cement on light and dark background would be similar on final color thin ceramic restorations.

Materials and Methods

The materials used in this study have been listed Table 1. Five glass-ceramic blocks (IPS Empress A1-HT; Ivoclar Vivadent, Schaan, Liechtenstein) were used in this study. Thin ceramic slices (8×8×0.8 mm) were

Table 1. The materials used in this study

Materials	Shade	Codes	Manufacturer	Composition	Type
Variolink N LC	Neutral	N	Ivoclar Vivadent, Schaan, Liechtenstein	UDMA,methacrylate, ytterbium trifluoride and spheroid mixed oxide, autopolymerizing initiators, light-polymerizing initiators, stabilizer, pigments	Light-cure
ESTECEM II	Universal	Un	Tokuyama Dental, Tokyo, Japan	Bis-GMA, TEGDMA, Bis-MPEPP, Peroxide, Camphorquinone and Silica-Zirconia Filler	Dual-cure
Ips Empress Material	A1	-	Ivoclar Vivadent, Schaan, Liechtenstein	Leucite-based Glass ceramic	-
Ips Natural Die	A1 A3,5	-	Ivoclar Vivadent, Schaan, Liechtenstein	-	Light-cure composite

obtained with a low-speed diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA) under continuous water cooling (n=40). A resin die material (IPS Natural Die Material; Ivoclar Vivadent) in two shades (light background: A1, dark background: A3,5) was used to prepare rectangular-shaped (8×8×4 mm) tooth-shaded background specimens to simulate underlying dental substrates. A metal mold was prepared to obtain the resin composites (8×8×4 mm). The metal mold was placed on the glass surface, and the composite material was added to the metal mold without air gaps. A new glass surface was placed on the metal mold to obtain a smooth surface. The resin composite materials were polymerized for 40 seconds (n=40). Then, each group was divided into two different shades of resin cement: N (Variolink Esthetic LC, N; Ivoclar Vivadent) and Un (ESTECEM II Un; Tokuyama Dental, Tokyo, Japan) (n=20).

One side of each ceramic and composite resin specimen was polished with 600-800-1,000-1,200 gradient silicon carbide abrasive papers sequentially (Leco VP 100; Leco Instrumente GmbH, Monchengladbach, Germany) under continuous water cooling for standardization. The thickness of each specimen was measured and controlled with a digital micrometer (Mitutoyo Corporation, Tokyo, Japan). All ceramic specimens were glazed with using Un Glaze Spray (Ivoclar Vivadent). Before cementation, 4.9% hydrofluoric acid (IPS Etching Gel; Ivoclar Vivadent) was applied to the ceramic surfaces for 60 seconds. After acid etching, etched surfaces were water-rinsed and air-dried. Then, silane (Monobond Plus; Ivoclar Vivadent) application was carried out onto the surfaces of the ceramic and die materials for 60 seconds.

Before cementation, baseline color measurements were carried out with a digital spectrophotometer (VITA Easyshade; VITA Zahnfabrik, Bad Säckingen, Germany) on a standard white background (L*=90.9, a*=0.3, b*=4.9). Resin cements were applied according to the manufacturer's instructions and light cured with an light-emitting diode light-curing device (Bluephase 1,200 mW/cm^2; Ivoclar Vivadent AG) for 120 seconds while 250 g of load applying (Figure 1) (13). After cementation, excessive resin cement was removed and measurements were carried out with a digital spectrophotometer on a standard white background.

The ΔE_{00} color formula was used to calculate the color differences between the baseline and the final measurements (14).

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)},$$

To decrease measurement uncertainty, the device was calibrated according to the manufacturer's instructions before and after measurement of each specimen. After the calibration was completed, the measurements were carried out at the center of each specimen by the same experienced investigator (Figure 2). The L*a*b* color measurements were made three times from each sample and recorded by taking the average values.

Statistical Analysis

According to a pilot study, for a power analysis with a 0.05 level and 80% power the required minimum sample size was n=40. Statistical analysis was performed using SPSS 19.0 for Windows software (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to determine normality, and the test results showed that the data were not distributed normally therefore Kruskal-Wallis was performed. The Mann-Whitney U test was performed to determine whether color differences were significantly different between each group. The significance level was set at $p<0.05$ ($p<0.05$).

Figure 1. Resin cements were carried out onto dark and light background according to the manufacturer's instructions

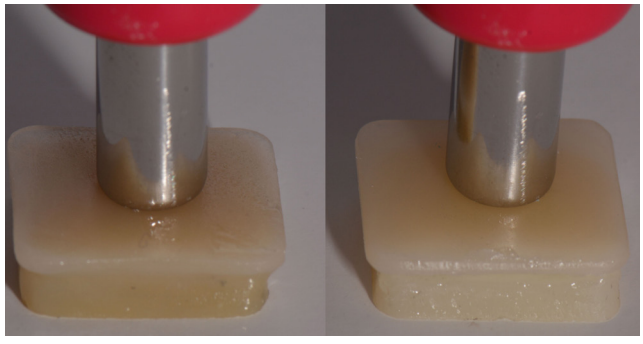

Figure 2. Measurements were recorded in CIELAB coordinates with using a spectrophotometer in dark and light substructure, respectively

Results

The Δ_{00} µm ± standard deviation values were shown in Table 2. There were no significant difference between Δ_{00} values in both the N (1.18±0.40) and Un (1.26±0.52) groups for the light background. Mean differences between N (0.74± 0.31) and Un (0.88±0.55) were insignificant for the dark background ($p>0.05$).

For the N group, there was a statistically significant difference between light (1.18±0.40) and dark (0.74±0.31) backgrounds ($p<0.05$). There was no statistically significant difference between light (1.26±0.52) and dark (0.88±0.55) backgrounds for the Un group. All Δ_{00} values were between acceptability and perceptibility color thresholds ($0.8<\Delta_{00}<1.8$) except for the value obtained from the dark background of the N (0.74±0.31) resin cement group ($\Delta_{00}<0.8$).

Discussion

In this study, the effect of Un and N shade resin cements on the final color changes of thin ceramic restorations on light and dark backgrounds was investigated using Δ_{00}. There was no significant difference between Δ_{00} value in both the N and Un groups for the light and dark backgrounds. However, considering the results obtained, it was seen that while the N cement was not able to mask the dark substructure, the Un cement could. Therefore, the null hypothesis was particularly rejected.

A statistically significant difference was observed between the light and dark backgrounds for the N cement group. This study found that the underlying tooth color can be reflected under 0.8 mm thick ceramic restorations and affect the final color, which is consistent with the findings of previous studies (4,15-17).

From a clinical standpoint, successful color matching is an important aspect for obtaining esthetic restorations and must be attained with the

Table 2. The mean Δ_{00} values and levels of significance according to differing cements and backgrounds

		Δ_{00}
Background (light)	N	1.18±0.40
	Un	1.26±0.52
Background (dark)	N	0.74±0.31
	Un	0.88±0.55

*p<0.05, N: Neutral, Un: Universal

optimal harmony of different variables. Ceramics type and thickness, adhesive resin cement, and underlying tooth color have an effect on the long-term color stability of esthetic restorations (16,18). Superior translucencies can be achieved with 0.3 mm to 1.0 mm thick, adhesively cemented ceramic laminate veneers with or without preparation. Thus, esthetically satisfying results can be obtained with ultra-thin ceramic restorations (2). However, resin cement polymerization can cause discoloration, which can negatively affect the final color beneath thinner and more transparent ceramic laminate restorations on dark underlying tooth structures (19,20). In the present study, it was concluded that the N shade cement applied with 0.8 mm thick ceramic restorations could not mask the underlying dark background. To overcome this problem clinically, use opaque and chromatic ceramic to cover the colorless substrate is recommended. Besides, clinicians should avoid to prefer minimal tooth preparation (4,8,15).

In the results of this present study, it was seen that Un shade resin cement, although clinically unsatisfactory, could mask dark background but it has not been found to be effective in achieving the final color (A1). A previous study evaluated the effect of different shades of resin cements (A1 and A3, opaque and translucent) on the final color of 1.0-mm A1-shade leucite reinforced ceramics, and it was observed that resin cements affect the final color of restorations (2). In another studies, the selected resin shades influenced the final color of leucite reinforced ceramics, and they could not be masked the dark background except for the opaque resin shade, and used resin shades made the ceramic specimens lighter or darker (7,15,21,22). Giti et al. (23) concluded that Un resin shade (A2) decreased the lightness of 0.5-mm thickness leucite reinforced ceramic. The results of another study also reported that Un shade of resin cement was not visually perceptible lithium disilicate ceramic in 1.5-mm thickness (14).

The Δ_{00} values obtained from the N resin cement group on dark background were under acceptability and perceptibility thresholds (Δ_{00}<0.8), but the others were between acceptability and perceptibility thresholds (0.8<Δ_{00}<1.8). These results have shown that the Un cement group could mask the dark background but was slightly higher than the acceptable level. From a clinical point of view, we think that using Un shade resin cement can change the underlying dark-colored background but cannot reach the desired shade.

In this study, the cement group Un showed higher Δ_{00} values in both light and dark backgrounds, but there was no statistically significant difference from the N cement group. This can be explained by the composition of various amounts of color ingredients in Un shade resin cement compared to N shade.

CIELAB is the most common system for color measurement in dental materials. Nevertheless, there are some shortcomings in the CIELAB color space system, especially about the hue value described by Munsell. In order to improve CIELAB (ΔEab), various color-difference formulas based on CIELAB have been developed, such as CMC, CIE94, and CIEDE2000 (Δ_{00}) (10). In color differences studies, the CIEDE2000 color-difference formula was found to be more accurate and represent the color differences perceived by the human eye (24,25). The value of Δ_{00} units showed the clinical influence of resin cements used on the final shade of the specimens. The results were evaluated according to the acceptability and perceptibility threshold (0.8<Δ_{00}<1.8) values. Such numerical values can be used as a control vehicle to guide the selection of esthetic dental materials and to understand and interpret visual and instrumental results that affect clinical performance (12).

ESTECEM II Un resin cement and leucite-based ceramic block were used in the current study because there are no previous *in vivo* studies evaluating the effect of the final color using those materials together. Besides this, further clinical studies considering the effect of Un shade resin cement on final color are needed.

The limitations of this study are that only two shades of luting cement were evaluated and no aging procedures were applied. Further studies should be conducted considering other shades of luting cement and changes recorded after aging procedures.

Conclusion

Within the limitations of this study, it can be concluded that background and luting cement color had significant effects on the final color of thin ceramic restorations. It was observed that the Un shade resin cement used in the study might mask the dark tooth structure when using 0.8 mm thickness

ceramic restorations. However, the results may not be clinically satisfying. Further clinical studies are needed in order to assess the effect of dual-curing Un resin cement, which was used in this study, on final color changes.

Ethics

Ethics Committee Approval: Since the materials used in this study do not related with any patient, ethical approval was not required.

Informed Consent: Since the materials used in this study do not related with any patient, informed patient approval was not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.P., Design: G.P., B.Y., D.R., Supervision: B.Y., Data Collection or Processing: D.R., Analysis or Interpretation: G.P., B.Y., Literature Search: G.P., D.R., Writing: G.P., B.Y., D.R., Critical Review: G.P., B.Y., D.R.

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Original Article / Özgün Araştırma

Salivary Interleukin-6 Levels in Patients with Periodontitis Stage IV

Evre IV Periodontitis Hastalarında Tükürük İnterlökin-6 Seviyeleri

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Abstract

Objective: The role of interleukin-6 (IL-6) in the pathogenesis of periodontal disease and tissue destruction at the periodontal site has been widely reported. This study aimed to evaluate the salivary IL-6 levels in patients with stage IV periodontitis.

Materials and Methods: The study included 28 patients who were diagnosed with periodontitis stage IV and the control group of 22 periodontally healthy patients. All the patients were systemically healthy. Saliva samples were collected, and clinical periodontal measurements, including probing depth (PD), clinical attachment level (CAL), papilla bleeding index (PBI), the percentage of sites with bleeding on probing (BOP) %, plaque index (PI) and calculus index (CI), were recorded. The unstimulated saliva of each patient was collected by a saliva collector, and all samples were analysed using the enzyme-linked immunosorbent assay method for the detection of IL-6.

Results: The mean value of salivary IL-6 in patients with periodontitis stage IV was 22.18±5.96 pg/mL. In the control group, the average measured value of IL-6 was 2.23±2.17 pg/mL. The periodontitis group had a significantly higher salivary IL-6 levels than the control group. A strong positive correlation was observed between the salivary IL-6 and clinical periodontal parameters (PD, CAL, PBI, BOP %, PI and CI) in patients with periodontitis stage IV ($p<0.0001$).

Conclusion: We demonstrated a statistically significant relationship between periodontal parameters and salivary IL-6 in patients with periodontitis stage IV. New studies are needed to accurately establish salivary IL-6 potential as a biomarker for periodontal disease monitoring, including all stages and grades of periodontitis.

Keywords
periodontitis, saliva, interleukin-6, ELISA

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Öz

Amaç: İnterlökin-6'nın (IL-6) periodontal hastalık patogenezi ve periodontal doku yıkımındaki rolü geniş çapta ele alınmıştır. Çalışmanın amacı, evre IV periodontitis hastalarının tükürüklerindeki IL-6 seviyelerini değerlendirmektir.

Gereç ve Yöntemler: Araştırmaya evre IV periodontitis teşhisi konulan 28 hasta ile 22 hastadan oluşan kontrol grubu dahil edildi. Hastaların tümü sistemik olarak sağlıklıydı. Tükürük örnekleri toplandı ve sondalama derinliği (SD), klinik ataşman seviyesi (KAS), papil kanama indeksi (PKİ), sondalamada kanama yüzdesi (SK %), plak indeksi (Pİ) ve diştaşı indeksini (Dİ) içeren klinik periodontal ölçümler kaydedildi. Her bir hastanın uyarılmamış tükürükleri, tükürük toplayıcı ile toplandı ve örneklerdeki IL-6 seviyesi enzim bağlı immünosorbent testi ile analiz edildi.

Bulgular: Evre IV periodontitis hastalarının ortalama IL-6 değeri 22,18±5,96 pg/mL idi. Kontrol grubunun ortalama IL-6 değeri ise 2,23±2,17 pg/mL idi. Periodontitis

grubunun tükürük IL-6 seviyesi kontrol grubundan daha yüksek bulundu. Evre IV periodontitis hastalarının tükürük IL-6 seviyeleri ile klinik periodontal parametreleri (SD, KAS, PKI, SK %, PI ve DI) arasında anlamlı ve güçlü pozitif korelasyonlar gözlendi (p<0,0001).
Sonuç: Çalışmamız, evre IV periodontitis hastalarının tükürük IL-6 seviyeleri ile klinik periodontal parametreleri arasında istatistiksel olarak anlamlı bir ilişki olduğunu ortaya koymuştur. Periodontitisin tüm evre ve derecelerini içeren periodontal hastalık takibinde, tükürük IL-6 seviyelerinin biyobelirteç olarak tam potansiyelini tespit etmek için yeni çalışmalara ihtiyaç vardır.

Introduction

Saliva is an biological fluid and according to recent data, it mirrors general health conditions and reflects various systemic changes in the body (1). Additionally to containing secretion of major and minor salivary glands it also contains several constituents that do not origin from salivary glands: Gingival crevicular fluid, derivates of blood and serum, expectorated bronchial and nasal secretions, viruses, fungi, bacteria, and bacterial products, various cells, electrolytes, immunoglobulins, proteins and enzymes, food debris and a small portion is gastro-esophageal reflux, etc. (2). This makes her an important diagnostic tool.

Although bacteria are the primary cause of periodontitis, the immune-inflammatory response of the host is responsible for the most destructive changes in periodontal tissue (3). A systematic review by Kc et al. (4) recognized interleukin-6 (IL-6) together with IL-1beta (IL-1β) as key biomarkers with accuracy that is acceptable for diagnostics of periodontitis. Several recent publications dealt with the potential diagnostic significance of saliva. It was assumed that saliva can be used not only for the diagnosis of oral diseases but also as a "body mirror" and can also be used to diagnose systemic illnesses (5). Saliva is an easy-to-reach body fluid that contains a wide range of significant proteins that are produced locally or transported through the blood vessels in the gingival tissue (6). Since many biomarkers in the saliva can be found in the blood, the use of saliva in the diagnosis is of great importance. The ideal biomarker must be sensitive, specific, prophetical, fast, economizing, non-invasive, and stable *in vivo* and *in vitro*. Compared to drawing blood, saliva sampling is a non-invasive and much more pleasing method for patients and is a diagnostic method suitable for children, young people, and the elderly. IL-6 is esteemed as a pro-inflammatory cytokine which acts on bone resorption in the presence of infections (7). The presence of IL-6 can be demonstrated by saliva analysis which can be a very important diagnostic method. The measurement of some biomarkers, including IL-6, can be confusing since the source of this cytokine can be local production by circulating neutrophils due to inflammation in periodontal tissues, or due to systemic inflammation and originating from the blood and circulation.

Salivary IL-6 has been measured using enzyme-linked immunosorbent assay (ELISA), Luminex multiplex assay, and enzyme immunoassay (EIA). In the study by Ramseier et al. (8) salivary IL-6 ranged from 22.1 pg/mL in patients with gingivitis to 88.7 pg/mL in patients with moderate to severe chronic periodontitis. Ebersole et al. (9) investigated the diagnostic potential IL-6 and mean IL-6 values obtained in the saliva samples were 3.30±2.32 pg/mL for healthy subjects and 35.57±48.17 pg/mL for patients with chronic periodontitis. Mean IL-6 levels in saliva in patients with chronic periodontitis (98.40±18.44 ng/L) was significantly higher than in controls (11.67±3.32; p=0.001) in a study by Nanakaly (10). In the study by Nagarajan et al. (11) the mean value of salivary IL-6 in patients with gingivitis was 3.9±5.9 pg/mL, and 12.1±10.2 pg/mL in periodontitis patients who had bleeding on probing (BOP) at >20% of sites, with >10% of sites with probing depth (PD) ≥4 mm and clinical attachment level (CAL) ≥2 mm.

Correlation of the obtained salivary IL-6 values in patients with periodontitis stage IV and healthy subjects is intended to determine the reliability of IL-6 in saliva as an inflammatory mediator for patients with periodontitis stage IV. We would like to evaluate salivary IL-6 levels in patients with stage IV periodontitis, and compare the results to earlier findings of salivary IL-6 in periodontitis. Reliability and precision of the results should give us guidelines for the possible use of salivary IL-6 as a biomarker in everyday practice.

Materials and Methods

Study Groups

The study protocol was in accordance with the local ethical guidelines and following the Helsinki Declaration of Human Rights and approved by the University in Sarajevo Ethics Committee of the Faculty of Dentistry (decision no: 02-3-4-189-9, date: 22.04.2014).

The study was conducted at a private dental practice in Sarajevo, Bosnia and Herzegovina. The study included patient volunteers who agreed to participate in the study. Patients were informed of the purpose and manner of conducting the study and gave their consent to participate in the study. Patients were selected by random sampling method using a software (Research Randomizer software). Patients were classified using the 2017 classification of periodontal and peri-implant diseases and conditions (12). The test group included 28 patients who were diagnosed with generalized periodontitis stage IV. The diagnosis was performed based on the history of the disease, and clinical examination. The control group consisted of 22 patients who did not have periodontal disease. The criteria for the healthy patient was age between 18-50 years, PD ≦3 mm, the percent of sites with papilla bleeding index (PBI) ≧2 less than 10%, no sites with PBI >4, and the absence of alveolar bone resorption. All patients were Caucasians, and smoking data were included in the patient's history. A sample size of 40 subjects (sample size analysis, d=2) was calculated with a confidence interval of 95%, α=0.05, and a strength of 80% using the standard deviation (SD) for the CAL parameter according to a study by Yue et al. (13).

Excluding factors were: History of periodontal therapy (non-surgical and surgical), the existence of systemic illnesses, presence of infectious diseases, need of antibiotic prophylaxis before examination, mental disability, use of antibiotic, corticoid or immunosuppressive therapy within the six months prior to the study, pregnancy, lactating women, minors, patients undergoing endodontic treatment, patients with periapical lesions, the use of antiseptics, and antimicrobial drugs.

Before taking the sample, patients gave written consent to participate in the study. History of disease and clinical examination data were entered in a specially prepared work chart. Following parameters were recorded: plaque index [PI, Silness and Loe (14)], PBI [Saxer and Mühlemann, (15)], calculus index [CI, Silness and Loe, (14)], tooth mobility, suppuration, PD, CAL, and the engagement of the furcation [Hamp et al. (16)]. All clinical parameters were registered by the same clinician, and World Health Organization periodontal probe was used in registration.

Sampling of Saliva

Before registration of the clinical parameters, samples of patients' saliva were collected. Saliva samples were collected for all patients on working days (Monday to Friday) between 9-10 AM.

A sample of unstimulated saliva was collected from the participants before periodontal treatment for the test group in a sterile vial of 5 mL (Thermo Scientific™, Thermo Fisher Scientific, USA) with the use of Saliva Collection Aid, by Salimetrics, USA. Saliva was collected on the volume base according to the modification of the method described by Navazesh and Kumar (17). Samples were collected from the patients in the control group without periodontal treatment required. Each participant rinsed with distilled water for one minute 10 minutes before the sampling to remove debris. This was done following the instruction of ELISA kit manufacturer Salimetrics, to minimize the possible effects of acidic or high sugar foods which can compromise assay performance by lowering sample pH and influencing bacterial growth.

After collecting, saliva samples were refrigerated within 30 minutes and frozen at -20 °C within 4 hours until further analysis.

Analysis of Saliva Samples

All samples were analyzed using the (Salimetrics 1-3602-Interleukin-6 Salivary Immunoassay Kit) ELISA/EIA manufactured by (Salimetrics), USA. We used ELISA sandwich immunoassay specially designed and validated for the quantitative measurement of salivary IL-6. Preparation of reagents, sample handling, and preparation procedures of IL-6 analysis in saliva samples were performed according to the manufacturer's instructions. The obtained results were expressed in optical density, using software and 4-parameter non-linear regression curves. The results were computed using (RayTo, Microplate Reader RT-6100). The obtained IL-6 values are expressed quantitatively in pg/mL.

Statistical Analysis

Continuous variables were presented as mean ± SD/median. ANOVA test and t-test were used for comparisons of means and proportions between the test group and the healthy control group. Pearson's correlation coefficient was used to assess the correlation of salivary IL-6 and clinical periodontal parameters. A two-tailed p<0.05 was considered to be statistically significant in all analyses. Empower stats software was used for data analysis.

Results

The study included patients 18-50 years old. The mean age of patients in the test group was 46±4.1 and for the control group 39±11.3 (p=0.0069). The test group was formed of 14 male and 14 female patients, and the control group was formed of 22 patients, 10 male and 12 female patients. P values for male-female ratio in study groups were p=0.7242 for male patients, and p=0.7230 for female patients.

Smoking was registered and 15 patients in the test group and 14 patients in the control group were confirmed to be smokers (p=0.4788). Average number of extracted teeth in test group was 6.57, and the average number of extracted teeth in the control group was 4.27 (p<0.0001). Average number of extracted multiple rooted teeth in the test group was 4.17, and in the control group 2.36 (p<0.0001).

Clinical periodontal parameters in the test group had the following values: PBI: 3.6±0.2, CI: 3.3±0.4, PD: 7.1±0.6, BOP %: 43.76±6.65, and CAL: 5.76±1.29. All clinical periodontal parameters of patients in the test group had a significant difference compared to the values of the clinical periodontal parameters in the control group. Table 1 summarizes the results of demographic and clinical periodontal parameters for both groups.

The mean value of salivary IL-6 in patients with periodontitis stage IV was 22.18±5.96 pg/mL and the average measured value of IL-6 in the saliva of the control group was 2.23±2.17 pg/mL (p<0.0001) (Figure 1).

Correlation Between Salivary IL-6 and Clinical Periodontal Parameters

We correlated the following clinical periodontal parameters with salivary IL-6: PI, PBI, CI, PD, BOP %, and CAL. The results show that there is a strong significant correlation between salivary IL-6 and clinical periodontal parameters in patients with periodontitis stage VI, and the results were statistically significant (Table 2).

Table 1. Demographic and clinical periodontal parameters of patients

Parameters	Test group (n=28)	Control group (n=22)	p-value
Age, year (mean ± SD)	46.3 ± 4.1	39.8 ± 11.3	p=0.0069
Gender			
Male (n)	14	10	p=0.7242
Female (n)	14	12	p=0.7230
PI	2.8 ± 0.3	0.3 ± 0.2	p<0.0001
PBI	3.6 ± 0.2	0.8 ± 0.4	p<0.0001
CI	3.3 ± 0.4	0.6 ± 0.2	p<0.0001
PD	7.1± 0.6	1.6 ± 0.6	p<0.0001
BOP (%)	43.76 ± 6.65	5.01 ± 2.32	p<0.0001
CAL	5.76 ± 1.29	0.02 ± 0.01	p<0.0001
Smoking			
Yes	15	14	p=0.4788
No	13	8	
IL-6	22.18 ± 5.96	2.23 ± 2.17	p<0.0001

PI: Plaque index (0-3), PBI: Papilla bleeding index (0-4), CI: Calculus index (0-4), PD: Pocket depth (mm), BOP (%): Bleeding on probing (%), CAL: Clinical attachment loss (mm), IL-6: Interleukin-6 in saliva (pg/mL), SD: Standard deviation

Table 2. Salivary IL-6 and clinical periodontal parameters correlation		
	Test group	Control group
Parameter	p-value	p-value
PI	p<0.0001	p=0.0002
PBI	p<0.0001	p=0.0041
CI	p<0.0001	p=0.0011
PD	p<0.0001	p=0,1965
BOP (%)	p<0.0001	p=0.0100
CAL	p<0.0001	p<0.0001
PI: Plaque index (0-3), PBI: Papilla bleeding index (0-4), CI: Calculus index (0-4), PD: Pocket depth (mm), BOP (%): Bleeding on probing (%), CAL: Clinical attachment loss (mm)		

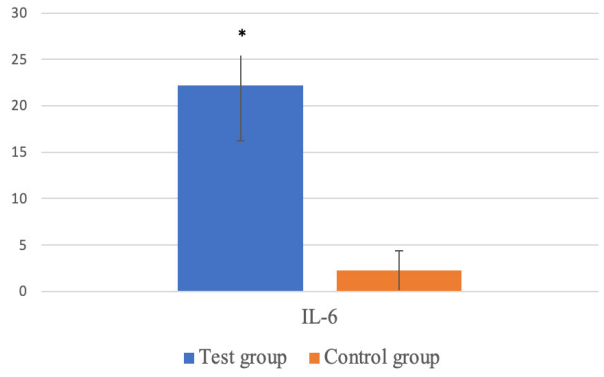

Figure 1. Salivary IL-6 levels (pg/mL) in study groups*

*P<0.0001, bar: Mean values of salivary IL-6 in study groups, line: Standard deviation values of salivary IL-6 in study groups

IL-6:interleukin-6

The results show that there is a highly significant difference between IL-6 in saliva and clinical periodontal parameters in the test group: PD (p<0.0001), BOP % (p<0.0001), CAL (p<0.0001). In the control group, the correlation of salivary IL-6 and clinical periodontal parameters showed no significant relation to PD (p=0.1965), while the correlation was significant for BOP % (p=0.0100) and CAL (p<0.0001).

Discussion

IL-6 has both pro-inflammatory and anti-inflammatory function and is a very important mediator of inflammation. It is, however, important to elucidate that IL-6 is a part of a complex network of cytokines included in the inflammatory response, and therefore a part of a possible complex of biomarkers. Several studies conclude that ILs are important as biomarkers to identify patients with periodontitis (13,18,19). This study does have its downsides; we did not include patients with gingivitis so we are not able to correlate and possibly distinguish levels of IL-6 for both gingivitis and periodontitis. Also, further studies would have to include other possible biomarkers for periodontitis in order to find a sensitive and specific set of biomarkers for diagnosis and monitoring of periodontitis.

We demonstrated a statistically significant difference between IL-6 levels in saliva of patients with periodontitis stage IV and the control group. Also, the correlation of IL-6 in the saliva of patients with periodontitis stage IV and clinical parameters (PI, PBI, CI, PD, BOP, and CAL) were statistically significant. In the Ebersole et al. (20) study of 2015, four biomarkers were included, including IL-6 in patients with healthy periodontal tissues, gingivitis patients, and patients with periodontal disease diagnosis. In this study, IL-6 was the highest in the saliva of patients with periodontitis (22.8±3.7 pg/mL) compared to gingivitis (6.3±2.7 pg/mL) and patients with healthy periodontal tissue (3.7±0.5 pg/mL). In the second study, Ebersole et al. (9) investigated the diagnostic potential of IL-1β, IL-6, matrix metalloproteinase-8, and salivary albumin, and compared the values obtained in healthy subjects and patients with chronic periodontitis. The values of all four observed biomarkers, including IL-6, were significantly increased in salivary patients with chronic periodontitis and concluded that biomarkers had significant diagnostic potential for periodontal disease. The mean IL-6 values obtained in the saliva samples in this study were 3.30±2.32 pg/mL for healthy subjects and 35.57±48.17 pg/mL for patients with chronic periodontitis. In this study, Ebersole et

al. (9) have had higher IL-6 values in patients with chronic periodontitis comparing to the values we had in our study. The values obtained for healthy patients, on the other hand, are similar to those in our study. In this study, Luminex multiplex assay was used for determination of IL-6 which may have impacted the differences in the results, and also reason may reside in the level of disease of the different study populations. The periodontitis group contained significantly more men, non-Caucasians, and smokers, and was significantly older than the controls.

Nagarajan et al. (11) study measured the values of four biomarkers including IL-6 in 40 gingivitis patients and 40 patients with periodontitis. The mean value and standard deviation of IL-6 in the saliva sample for patients with periodontitis is 12.1±10.2 pg/mL. A study by Nanakaly (10) published in 2016 demonstrated higher salivary IL-6 levels in patients with periodontitis compared to healthy subjects. The values obtained in this study were consistent with Costa et al. (21) findings, which also demonstrated a significantly higher IL-6 value in saliva in patients with periodontitis compared to healthy patients. Significantly higher values of IL-6 in the saliva sample in patients with chronic periodontitis compared to the healthy control group were also proved by Geng et al. (22).

Higher levels of IL-6 in the saliva of patients with periodontal disease were found in a study by Teles et al. (23). In their study of 118 patients, of whom 74 were patients with chronic periodontitis, the level of IL-6 was also measured by the ELISA method. The results obtained in this study were not statistically significant because the difference in IL-6 in patients with chronic periodontitis and healthy subjects was low. A possible explanation for the discrepancy between the results might reside in the level of disease of the two different study populations. Also, in this study used cytokine levels were determined using a multiplexed bead immunoassay using Luminex, and this methodology difference used for the quantification of the IL-6 (ELISA vs. Luminex) might also have impacted the difference in the results.

The results of Ramseier et al. (8) agree with higher IL-6 in the saliva of patients with periodontitis than those in healthy subjects but the difference was small and obtained results were not statistically significant. In this study, saliva samples were supplemented with a proteinase inhibitor combination of 1% aprotinin and 0.5% phenylmethylsulphonyl fluoride prior to storage at -80 °C, which is different from our study. Also, the levels of IL-6 were measured by using protein microarray which may also be a reason for the difference in the results.

A study by Kc et al. (4) states that IL-6 is a strong salivary biomarker for periodontal inflammation with a sensitivity range of 52-80%, and specificity of 48-87%. Since periodontitis is an episodic disease with various biological stages and cyclic nature studies may have included patients with different stages which may be a reason for a different expression of IL-6 biomarker.

We demonstrated a statistically significant correlation of IL-6 values in the saliva of patients with periodontitis stage IV and periodontal parameters. Javed et al. (24) also demonstrated a statistically significant association of IL-6 levels in saliva and clinical parameters including PBI and found that IL-6 levels increased proportionally to the severity of periodontal disease. A study by Teles et al. (23) did not demonstrate a significant difference between levels of any of the 10 cytokines tested they tested and reported only weak statistically significant associations of mean clinical parameters and mean salivary levels of IL-8 and IL-10, casting a doubt on salivary cytokines as a biomarker for periodontitis. Ng et al. (25) reported a significant relation of salivary IL-6 and loss of alveolar bone, and in the study by Ebersole et al. (9), IL-6 levels were significantly positively correlated with BOP frequency in the population.

In our study, higher IL-6 values were obtained in patients with severe teeth mobility and X-ray visible resorptive changes in bone tissue. This fact is also corroborated by the evidence of Kurihara et al. (26) who have shown that IL-6 locally produces osteoclasts, which is a very important factor in the differentiation of these cells, and the role of osteoclast in bone resorption is known to us. These facts are also consistent with the findings of other studies (27,28) that speak of higher values of IL-6 as biomarkers in people with periodontal disease. The presence of plaque and dental concrements, in particular lipopolysaccharide activity on the tissue results in the activation of monocyte/T-lymphocytes. Their activation leads to increased cytokine secretion, including the IL-6 produced by multiple cells involved

in the inflammatory reaction of the organism, and periodontal disease is precisely the result of this type of reaction. These studies suggest that there are contradictions in the obtained results and measured values of IL-6 in patients with chronic periodontitis and IL-6 levels in patients compared to healthy controls. However, the IL-6 level correlation in the saliva of patients with periodontitis stage IV and the clinical parameter PBI tells us that there is a link that should be examined further. Possible directions for further investigation of IL-6 and periodontal disease bond could be in the measurement of IL-6 in the gingival crevicular fluid of patients with all grades of periodontitis using the new classification.

The limitations of this study are that the study was performed only in patients with generalized stage IV periodontitis. Patients were not divided according to the grade of the disease, which in potential studies with a larger number of participants and inclusion of all grades of the disease, could provide data on the potential impact of the disease on systemic health or the patient's response to standard therapy of periodontitis.

Conclusion

We demonstrated a statistically significant relationship between periodontal parameters and salivary IL-6 in patients with periodontitis stage IV. This data support earlier findings of IL-6 as one of the salivary biomarkers which may be useful in monitoring the current state of the disease, the effectiveness of the treatment, and possibly predict the progression of periodontal disease. But before it could be used as a tool in clinical practice, more data on salivary IL-6 in periodontitis is needed. Since periodontitis is an episodic inflammatory disease with various biological stages and cyclic nature, studies must include patients with different stages and grades of periodontitis. We may expect different expression of IL-6 biomarker in different stages of inflammation.

Ethics

Ethics Committee Approval: The study protocol was in accordance with the local ethical guidelines and following the Helsinki Declaration of Human Rights and approved by the University in Sarajevo Ethics Committee of the Faculty of Dentistry (decision no: 02-3-4-189-9, date: 22.04.2014).

Informed Consent: Patients were informed of the purpose and manner of conducting the study and gave their consent to participate in the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Z.H., E.P., M.H., Design: Z.H., E.P., S.H., Supervision: E.P., M.H., Fundings: Z.H., M.H., Materials: M.G.V., S.H., Data Collection or Processing: M.G.V., S.H., Z.H., Analysis or Interpretation: Z.H., E.P., Literature Search: M.G.V., Writing: Z.H., Critical Review: M.G.V., S.H.

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Original Article / Özgün Araştırma

Effect of an MDP-containing Primer on the Bond Strength Between Ten Different Resin Cements and Air-abraded Monolithic Zirconia

MDP İçerikli Primerin On Farklı Rezin Siman ve Kumlanmış Monolitik Zirkonya Arasındaki Bağlanma Dayanımına Etkisi

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Air-abrasion, MDP, monolithic zirconia, primer, resin cement

Anahtar Kelimeler
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Abstract

Objective: The aim of the present study was to evaluate the shear bond strength (SBS) of different self-adhesive luting systems to airborne-particle-abraded monolithic zirconia ceramic, with or without the application of a 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based ceramic primer.

Materials and Methods: A total of 200 monolithic zirconia specimens were prepared and air-abraded using 50-µm aluminum oxide particles under a pressure of 2.5 bar. Ten different self-adhesive resin cements were then bonded to the zirconia specimens with or without the use of an MDP-containing primer. After 24 h of water storage, all of the resin-bonded samples were thermocycled 5,000 times (5-55 °C). The SBS of the specimens was measured using a universal testing machine, and failure types were examined under a stereomicroscope. The data were statistically analyzed using two-way ANOVA and Tukey's honestly significant difference tests.

Results: Statistical analyses revealed significant differences between the different resin cement groups ($p<0.001$). All of the primer-applied groups showed statistically higher SBS values than the non-primed groups ($p<0.05$). The SBS values of primer-applied groups ranged between 8.56±0.91 and 16.08±0.67 MPa. Adhesive failures were more predominant in the nonprimed groups than in the primed groups for each resin cement tested. MDP-based resin cements used did not result in the highest bond strengths.

Conclusion: In the case of every self-adhesive resin cement tested, application of an additional MDP-containing priming agent yielded enhanced bond strength to air-abraded monolithic zirconia specimens. However, no association between the MDP content of the resin cements and the SBS values was observed.

Öz

Amaç: Bu çalışmanın amacı kendinden adezivli farklı yapıştırma sistemlerinin kumlanmış monolitik zirkonyaya bağlanma dayanımını (SBS) metakriloiloksidesil dihidrojen fosfat (MDP) esaslı seramik primeri uygulaması yaparak veya yapmadan değerlendirmektir.

Gereç ve Yöntemler: Toplamda 200 adet monolitik zirkonya örneği hazırlandı ve 50-µm alüminyum oksit kumu kullanılarak 2,5 bar basınç altında kumlandı. Daha sonra, 10 farklı kendinden adezivli rezin siman MDP içerikli primer uygulaması yapılarak veya yapılmadan zirkonya örneklere bağlandı. Rezin uygulanmış örneklere 24 saat suda bekleme süresinden sonra 5.000 termal döngü (5-55 °C) uygulandı. Örneklerin SBS değerleri evrensel bir test makinesi kullanılarak ölçüldü. Başarısızlık tipleri stereomikroskop altında incelendi. Veriler iki-yönlü ANOVA ve Tukey dürüstçe anlamlı fark testleri kullanılarak istatistiksel olarak analiz edildi.
Bulgular: İstatistiksel analizler farklı rezin siman grupları arasında önemli farklılıklar olduğunu ortaya koymuştur ($p<0,001$). Bütün primer uygulanmış gruplar, primer uygulanmamış olanlara göre daha fazla SBS değerleri göstermiştir ($p<0,05$). Primer uygulanan grupların SBS değerleri 8,56±0,91 MPa ve 16,08±0,67 MPa arasında değişmiştir. Her bir rezin siman için primer uygulanmayan gruplarda primer uygulananlara göre adeziv başarısızlık daha fazla görülmüştür. MDP esaslı rezin simanların kullanımı ile en yüksek bağlanma dayanımı elde edilmemiştir.
Sonuç: MDP içerikli primer ajanı uygulaması ile test edilen bütün rezin simanlar için önceden kumlanmış monolitik zirkonya örneklere daha fazla bağlanma dayanımı elde edilmiştir. Ancak, rezin simanların MDP içeriği ile SBS değerleri arasında bir ilişki bulunmamıştır.

Introduction

In recent years, use of the yttria-tetragonal zirconia polycrystal (Y-TZP) has become widespread as a metal-free alternative in fixed dental prosthesis due to its superior mechanical properties (1,2). Depending on the specific composition of sintered zirconia ceramics, fracture strength can be >1.000 MPa (3,4). Computer aided design/manufacturing technologies have resulted in ease-of-use with this highly crystalline material, allowing the fabrication of fitting frameworks (5,6). Covering these high-strength Y-TZP infrastructures with veneering ceramics that offer improved aesthetics is a frequently used process (7,8). However, some studies have reported that such a multilayered structure may cause some major problems including chipping, cracking, or delamination of veneering porcelain (8-10). To overcome these challenges, monolithic zirconia has recently been considered a viable alternative in fabricating both tooth and implant-supported full-contour ceramic restorations (11,12). Another advantage of monolithic zirconia materials was shown in an earlier study, with the results indicating that such restorations can withstand forces in the molar region even at a reduced thickness. Therefore, limiting the abutment preparation can preserve both the tooth substance and the axial height, promoting retention and resistance (12).

The success of zirconia-based restorations is highly dependent on achieving a reliable adhesion between the zirconia and resin cement. A strong adhesion of luting cements to the restoration improves marginal adaptation, prevenents microleakage, and increases retention where sufficient mechanical retention does not exist (13). Unfortunately, unlike glass ceramics, zirconia is an acid-resistant and non-etchable material (14) because of its glass-free, polycrystalline microstructure (15), and therefore requires more aggresive treatment methods (16). Airborne-particle abrasion, one of the common surface treatment methods for zirconia, creates micron-sized rough areas that provide an increased surface area required for micro-mechanical interlocking (17,18). The effects of this treatment are still controversial with some studies showing a strength-reducing effect that may be related to deep surface flaws which act as stress concentrators (19,20); on the contrary, others have reported a strengthening effect which may be due to a compressive layer formed via tetragonal to monoclinic phase transformation (4,21). This stress-induced transformation leads to a volume expansion which generates compressive stresses, thereby closing the crack tip and preventing further crack propagation (2). In fact, the relative importance of these countervailing effects may depend on the material microstructure and the severity of the air-abrasion treatment (4,19). Therefore, to minimize possible surface damage, application of air-abrasion treatments at moderate or low pressures has been recommended (22-24).

In addition to damage-inducing effects, surface roughening using airborne-particle abrasion may not always produce a reliable bond between resin and zirconia (16,25). Application of a primer onto the zirconia surface can improve the resin-bond strength, via chemical interaction between the ceramic surface and the applied resin cement (23,25,26). The 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer has been used by several investigators for this purpose (25-30), with effective bonding between the MDP acidic groups (phosphoric acid) and the oxide layer of the zirconia.

It was previously noted that chemical bonding to oxide ceramics obtained through the use of primers promotes long-term success under clinical conditions (22). However, the findings of another study indicated that without airborne-particle abrasion, MDP-containing materials were not capable of maintaining durable long-term adhesion (29). Overall, limited information is available regarding the use of MDP-containing primers in combination with air-particle abrasion. Therefore, to guide clinical practice, it would be useful to assess whether the use of an MDP primer applied to the airborne-particle-abraded zirconia surface is beneficial compared to air-abrasion treatment alone. Clearfil ceramic primer plus, a commercially available priming agent, contains a silane bi-functional molecule; however, it is not well-understood if MDP in combination with silane has a synergistic or antagonistic bond-promoting effect (31).

The choice of the most proper cement is a prerequisite for efficient bonding between resin and zirconia (32). Conventional resin cements require independent pretreatment procedures such as etching, priming, and bonding to achieve adequate adhesion; therefore, this multi-step cementation procedure is technique-sensitive, unpredictable, and time-consuming. To simplify the process, self-adhesive resin cements that rely on a single-step process have been suggested for luting of zirconia-based restorations (18,32,33). The resin matrix of these systems contains multifunctional acid methacrylates that react with the substrate to improve adhesion (32). In addition, various self-adhesive resin cements consist of phosphate monomer, including MDP. Although manufacturers suggest that clinicians may apply self-adhesive resin cements to Y-TZP without an additional primer (18,34), there is no sufficient information regarding the bonding efficiency of phosphate monomers in self-adhesive resin cements (34). Further, because of the limited information available regarding the use of MDP-based primers on zirconia combined with self-adhesive resin cements, investigations in this area are necessary.

The purpose of the present study was to investigate the shear bond strength (SBS) of different self-adhesive resin cements to air-abraded monolithic zirconia ceramics with or without application of an MDP-containing ceramic primer. The two null hyphotheses were that (i) there would be no difference between the SBS values of different resin cements and (ii) the application of MDP-containing primer would not change the SBS values.

Materials and Methods

Preparation of Zirconia Specimens

A total of 200 rectangular-shaped specimens were cut from a monolithic Y-TZP ceramic block (Zenostar T, Wieland Dental GmbH, Pforzheim, Germany) using a water-cooled low speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) and polished manually with 600, 1,000, and 1,200-grit silicon carbide abrasive papers (English Abrasives & Chemicals Ltd., London, UK) under running water to obtain standardized smooth surfaces. Next, all the specimens were sintered to full density following the manufacturer's instructions. 2-mm-thick slices were carefully embedded in autopolymerysing acrylic resin, and one surface of the sample was left uncovered to adhere to the resin cement. The zirconia surfaces were airborne-particle-abraded with 50-µm Al_2O_3 particles (Mega Strahlkorund, Mega Dental, Budingen, Germany), under a 2.5-bar pressure for 15 s. The samples were placed at a distance of 10 mm from the handpiece of the sandblaster unit (Bego Easyblast, Bego, Germany) during the air-abrasion treatment. All the surface-treated zirconia specimens were cleaned ultrasonically with 96% isopropanol for 180 s in an ultrasonic cleaner (Whaledent Biosonic, Whaledent Inc., New York, USA) and air-dried to remove the debris of aluminum oxide particles from the ceramic surface. The specimens were then randomly divided into 20 subgroups, each containing 10 specimens, based on the 10 different self-adhesive resin cements combined with or without primer application. The manufacturers and compositions of the resin materials used in the study are summarized in Table 1.

Bonding, Thermocycling, and Shear Bond Strength Test Procedures

First, an MDP-containing primer (Clearfil Ceramic Primer Plus, Kuraray Noritake, Okayama, Japan) was applied on one half of the air-abraded zirconia specimens in each resin cement group using an applicator microbrush, and the adherent surfaces were dried sufficiently using oil-free air. Subsequently, specific teflon molds possesing a central cylindirical

Table 1. List of resin cements and their characteristics

Self-adhesive resin cement	Manufacturer	Main composition
RelyX U200	3M ESPE, St.Paul, MN, USA	Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, alkaline (basic) fillers; silanated fillers; initiator components; stabilizers; pigments; rheological additives
TheraCem	BISCO, Schaumburg, IL, USA	Calcium base filler, glass filler, 10-MDP, bisphenol A diglycidylmethacrylate, dimethacrylates, 2-hydroxyethyl methacrylate, ytterbium flüoride, initiator, amorphous silica
Panavia SA Cement Plus	Kuraray Noritake, Okayama, Japan	10-MDP, Bis-GMA, TEGDMA, HEMA, hydrophobic aromatic dimethacrylate; hydrophobic aliphatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, di-camphorquinone, peroxide, catalysts, surface treated sodium fluoride, accelerators, pigments
G-Cem LinkAce	GC Corporation, Tokyo, Japan	UDMA, dimethacrylate, fluoroalumino silicate glass, initiator, pigment, silicone dioxide, inhibitor
PermaCem 2.0	DMG, Hamburg, Germany	Barium glass, Bis-GMA, pigments, additives, catalysts
BisCem	BISCO, Schaumburg, IL, USA	Bis-GMA, uncured dimethacrylate monomer, glass filler, phosphate acidic monomer
SmartCem 2	Dentsply Caulk, Milford, DE, USA	UDMA, di- and tri-methacrylate resins, phosphoric acid modified acrylate resin, barium boron fluoroaluminosilicate glass, organic peroxide initiator, camphorquinone photoinitiator, phosphene oxide photoinitiator, accelerators, butylatedhydroxytoluene, UV stabilizer, titanium dioxide, iron oxide, hydrophobic amorphous silicon dioxide
Maxcem Elite	Kerr Corporation, Orange, CA, USA	GPDM, co-monomers (mono-, di-, and tri-functional), proprietary self-curing redox activator, methacrylate monomers, water, acetone, and ethanol, inert minerals and ytterbium fluoride
ZenitCem	President Dental, Munich, Germany	Barium glass, Bis-GMA, pigments, additives, catalysts
Bifix SE	Voco GmbH, Cuxhaven, Germany	UDMA, Bis-GMA, Gly-DMA, phosphate monomers, initatiors, stabilizer, glass

UDMA: Urethane dimetacrylate, 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate, Bis-GMA: Bisphenol A glycidyl methacrylate, TEGDMA: Triethyleneglycol dimethacrylate, HEMA: Hydroxy-ethyl methacrylate, GPDM: Glycero-phosphate dimethacrylate, Gly-DMA: Glycerol dimethacrylate

chamber (inner diameter, 3 mm; height, 3 mm) were placed on the zirconia surface and mixed self-adhesive dual-curing resin cements were applied into the molds following the manufacturers' instructions. Self-adhesive resin cements were light polymerized for 40 s with a light-emitting diode curing unit (1,200 mW/cm^2, Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein). After the bonding procedures, the teflon molds were gently removed from the bonded specimens. All the resin-bonded zirconia specimens were stored in distilled water at 37 °C for 24 h and then thermocycled 5,000 times between 5 and 55 °C with a dwelling time of 30 s at each temperature. The SBS of the specimens were measured using a knife edge rod mounted on a universal test machine (TSTM 02500, Elista Ltd., İstanbul, Turkey) at a crosshead speed of 1 mm/min. The shear load at failure was recorded and the SBS was calculated using the following formula:

SBS (MPa) = Failure Load (N)/Area (mm^2)

Finally, the failure modes were examined under a strereomicroscope (Olympus SZ40; Olympus Optical Co., Tokyo, Japan). Failure types of the tested specimens were classified as adhesive, mixed, or cohesive.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov and Levene tests were used to assess normal distribution of the SBS data and homogeneity of variances, respectively. The SBS data were statistically analyzed using two-way analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) test and independent sample t-tests were used for pairwise comparisons. The significance level was set at α=0.05 for all statistical tests.

Results

The two-way ANOVA revealed an individual significant effect of both primer application and different self-adhesive cements on the SBS, as shown in Table 2 ($p<0.001$). The mean and standard deviation values, and comparisons for the subgroups are shown in Table 3. Additionally, a box-plot graph is shown, with the distributions of SBS values including median, minimum, and maximum values of the groups (Figure 1). Primer application significantly increased the shear bond values in all cements ($p<0.001$). The results of Tukey's HSD showed that Maxcem Elite had the highest SBS values in both primer-applied and non-applied groups ($p<0.05$), whereas the SBS values of SmartCem 2, Bifix SE, and RelyX U200 cements were lower than those of the other cements ($p<0.05$). Panavia SA Cement Plus had higher and TheraCem had lower SBS values compared to G-Cem LinkAce in the groups without primer application ($p<0.05$), but no statistically difference was found between SBS values of these cements in primer-applied groups ($p>0.05$).

As shown in Figure 2, adhesive and mixed failure types were evident in all experimental groups, whereas no cohesive failure of the resin cement was observed. Mixed failures were more predominant in primed groups than in non-primed groups for each resin cement. In the primer-applied Maxcem Elite group, adhesive failure occurred in only one specimen, while non-primed Bifix SE and SmartCem 2 showed the highest adhesive failure rates, in nine specimens.

Discussion

The present study was designed to compare the SBS of different self-adhesive resin cements to a monolithic zirconia ceramic with or without application of a MDP-containing primer. The two null hypotheses were rejected because use of different resin cements or use of primer application significantly changed the SBS values. Primer application resulted in higher SBS values for each resin cement.

All the zirconia ceramic surfaces were subjected to airborne-particle abrasion in the study. It has

Table 2. Summary of two-way ANOVA results

	Type III sum of squares	df	Mean square	F	p
Cement	1205.216	9	133.913	146.953	<0.001
Primer	881.790	1	881.790	967.659	<0.001
Cement x Primer	48.637	9	5.404	5.930	<0.001

P<0.05 indicates statistically significance, df: degrees of freedom

Table 3. Mean and standard deviation values and group comparisons

	Primer +			Primer -		
	Mean	SD	*	Mean	SD	*
SmartCem 2	8.56	0.91	A, a	4.03	0.70	A, b
Bifix SE	8.72	0.70	A, a	4.23	0.48	A, b
RelyX U200	9.44	0.90	A, a	4.20	0.88	A, b
Zenitcem	11.09	0.91	B, a	8.72	0.56	CD, b
TheraCem	12.67	1.31	C, a	6.59	0.75	B, b
G-cem LinkAce	12.77	1.09	C, a	8.23	0.99	C, b
Panavia SA Cement Plus	12.97	1.36	C, a	9.81	1.01	D, b
PermaCem 2.0	13.11	1.30	C, a	9.32	1.19	CD, b
BisCem	13.79	0.86	C, a	9.91	1.15	D, b
Maxcem Elite	16.08	0.67	D, a	12.18	0.73	E, b

SD: Standard deviation

*Same uppercase letters in the same column (Tukey's HSD, $p>0.05$) and same lowercase letters in the same row (Independent samples t-tests, $p>0.05$) denote subgroups that were not significantly different

been reported that airborne-particle abrasion under relatively higher pressures might compromise the mechanical strength of the zirconia ceramic, and that to avoid possible excessive surface damage, zirconia should be abraded using 50-µm particles with a pressure of 2.5 bar or less (22). The alumina blasting parameters used in the present study have also been used in other studies (17,23,24,35), and allow sufficient surface roughening and cleaning (22). Such surface treatment may increase the surface area, surface energy, and wettability, thus facilitating the resin to flow into the surface (6,16). Moreover, the

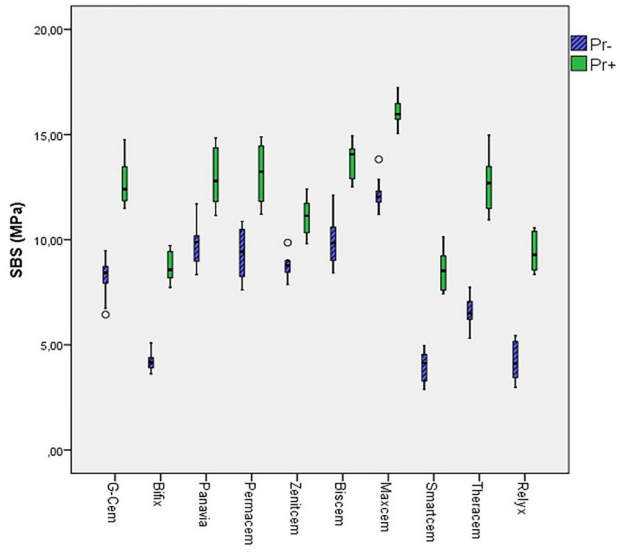

Figure 1. Box-plot graph based on the SBS values of 20 subgroups (n=10). Data are presented as median and 1st and 3rd quartile. The median is shown with a horizontal line within the box. The maximum and minimum values are illustrated with the upper and lower strokes. 0 marks outliers

SBS: Shear bond strength

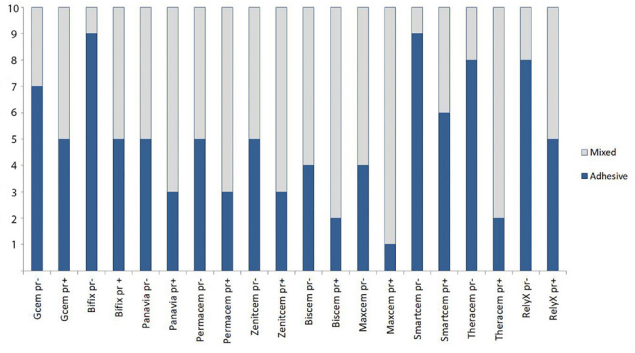

Figure 2. The failure types in each group. The vertical axis of the graph indicates the number of specimens and the bars represent the ratio between the failure types

abrasion process may generate hydroxyl groups on the Y-TZP surface that enable chemical bonding (34). In one clinical trial (36), air-abrasion was not performed and MDP-containing luting cements and primers were used on the as-machined zirconia surface; the authors reported a debonding rate of 13.3% over an observation time of 53 months. Similarly, findings of a study by de Souza et al. (29) revealed that the application of MDP-containing primers and adhesives to a non-airborne-particle-abraded zirconia surface increased the initial resin bond strength but no stable bonding was present after six months of water storage. Therefore, omitting the abrasion process appears to increase the risk of debonding when using a MDP monomer. In a meta-analysis of bonding to zirconia, Inokoshi et al. (31) also concluded that the combination of mechanical and chemical pre-treatments contributed to durability of resin bonding. Additionally, the study emphasized that application of MDP-based primer after Al_2O_3 blasting may lead to increased aging resistance of the cement-zirconia bond (31), but that further investigation is needed.

MDP-based ceramic primer application significantly increased the SBS of all self-adhesive resin cements used in the present study. Further, increased ratios of mixed failure types observed in primer-applied groups supported the findings. The positive influence of priming may be due to enhanced physicochemical interaction between resin and zirconia (37), and also due to the increased wettability of the zirconia surface (31). Phosphoric groups in MDP yield certain chemical reactions with hydroxyl groups of zirconia (37,38), while the decyl group in MDP prevents water penetration at the interface between the dihydrogen phosphate and metal oxide layers (39). This was confirmed in a recent study by Yang et al. (30), in which the durability of resin bonding was evaluated and a remarkable reduction in SBS in all experimental groups was seen after long-term artifical aging, with the exception of groups treated with an MDP-based zirconia primer. In another investigation, Yagawa et al. (26) concluded that application of primers containing MDP ensured durable bond strength after 5,000 thermocycles; the authors also reported that MDP-based primers provide higher bond strengths than priming agents containing carboxylic monomer (4-META). In agreement with the results of the present study, those in a study by Kitayama et al. (6) indicated

that primers containing a phosphate ester monomer were effective in improving bonding of resin cements to zirconia. On the other hand, the authors also concluded that even without primer application, the MDP-containing resin cement was effective in bonding. Another study conducted by Wegner and Kern (40) on two-year tensile bond strength showed that the functional phosphate ester groups in MDP yielded water-resistant chemical adhesion with the zirconia; MDP-based resin cements are recommended in clinical practice based on their results. However, these findings are not consistent with our results, because there was no association between the MDP content of resin cements and the bond strength values. The results of the present study indicate that MDP-containing self-adhesive resin cements (TheraCem and Panavia SA Cement Plus) did not have the highest SBS values. Similarly, in a previous study, de Souza et al. (41) reported that application of an MDP-containing primer may increase the bond strength; however, an MDP-based luting system did not increase bond strength to zirconia; in addition, the aged specimens showed lower values. In agreement, Zhao et al. (25) also showed no positive effect of the presence of MDP in resin cement on SBS values after long-term aging. As stated in earlier studies, the lack of correlation between MDP content of resin cement and higher bond strength values may be related to the concentration of MDP in the resins and differences in viscosities of the cements (29,42). In another study, de Souza et al. (29) compared the bond strength of MDP-based resin cement with that of a non-MDP-containing resin cement. The similarity in bond strength values between these resin cements have been attributed to relatively higher viscosity of the MDP-containing resin cement used in the above study. Resin cements with low viscosity can easily flow into the microporosities of the air-particle abraded zirconia surface; thus, a larger adhesive surface can be obtained (42). The SBS values showed significant differences among the 10 different commercially available resin cements in the present study. Regardless of the primer application, Maxcem Elite showed higher SBS values and Smart CEM showed lower SBS values compared to the others. As discussed previously, other factors that may influence bonding capacity include mechanical properties, wetting capacity, and variation in chemical composition of different luting cements (29). In addition, Thompson et al. (16) highlighted the fact that the composition of zirconia ceramics could influence the resin bond strength. The present study mainly focused on the effect of priming combined with different resin cements and therefore, a single standardized monolithic zirconia was used as the ceramic material to understand these effects more clearly.

Thermocycling is a frequently used *in vitro* study method because it may influence bonding, especially in high-strength ceramics (26,40). This method can imitate clinical conditions by inducing thermal stresses and hydrolytic effects (43). Therefore, in the present study, 5,000 thermal cycles in water beween 5-55 °C were done. Even if these parameters were considered as an appropriate aging regimen as reported in a previous study (26), long-term aging conditions should also be investigated. The other limitation of our study was that only Clearfil Ceramic Primer was used as MDP-containing primer. This primer is known to contain a silane agent. Future studies should be focussed on other types of primers that have different compositions, or on usage of such silane-containing MDP-based primers in combination with tribochemical silica-coating techniques. Finally, the *in vitro* design of this study may not fully represent the intra-oral conditions. For instance, the resin cement thickness formed in clinical conditions could not be simulated, which is also an important factor in bonding efficiency (16). Therefore, further investigations are needed to understand the effects of zirconia primers on the bonding of different resin cements in both *in vivo* and *in vitro* conditions using different materials, test designs, and aging conditions.

Conclusion

Under the conditions of the present *in vitro* study, the following conclusions can be made:

1) Application of an MDP-containing primer was found to be effective in improving the SBS of resin cements to airborne-particle-abraded zirconia.

2) Regardless of primer use, SBS values of various self-adhesive resin cements were significantly different from each other.

3) There was no association between the MDP content of the resin cements and the SBS values because MDP-based resin cements used did not result in the highest bond strengths.

4) Mixed/adhesive failure type ratios for each resin cement were increased due to the application of an MDP-based primer.

Ethics

Ethics Committee Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: For this type of study, informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.T.Y., M.B.D., T.G., Design: Y.O., M.T.Y, M.B.D., Supervision: M.T.Y., Y.O., Fundings: M.T.Y., Data Collection or Processing: T.G., M.T.Y., M.B.D., Analysis or Interpretation: Y.O., M.T.Y., M.B.D., T.G., Literature Search: Y.O, M.B.D., T.G., Writing: Y.O., Critical Review: Y.O., M.T.Y., M.B.D., T.G.

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Original Article / Özgün Araştırma

Effect of Different Heat-treated Nickel-Titanium Files on Vertical Root Fracture Resistance

Farklı Isıl İşlem ile Üretilmiş Nikel-Titanyum Eğelerin Vertikal Kök Kırık Direncine Etkisi

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Abstract

Objective: This study aimed to evaluate the fracture resistance of teeth instrumented with rotary files manufactured from different heat-treated nickel-titanium (NiTi) wires.

Materials and Methods: One hundred extracted single-canal mandibular premolar teeth were sectioned, leaving a standard root length of 13 mm. Samples were matched with respect to the buccolingual-mesiodistal diameters. They were randomly divided into four experimental groups and one control group (n=20): Control group, no preparation or obturation; ProTaper Universal (PTU) group, prepared with the PTU system as a universal NiTi; Twisted File (TF) group, prepared with the TF system as an R-phase treatment; ProTaper Next (PTN) group, prepared with the PTN system as an M-Wire thermal treatment; and HyFlex Controlled Memory (HCM) group, prepared with the HCM system as a CM-Wire thermal treatment. After preparations, samples were obturated with gutta-percha and sealer. Samples were subjected to a vertical loading force of 1 mm/min until fracture occurred. Data were subjected to statistical analysis using One-way ANOVA (p=0.05) and Tukey tests.

Results: Although the control group had the highest root fracture resistance value, the differences between the control group and HCM group was not significant (p>0.05). Except for the control group, the differences among the study groups were not significant (p>0.05).

Conclusion: Different heat treatments of instruments have no effects on vertical root fracture resistance.

Öz

Amaç: Bu çalışmanın amacı, farklı ısıl işlem görmüş nikel-titanyum (NiTi) tellerinden üretilen döner eğeler ile prepare edilen dişlerin kırılma direncini değerlendirmektir.

Gereç ve Yöntemler: Yüz adet tek kanallı mandibular premolar diş standart kök uzunluğu 13 mm olacak şekilde separe edilmiştir. Örnekler mesiodistal ve bukkolingual boyutları benzer olacak şekilde seçildi (p>0,05). Örnekler 4 çalışma grubu ve 1 kontrol grubu olacak şekilde rastgele dağıtıldı (n=20): Kontrol grubu; preparasyon ve obturasyon yapılmadı. Grup ProTaper Universal (PTU); PTU sistemi, grup Twisted File (TF); TF sistemi, grup ProTaper Next (PTN); PTN sistemi, Grup HyFlex

Controlled Memory (HCM); HCM sistemi kullanılarak örneklerin preparasyonu tamamlandı. Preparasyonların ardından örnekler güta perka ve kanal patı ile dolduruldu. Örneklere kırık oluşana kadar 1 mm/dk hızında vertikal yükleme yapıldı. Elde edilen verilerin istatistiksel analizi Tek yönlü varyans analizi ve Tukey testi ile gerçekleştirildi (p=0,05).
Bulgular: Kontrol grubu en yüksek kırık direnci değerini gösterirken grup HCM ile aralarında istatistiksel olarak anlamlı bir fark bulunamadı (p>0,05). Çalışma gruplarının aralarında da anlamlı bir fark bulunamadı.
Sonuç: Eğelere uygulanan ısıl işlemlerdeki farklılığın vertikal kök kırık direnci üzerine etkisi bulunmamaktadır.

Introduction

One of the most severe complications related with the root canal treatment is the vertical root fractures and they were reported to frequently cause tooth extraction (1). The main reasons for the higher fracture susceptibility of the teeth, which have endodontic treatment history, are the cumulative loss of tooth structure from the caries, as well as the trauma and the restorative and endodontic procedures (2). It is known that many predisposing factors increase the predilection of the root-filled teeth to vertical root fractures (VRFs). Some of these predisposing factors are the removal of an excessive amount of dentine during the operative procedures or root canal preparation (3), post-space preparation (4), and the dentinal defects emerging in these procedures.

It was concluded that, the root canal preparation performed by using nickel-titanium (NiTi) instruments induced more dentinal damage when compared to the level of damage caused by the hand files (5). In the procedures performed by using NiTi instruments, a force is inevitably applied on or around the apex. As a result of these forces, the strain on the dentinal walls increases and, thus, the dentinal micro-crack occurs (6). In previous researches (6,7) it was reported that different levels of damages on the root canal wall were observed when different root canal shaping instruments were utilized.

Many different NiTi rotary file systems have been introduced to the market by different companies. These instruments have different characteristics in terms of the design of cutting blades, as well as the body taper and tip configuration. Moreover, different thermal treatments are applied in order to improve the characteristics of NiTi instruments such as the cyclic fatigue resistance, shaping efficiency, and transportation amount. In the production of Twisted File (TF; SybronEndo, Orange, CA), which has been developed in year 2008, three methods are combined in the production process. These methods are heat treatment (R-phase), twisting the metal wire, and special surface conditioning. ProTaper Next (PTN; Dentsply Sirona, Ballaigues, Switzerland) systems are produced by making use of M-Wire technology and these instruments have an off-centered rectangular design, as well as the progressive and regressive percentage tapers on a single file. It was reported that the snake-like motion minimized the contact between the instrument and dentin and, thus, reduced the screw effect, dangerous taper lock, and torque on the files (8). Introduced to the market in year 2011, the HyFlex Controlled Memory (HCM; Coltene-Whaledent, Allst€atten, Switzerland) is a NiTi rotary system is made of CM alloy. The CM alloy used in the production of NiTi files are of no shape memory, which is seen in traditional NiTi files, and the thermo-mechanical method employed in manufacturing the HCM instruments aims to improve the flexibility of traditional NiTi files (9).

In the recent years, different researchers investigate the effects of different rotary file systems (10,11), preparations performed with different tapers (12,13), and different kinematics (14) on the vertical root fracture of the teeth, which have root canal treatment history. In the present study, it is aimed to examine the effects of various instruments, which have been exposed to heat treatments, on the fracture resistance of the roots, which have endodontic treatment history. The null hypothesis of the present study is that different heat treatments would have no effect on the vertical root fracture resistance.

Materials and Methods

For the study Ethics Committee approval was received by Ondokuz Mayıs University (decision no: KAEK 2014/663, date: 24.04.2014). A hundred mandibular premolar teeth, which were extracted for orthodontic reasons, colected and stored in saline solution for the study. The teeth were examined radyografically. Single and straight roots with matured

apex were determinated and included the study. Under water cooling, coronal parts of the teeth were removed from the roots with diamond coated bur. The root lengths were standardized to 13 mm. Roots examinated with stereomicroscope at x10 magnification and undamaged roots were included the study. For standardization, dimensions of roots at the coronal ends which range from 4-6 mm in buccolingual (BL) aspects, 2-4 mm in mesiodistal (MD) aspect were choosed (15). All samples were randomly divided into five groups (n=20). Analysis of variance test performed to evaluate size differences of the roots and no significant differences were found [BL (p=0.687), MD (p=0.999)] between groups. All root canals were checked for any calsifications or blockage with a size 15 K-file. Then root canal preparations were performed with different NiTi systems.

Root Canal Preparations

The study groups are given in Table 1.

In control group, no instrumentation or obturation was performed.

Group ProTaper Universal (PTU): The root canals were englarged by single experienced operator with the PTU rotary system driven by a torque-controlled endodontic motor VDW Gold (VDW, Münih, Germany) according to the manufacturer's instructions. The SX file was used with a brushing motion at coronal part of the root; then S1, S2 files were placed in the root canal and withdraw the file once the working length (WL) is reached. The root canals were prepared with F1 (20/.07), F2 (25/.08), F3 (30/.06), and F4 (40/.06) files respectively with a gentle in-and-out motion.

Group TF: The root canals were prepared with the TF system according to the manufacturer's instructions, using VDW Gold. The TF files (20/.04), (25/.06), (25/.08), (30/.06), (35/.06), and (40/.04) were used to the WL with a gentle in-and-out motion at 500 rpm and 4 Ncm torque.

Group PTN: The root canals were prepared with the PTN system using at 300 rpm and 2 Ncm torque using a VDW Gold. The SX file was used at coronal part of the root with a brushing motion. The root canal preparation was performed up to X4 at the WL with a gentle in-and-out motion. The files were used respectly; X1 (17/.04), X2 (25/.06), X3 (30/.06), and X4 files (40/.06).

Group HCM: The HCM system were used in a gentle in-and-out motion with a rotational speed of 500 rpm and 250 g/cm torque. The HCM were used in the sequence of 25/.08 (two thirds of the WL), 25/.06, 30/.06, and 40/.04 (the full WL).

During preparation, each sample was irrigated with 5 mL of 2.5% sodium hypochlorite with a side vented needle. After instrumentation, the root canals were irrigated with 2 mL of 17% ethylenediaminetetraacetic acid for 3 min, and the final irrigation was performed by 5 mL 2.5% sodium hypochlorite. The root canals dried with their respective paper points. The root canal obturation was performed using the single cone technique with competible gutta percha systems and

Table 1. Thermal treatment methods and NiTi files included in the study

Groups	Thermal treatment methods	NiTi system and manufacturer
Group PT	Universal NiTi	ProTaper Universal; Dentsply, Sirona
Group TF	R-phase	Twisted File; SybronEndo
Group PTN	M-Wire	Protaper Next; Dentsply, Sirona
Group HCM	Controlled Memory	HyFlex CM; Coltene-Whaledent

NiTi: Nickel-titanium, PT: ProTaper, TF: Twisted File, PTN: ProTaper Next, HCM: HyFlex Controlled Memory, CM: Controlled Memory

Table 2. Fracture loads of the roots as mean, maximum, and minimum Newton

		NiTi System			
	Control	ProTaper Universal	Twisted File	ProTaper Next	HyFlex CM
Mean	143.94±32.7[b]	105.31±20.07[a]	111.21±26.21[a]	102.38±20.20[a]	121.57±43.29[ab]
Maximum	206.96	138.68	157.99	145.68	237.99
Minimum	83.02	72.59	69.16	69.63	60.73

*Different superscript letters indicate a significant difference (p<0.05). CM: Controlled Memory, NiTi: Nickel-titanium

resin based sealer; AH Plus (Dentsply, De Trey, Kontanz, Germany). The coronal excess of the specimen was sealed with temporary filling material (3M ESPE, St. Paul, MN, USA), and the roots were kept in 100% humidity for 2 weeks.

Fracture Resistance Test

Acrylic resin blocks (25 mm high and 10 mm in diameter) were prepared using cylindrical plastic molds with self-cured acrylic resin (Imicryl, Konya, Turkey). The apical root ends were embedded vertically in 4 mm of the acrylic resin, exposing 9 mm of the coronal portion of each root (Figure 1) (10). For preventing dehydration, the roots were kept wet with a wet towel until strength test. Acrylic blocks including specimens were placed in a universal testing machine (Autograph AG-X; Shimadzu Corporation, Kyoto, Japan). A compressive vertical loading at a speed of 1 mm/min was applied. The force at which the fracture occurred was recorded in Newtons.

Statistical Analysis

SPSS (SPSS Version 20, SPSS Inc, Chicago, IL) software was used for statistical analysis. Descriptive statistics, Shapiro-Wilk, One-way ANOVA and Tukey analysis were performed. The results for $p<0.05$ was considered statistically significant.

Results

Table 2 summarizes the means and standard deviations and minimum and maximum fracture loads of the experimental groups. Group HCM was the most resistant to fracture (121.57±43.29N), and group PTN was the least resistant (102.38±20.20N). The mean of the fracture values is shown graphically in Figure 2.

Discussion

The diagnosis and treatment of the vertical root fracture are very difficult and it may require the extraction of the tooth (2,16,17). In literature, there are various studies carried out on the instrument diameters, different kinematics, and root canal filling methods influencing the vertical root fracture. However, there is no study examining the heat treatments applied to the instruments. For this reason, the present study aims to compare the VRF resistances of the teeth by making use of various NiTi files, which have been exposed to different thermal procedures. In the present study, there were no significant difference between PT, TF, PTN and HCM ($p\geq0.05$). Thus, the null hypothesis was accepted.

In the present study, it was aimed to maximally standardize all of the controllable variables. The BL and MD widths of the samples were examined and included those that were not statistically different between them ($p>0.05$). As stated in previous studies (10,11,15), the differences between the dimensions of the roots influence the residual dentin thickness that will be seen after the instrumentation by using different tapers. Thus, the apical diameters of all the root canals were enlarged to ISO #40 (10-13,18). Root canal preparations were completed by selecting different heat-treated files with this apical diameter (PTU 40/.06, TF 40/.04, PTN 40/.06, HCM 40/.04). This situation created a difference in the taper of the preparations. Actually, one of the factors affecting the predisposition to vertical root fracture is the diameter of the prepared canal. As reported in previous studies

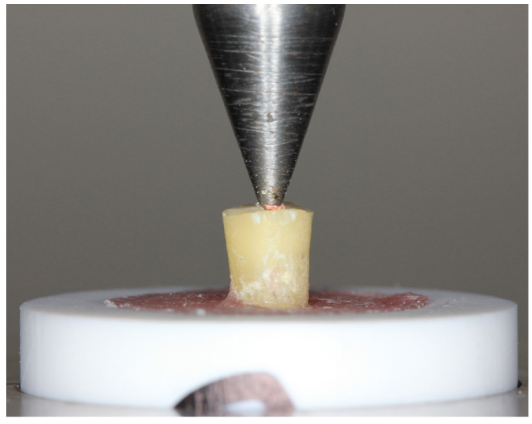

Figure 1. A specimen prepared for Instron machine to test the fracture strength

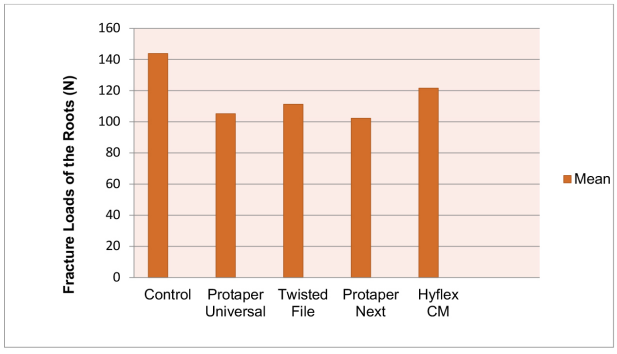

Figure 2. Fracture loads of the roots as mean
CM: Controlled Memory, N: Newton

(3,4,19,20), it is known that the fracture resistance of the teeth, which have endodontic treatment history, is directly proportionate to the amount of remaining sound tooth structure. Wilcox et al. (19) reported that the preparation performed by using hand files or the lateral condensation does not damage the tooth but the tooth would be more inclined to fracture when an excessive amount of dentin is removed. Krikeli et al. (13) examined the preparations performed using different tapers in terms of the vertical fracture resistance, and authors reported that the roots enlarged using .06 taper had less resistance to the fracture than in the control group, whereas there was no significant difference between the control group and the other groups, in which the enlargement was performed using smaller tapers, in terms of fracture resistance.

On the contrary in the present study; although both HCM and TF have a .04 taper, there is no difference between the HCM group and control group, while there is a difference between the TF group and the control group. This difference may be due to the difference in material between files. Abou El Nasr et al. (14) in the study comparing the effect of the material used and kinematics on fracture resistance, reported that the material difference had an effect on fracture resistance. In addition, M-wire and Cm-Wire wires are more flexible (21) and it has been reported in studies that HCM has a better centering ability compared to TF (22,23). Thus, the original form of the root canal is protected and the increase in the risk of vertical fracture might be eliminated by preventing the formation of thin dentin zones (20,24). In the present study, the statistically insignificant difference between HCM system and control group ($p \geq 0.05$) may arise from the .04 taper and CM heat treatment.

In many studies, it was emphasized that the NiTi instruments may lead to cracks in the root dentine (6,7,25) and this may cause the vertical root fractures. It is believed that these micro-cracks, which are seen on the dentinal walls after the preparation, create stress accumulation zones on the dentinal walls and cause VRF formation when an external force is applied (26). In previous studies, it was emphasized that the taper of files might be the most important factor playing role in the formation of dentinal cracks (27,28). In addition to the studies; reporting that different file systems caused micro-cracks but HCM system did not (7,29), also there is a study reporting that HCM caused less micro-crack formation than PTU and PTN systems did (30). In the present study, it was found that there is no statistically significant difference between HCM system and control group ($p \geq 0.05$). When compared to the other groups in this study, HCM system created less or no micro-crack. In the recent studies, it was reported that there were micro-cracks before the root canal preparation (7,31) but the number of micro-cracks increased after the preparation (32). De-Deus et al. (33) reported that the micro-cracks do not exist in the teeth in the intraoral environment but they form under the storage conditions, whereas Shemesh et al. (34) reported that the dehydration played an effective role in the formation of dentinal micro-cracks. Further studies examining the formation of micro-crack and its relationship with the vertical fracture are need.

Conclusion

Within the limitations of the present study, preparations of roots with PTU, TF, and PTN reduced the vertical fracture resistance of roots when compared with the control group. In addition fracture resistance of preparation of roots with HCM did not showed differences from control group, significantly. Further studies are warranted to evaluate the effect of heat treatment of NiTi files on the mechanical properties of endodontically treated teeth.

Ethics

Ethics Committee Approval: For the study Ethics Committee approval was received by Ondokuz Mayıs University (decision no: KAEK 2014/663, date: 24.04.2014).

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.F., E.K., Design: F.F., E.K., Supervision: E.K., Fundings: F.F., E.K., Materials: F.F., Data Collection or Processing: F.F., E.K., Analysis or Interpretation: F.F., E.K., Literature Search: F.F., Writing: F.F., Critical Review: E.K.

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Original Article / Özgün Araştırma

The Effects of Manual and Powered Brushing with a Tooth Brush on Surface Roughness Alteration of Different Resin and Glass Ionomer-based Restorative Materials: An *In Vitro* Study

Manuel ve Elektrikli Diş Fırçalamanın Rezin ve Cam İyonomer Esaslı Farklı Restoratif Materyallerin Yüzey Pürüzlülüğünün Değişimi Üzerine Olan Etkileri: Bir İn Vitro Çalışma

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Abstract

Objective: This study aimed to present a comparative evaluation of the effects of manual and powered brushing with a tooth brush on surface roughness of different resin and glass ionomer-based restorative materials.

Materials and Methods: A total of 160 discs were prepared from four different restorative materials (resin, compomer, high-viscosity glass ionomer and conventional glass ionomer-based materials). Half of the specimens from each group were brushed manually, and the other half were power-brushed. The surface roughness (Ra) values before and after brushing (ΔRa) were measured, recorded and statistically analysed. Student's t-test, Mann-Whitney U and Kruskal-Wallis H tests were used for statistical comparisons. The statistical significance level was determined as 5%.

Results: An increase in the surface roughness was observed in all restorative materials after manual and powered brushing. However, no statistically significant difference was observed among the different restorative materials in terms of the increase in roughness ($p>0.05$). Additionally, no statistically significant difference was found between manual and electric brushing in terms of the increase in surface roughness of the restorative materials ($p>0.05$).

Conclusion: The restorative materials examined in this study did not have any superiority or disadvantage over each other in terms of the increase in surface roughness after manual and powered brushing. In addition, as powered brushing does not lead to extra roughness compared with manual brushing and owing to its other advantages, the use of powered toothbrushes can be recommended for improving oral hygiene in children.

Öz

Amaç: Bu çalışma, manuel ve elektrikli diş fırçalamanın farklı rezin esaslı ve cam iyonomer esaslı restoratif materyallerin yüzey pürüzlülüğüne olan etkilerinin karşılaştırmalı olarak değerlendirilmesini sunmayı amaçlamıştır.

Gereç ve Yöntemler: Dört farklı restoratif materyale ait (rezin, kompomer, yüksek vizkoziteli cam iyonomer ve geleneksel cam iyonomer esaslı materyaller) 160 adet restoratif materyal diskleri hazırlanmıştır. Her gruba ait örneklerin yarısı manuel, diğer yarısı da elektrikli fırça ile fırçalanmıştır. Fırçalama öncesi ve sonrası yüzey pürüzlülük değerleri (Ra) ölçülmüş, kaydedilmiş ve pürüzlülük değişim (ΔRa) değerleri istatistiksel olarak analiz edilmiştir. İstatistiksel karşılaştırmalarda, Student t-test, Mann-Whitney U ve Kruskal-Wallis H testleri kullanılmıştır. İstatistiksel anlamlılık düzeyi %5 olarak alınmıştır.
Bulgular: Hem manuel hem de elektrikli fırçalamadan sonra, tüm restoratif materyallerde, yüzey pürüzlülüğünde artış bulunmuş ancak restoratif materyaller arasında pürüzlülük artışı açısından istatistiksel olarak anlamlı bir farklılık tespit edilmemiştir (p>0,05). Ayrıca, restoratif materyallerin yüzey pürüzlülüğü artışı açısından, manuel ve elektrikli fırçalama arasında istatistiksel olarak anlamlı bir farklılık tespit edilmemiştir (p>0,05).
Sonuç: Hem manuel hem de elektrikli fırçalama sonrası, yüzey pürüzlülüğü artışı açısından restoratif materyallerin birbirlerine üstünlüğünün ya da dezavantajının olmadığı sonucuna varılmıştır. Ayrıca, elektrikli fırçalamanın manuel fırçalamaya oranla ekstra pürüzlülük yaratmamış olması ve sunduğu diğer avantajlar göz önüne alınarak, çocuklarda oral hijyenin sağlanmasında elektrikli fırçaların kullanımı önerilebilir.

Introduction

Despite all the advances in dental caries prevention, caries formation is still a major clinical problem which increases the need for the restoration of dental structures and restorative materials (1-4). Many different materials are used in restorative treatments in pediatric dentistry such as composites, polyacid-modified composite resins (compomers) and glass ionomer-based materials. The resin-containing materials are preferred due to its high adhesive and aesthetic properties, while glass ionomer-based materials are preferred due to their easy-to-handle properties, anti-caries features, fluoride release/fluoride charging abilities (1,2,5). On the other hand, high viscosity glass ionomers produced for atraumatic restorative treatment (ART) approach, which is especially carried out in outreach situations, are among the materials offered to the clinician as a subgroup of glass ionomer materials (6,7). Nowadays, with coronavirus disease-2019 (COVID-19) pandemic, minimal intervention dentistry (MID) approach, and ART-which is a part of the MID approach- are adopted for reducing the amount of aerosol formation and the chair time. Considering both these aspects and other advantages, the use of glass ionomer-containing materials is as much used as resin-containing materials in pediatric dentistry clinics (2,7,8).

The most effective method to prevent dental caries and periodontal diseases is toothbrushing with an appropriate dentifrice. The aim of using dentifrice is to enhance chemical plaque removal effectiveness in addition to mechanical cleaning (4,9,10). On the other hand, abrasive substances consisting of insoluble inorganic compounds are added to dentifrices in order to effectively clean the dental surfaces and remove stains (9,11). However, since dentifrices contain abrasives, it is known that toothbrushing causes an increase in the surface roughness of dental restorative materials (11-13). Although it is recommended that the amount of abrasive material is less in dentifrices for children, it is an inevitable fact that abrasives increase the surface roughness (13,14). On the other hand, the increase in surface roughness after toothbrushing increases the dental plaque accumulation, staining due to pigmentations and the risk of dental caries formation (13,15,16).

Powered toothbrushing is as common as manual toothbrushing in children's daily oral care. The use of powered toothbrushes tends to increase especially due to deep cleaning abilities at the gingival margins and in hard-to-reach interdental areas (17-19). On the other hand, the use of powered toothbrushing in children assist to overcome the problems of lack of motivation and poor brushing technique (20). It has also been shown that the use of powered toothbrushing in both primary and permanent teeth is more effective than manual toothbrushing in children (21) and therefore powered brushing is recommended to increase oral health status in children (19).

In the literature, only a limited number of studies have examined the effects of both manual and powered toothbrushing on the surface roughness of restoratives. Therefore, this study aimed at presenting a comparative evaluation of the effects of manual and powered toothbrushing on surface roughness alteration in different resin and glass ionomer-based restorative materials under *in vitro* conditions. The null hypothesis tested in the present study was that there would not be statistically difference between

both different restorative materials and brushing procedures regarding surface roughness alteration.

Materials and Methods

Study Design and Preparation of the Specimens

This study has followed the CRIS guidelines for *in vitro* research as discussed in 2014 concept note (22). A total of 160 restorative disc-shaped specimens of 4 different types of restorative materials were prepared according to manufacturer's recommendations (n=40). Restorative materials included in this study were nano-hybrid composite (Clearfil Majesty Posterior), polyacid-modified composite resin (Dyract XP), high viscosity glass ionomer (Equia Fil), and conventional glass ionomer (Ionofil Molar) (Table 1). Teflon-based ring molds were used to prepare restorative disc-shaped specimens (diameter: 10 mm × height: 2 mm). Disc-shaped restorative specimens were polished via polishing discs (Sof-Lex, 3M ESPE, St. Paul, MN, USA) by a handpiece at 15,000 rpm for 10 seconds. Then, the specimens were hydrated in distilled water at 37 °C for 24 hours.

Forty samples in each restorative material group were assigned to the 2 subgroups (n=20) of manual (Oral-B Stages 3 Manual Toothbrush, aged 5-7, Oral B, USA) and powered (Oral B Junior Powered Toothbrush, aged 6+, Oral B, USA) toothbrushes.

Brushing Procedures

For both manual and powered brushing, disc-shaped restorative material samples were brushed with 2 mL of same dentifrice (Oral-B Stages Kids Dentifrice, aged 5-7, Oral B, USA) for 2 minutes every day to simulate home brushing procedures. All the specimens were brushed every day at 12-hour intervals for 90 days. The specimens were washed under tap water after brushing and immersed in distilled water at 37 °C until the next brushing. After the brushing process was completed, surface roughness was measured. Specimens were brushed by the same operator (A.D.) at the same motion force of brushing.

Surface Roughness Measurements

Surface roughness measurements were performed via a profilometer (Perthometer M2, Mahr, Germany). For the surface roughness measurement, the Ra value read on the profilometer device was used. Three consecutive measurements were performed on the surface of all the samples and the mean values were recorded. Surface roughness (Ra) (μm) of the specimens were measured before (baseline: Ra_B) and after (final: Ra_F) both brushing procedures. After 90 days of the brushing procedure, surface roughness alteration (ΔRa) was measured based on the differences between Ra_F and Ra_B values. Subsequently, surface roughness alteration (ΔRa) values for each restorative material and brushing method were analyzed statistically. The equation for surface roughness alteration measurement is given below:

$\Delta Ra = Ra_F - Ra_B$

Statistical Analysis

SPSS 11.5 software was used to analyze the study findings. As descriptive, mean ± standard deviation (SD) and median (minimum-maximum) were used for quantitative variables, and (%) for qualitative variables. In terms of the quantitative variable, whether there is a difference between categories of qualitative variable with two categories was analyzed using Student's t-test if normal distribution assumptions were provided, and Mann-Whitney U test if not. In terms of the quantitative variable, whether there is a difference between categories of qualitative variable with more than two categories was analyzed using the Kruskal-Wallis H test, since normal distribution assumptions were not provided. Statistical significance level was taken as 5%.

Results

After manual toothbrushing, no statistically significant difference was found between the restorative materials in terms of surface roughness alteration (ΔRa) (p=0.279). The mean ± SD values of the surface roughness alteration (μm) of all the restorative materials were 0.11±0.04, 0.12±0.04, 0.10±0.06 and 0.10±0.05, respectively (Table 2). Accordingly, after manual toothbrushing, an increase in surface roughness (ΔRa>0) occurred in all restorative materials examined in the present study.

After powered toothbrushing, no statistically significant difference was found between the restorative materials in terms of surface roughness alteration (ΔRa) (p=0.813). The mean ± SD values of the surface roughness alteration (μm) of all the restorative materials were 0.11±0.08, 0.09±0.05, 0.10±0.04 and 0.09±0.04, respectively (Table 2).

Accordingly, after powered toothbrushing, an increase in surface roughness (ΔRa>0) occurred in all restorative materials examined in the present study.

For all restorative material groups, no statistically significant difference was found between manual and powered brushing procedures in terms of surface roughness alteration (ΔRa) (p=0.820, p=0.174, p=0.959 and p=0.564, respectively) (Table 3).

Discussion

Despite all the improvements regarding the oral health status of children, dental caries is still an important oral health problem, especially in socioeconomically-deprived populations (3,4). To intercept dental caries and periodontal diseases, removing microbial dental plaque and maintaining the oral hygiene are important (4,9,23). In toothbrushing procedures, dentifrices are used to remove the dental plaque chemically in addition to the mechanical cleaning of the toothbrush (9,10,24). However, due to the mechanical movement/action of the bristles of toothbrushes and the effect of the abrasives in the dentifrices, the surface roughness of the dental hard tissues and the restorative materials increases (10,13). The increased surface roughness accelerates dental plaque accumulation, caries formation and staining caused by pigmentation. In this respect, it is also important to detect the increase in surface roughness of restorative materials after toothbrushing (13,15,16). Based on this view, the present study aimed to assess the effects of different

Table 1. Material type and its compositions of restorative materials used in the present study

Type of restorative material	Commercially brand name	Composition	Manufacturer company
Nano-hybrid composite	Clearfil Majesty Posterior	Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate	Kuraray Medical Co, Tokyo, Japan
Polyacid-modified composite resin	Dyract XP	UDMA Strontium-fluoro-silicate glass, strontium fluoride, TCB resin, photoinitiator and stabilizers	Dentsply, DeTrey, Konstanz, Germany
High viscosity glass ionomer	Equia Fil	Strontium fluoroalumino-silicate glass, polyacrylic acid, aqueous polyacrylic acid	GC Corporation, Tokyo, Japan
Conventional glass ionomer	Ionofil Molar	Water, pure polyacrylic acid, tartaric acid, aluminofluorosilicate glass and pigments	Voco, Cuxhaven, Germany

Table 2. The mean ± SD and median (min-max) values of surface roughness alteration (ΔRa) (μm) for each restorative material group and results of statistical comparisons

Manual brushing				Powered brushing			
Restorative materials	Surface roughness alteration (ΔRa) (μm)		p-value	Restorative materials	Surface roughness alteration (ΔRa) (μm)		p-value
Clearfil majesty posterior	Mean ± SD	0.11±0.04	0.279[a]	Clearfil majesty posterior	Mean ± SD	0.11±0.08	0.813[a]
	Median (min-max)	0.10 (0.05-0.19)			Median (min-max)	0.10 (0.01-0.28)	
Dyract XP	Mean ± SD	0.12±0.04		Dyract XP	Mean ± SD	0.09±0.05	
	Median (min-max)	0.12 (0.06-0.24)			Median (min-max)	0.09 (0.01-0.19)	
Equia Fil	Mean ± SD	0.10±0.06		Equia Fil	Mean ± SD	0.10±0.04	
	Median (min-max)	0.10 (0.01-0.23)			Median (min-max)	0.09 (0.03-0.19)	
Ionofil molar	Mean ± SD	0.10±0.05		Ionofil molar	Mean ± SD	0.09±0.04	
	Median (min-max)	0.10 (0.03-0.22)			Median (min-max)	0.09 (0.02-0.17)	

[a]Kruskal-Wallis H test, SD: Standard deviation, min: Minimum, max: Maximum

Table 3. The statistical comparison between manual and powered brushing in terms of surface roughness alteration (ΔRa) for each restorative material

Restorative materials	Manual brushing		Powered brushing		p-value
	Surface roughness alteration (ΔRa) (μm)		Surface roughness alteration (ΔRa) (μm)		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Clearfil majesty posterior	0.11±0.04	0.10 (0.05-0.19)	0.11±0.08	0.10 (0.01-0.28)	0.820[b]
Dyract XP	0.12±0.04	0.12 (0.06-0.24)	0.09±0.05	0.09 (0.01-0.19)	0.174[b]
Equia Fil	0.10±0.06	0.10 (0.01-0.23)	0.10±0.04	0.09 (0.03-0.19)	0.959[a]
Ionofil molar	0.10±0.05	0.10 (0.03-0.22)	0.09±0.04	0.09 (0.02-0.17)	0.564[a]

[a]Student's t-test, [b]Mann-Whitney U test, SD: Standard deviation, min: Minimum, max: Maximum

toothbrushing procedures on the changes in surface roughness of restorative materials. In this respect, this study investigated the changes/alterations in surface roughness of restorative materials (ΔRa) that occur with toothbrushing rather than the roughness levels before or after brushing.

Although many materials have been used in restorative treatments in pediatric dentistry, resin-containing composite, compomer and glass ionomer-containing materials are among the most preferred (1,2,5). While resin-based materials such as composites and compomers are among the materials that are frequently used due to their superior adhesive and aesthetic properties, glass ionomer-based restorative materials are preferred due to their chemical adhesion to dental hard tissues, fluoride releasing/fluoride reservoir features and easy-to-handle properties (1,2,25,26). Moreover, high viscosity glass ionomers are preferred due to their high compressive strength and wear resistance in addition to the advantages offered by traditional glass ionomer materials. Also, high viscosity glass ionomers are used in ART approach based on the MID philosophy that reduces aerosol formation especially during the COVID-19 pandemic (1,2,7,8). Consequently, due to frequent use of resin and glass ionomers in routine clinical practice and outreach situations (especially in ART technique), our study included these restorative materials.

Powered toothbrushing is effective in removing microbial dental plaque in adults as well as children (18,20,27,28). On the other hand, manual brushing requires more pressure than powered brushing. In addition, powered brushing may be more effective in improving the oral health of individuals with physical or mental disabilities, since it requires less hand movement and motor coordination skills (17,19,29,30). In a pilot study, Durhan et al. (19) stated that powered toothbrushing showed more reduction in dental plaque than manual brushing procedure. In line with above-mentioned properties of powered toothbrushing, which was recommended for use by the other studies in the literature, we included powered toothbrushing in this study procedure in order to investigate its effect on the surface roughness of restorative materials and to be able to make recommendations in this respect. Both manual and powered toothbrushes selected for use in this study were suitable for children. Bristle properties of manual and powered toothbrushes were approximately similar.

Physical removal of the dental plaque in toothbrushing is achieved by the use of a toothbrush and dentifrice containing abrasive particles (14,31). On the other hand, the cleaning process on tooth surfaces depends on factors such as type, morphology and particle size of the abrasive contents (14,32). Many of the benefits provided by abrasive particles are desired in adult pastes regarding plaque removal. However, in children, the amount of abrasive should be balanced in a way that can provide effective cleaning and plaque removal, but not damage the developing

tooth surfaces (14). On the other hand, as the abrasive content of dentifrices changes, the alterations occur in surface roughness (13). Therefore, in our study, since we investigated to what extent toothbrushing affects the increase in surface roughness of the restorative materials, dentifrices suitable for children in terms of abrasive properties and other features were used in our study.

According to the findings of the present study, surface roughness increase was detected in all the materials both after manual and powered toothbrushing. However, no significant difference was found between the amounts of rise in surface roughness in all the restorative materials both after manual and powered brushing procedures. Accordingly, it can be stated that both toothbrushing methods cause similar increase in surface roughness in all the restorative materials used in this study. Pala et al. (33) reported that after powered brushing with two different dentifrices, similar roughness values were found in their study without any statistically significant difference in composite (Clearfil Majesty Posterior) and compomer (Dyract XP) materials. Also, similar to our study, Mondelli et al. (34) reported that several different commercially available compomer restorative materials (Dyract, Dyract AP, Compoglass F) showed surface roughness increase without statistically significant difference between dental composites (Z100 and Silux Plus) after simulated toothbrushing. On the other hand, Dudás et al. (13) reported that the increase in surface roughness of glass ionomer-based restorative material was the highest compared to composites, in contrast with the findings of the present study. This difference can be attributed to the different effects of dentifrices on surface roughness of restorative materials, since the dentifrices used in the studies were different. On the other hand, considering the fact that there are not many studies comparing the increase in roughness on the surfaces of different restorative materials after tooth brushing, we attribute the statistically similar increase in surface roughness in our study to the use of pediatric dentifrice with low abrasive content. If this analysis was performed with dentifrices with higher abrasive particle sizes or whitening effect, the results might have been different, and in this respect, further studies are needed to investigate effects of different pastes containing different abrasives. Consequently, considering that the similar surface roughness increases of high viscosity glass ionomers compared to the other materials examined in this study, it is possible to recommend the use of the high viscosity glass ionomer materials in ART applications, which has gained popularity in clinics due to reducing aerosol amount during the COVID-19 pandemic. Moreover, one of the limitations of this study was the 3-month brushing period. Considering the serving period of restorative materials in oral cavity, further studies that use a brushing simulator are required in order to show the changes in the surface roughness of the restorative materials in longer term.

In the present study, no statistical difference was found between manual and powered brushing procedures in terms of surface roughness alterations in different restorative materials. Although there are not enough studies in the literature that compare manual and powered brushing in this respect in restorative materials, the absence of statistically significant difference was attributed to the similar hardness of the bristles in toothbrushes. Powered brushes have advantages such as effective cleaning of inaccessible areas especially gingival margins and interproximal areas, effective plaque removal properties and providing personalized tracking and feedback features in more advanced models (18). Moreover, pediatric patients lack motivation, compliance and adequate manual dexterity in toothbrushing. In this respect, powered toothbrushes help children overcome their lack of motivation, solve brushing problems, circumventing the need for good manual dexterity (18,20,35). Also, in a randomized clinical study, Davidovich et al. (18) reported that powered toothbrushing provided superior plaque reduction in children than manual toothbrushing. In this respect, considering both the mentioned advantages of powered brushing and the absence of extra surface roughness in all the restorative materials after powered toothbrushing compared to manual brushing in this study, powered toothbrushing systems can be recommended for providing and maintaining oral hygiene in children.

Conclusion

The null hypothesis that there would not be statistically differences between both different restorative materials and brushing procedures

regarding surface roughness alteration was accepted. Within the limitations of this study, after both manual and powered brushing, it is possible to concluded that one of the restorative materials has no advantage or disadvantage over the others in terms of surface roughness increase. In addition, since manual and powered brushing procedures showed no significant difference in surface roughness alteration, the use of powered brushes are recommended children, considering the other advantages.

Ethics

Ethics Committee Approval: Ethics committee approval is not required for this study.

Informed Consent: For this type of study, informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.D., N.B., Design: A.D., N.B., Supervision: A.D., N.B., Fundings: A.D., N.B., Materials: A.D., N.B., Data Collection or Processing: A.D., N.B., Analysis or Interpretation: A.D., N.B., Literature Search: A.D., N.B., Writing: A.D., N.B., Critical Review: A.D., N.B.

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Original Article / Özgün Araştırma

Frequency, Locations and Histopathological Features of Polyps Detected in Upper Gastrointestinal System Examination and Its Association with *Helicobacter Pylori* Infection

Üst Gastrointestinal Sistem İncelemesinde Saptanan Poliplerin Sıklığı, Yerleşim Yerleri, Histopatolojik Özellikleri ve Helicobacter Pylori Enfeksiyonuyla Birlikteliği

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Abstract

Objective: Gastric polyps are often detected incidentally during endoscopic examination performed for a different purpose. Hyperplastic polyp is the most common type of polyp in the stomach. This study aimed to determine the frequency, locations and histopathological features of polyps detected in gastroscopic procedures performed at the Gastroenterology Department of Aydın State Hospital and their relationship with *Helicobacter pylori (H. pylori)* infection.

Materials and Methods: Data of 129 patients with polyps during 8,787 gastroscopic procedures performed between July 2016 and January 2020 at the Gastroenterology Department were retrospectively analysed. The size, location and histopathological features of the polyps, *H. pylori* positivity, presence of intestinal metaplasia and gastric atrophy and use of proton pump inhibitors were recorded.

Results: Polyps were localised in the corpus in 43 (33.3%) patients, in the antrum in 36 (27.9%) patients and in the fundus in 20 (15.5%) patients. On histopathological examination, hyperplastic polyp was detected in 84 (65.4%) patients, fundic gland polyp in 19 (14.7%) patients, adenomatous polyp in 11 (8.5%) patients, squamous papilloma in 6 (4.7%) patients and inflammatory polyp in 5 (3.9%) patients. *H. pylori* positivity was significantly higher in hyperplastic polyps compared to other polyps (40.8% vs 23.4%) (p=0.04), while it was found to be significantly less in fundic gland polyps compared to other polyps (15.8% vs 41.8%) (p=0.03).

Conclusion: In this study, hyperplastic polyp is the most common type of polyp found on gastroscopic examination. While *H. pylori* positivity rate was significantly higher in hyperplastic polyps, it was significantly less in fundic gland polyps. Fundic gland polyps are more common with long-term use of proton pump inhibitors. In the literature, the histopathology of polyps and the relationship of *H. pylori* infection show similarities.

Öz

Amaç: Gastrik polipler, sıklıkla farklı bir nedenle yapılan endoskopik inceleme esnasında tesadüfen saptanırlar. Midede en sık rastlanan polip türü hiperplastik poliptir. Bu çalışmanın amacı, Aydın Devlet Hastanesi Gastroenteroloji Bölümü'nde yapılan gastroskopi işlemlerinde saptanan poliplerin sıklığını, yerleşim yerlerini, histopatolojik özelliklerini ve *Helicobacter pylori (H. pylori)* ile ilişkilerini saptamaktır.

Gereç ve Yöntemler: Gastroenteroloji bölümünde Temmuz 2016 ve Ocak 2020 tarihleri arasında yapılan 8.787 gastroskopi işlemi esnasında polip saptanan 129 hastanın verileri retrospektif olarak incelenmiştir. Poliplerin boyutu, yeri, histopatolojik özellikleri, *H. pylori* pozitifliği, intestinal metaplazi ve gastrik atrofi varlığı ve hastaların proton pompa inhibitörü kullanım durumları kaydedilmiştir.

Bulgular: Polip lokalizasyonunun 43 (%33,3) hastada korpusta, 36 (%27,9) hastada antrumda, 20 (%15,5) hastada fundusta olduğu tespit edilmiştir. Histopatolojik incelemede 84 (%65,4) hastada hiperplastik polip, 19 (%14,7) hastada fundik gland polipi, 11 (%8,5) hastada adenomatöz polip, 6 (%4,7) hastada skuamöz papillom, 5 (%3,9) hastada enflamatuvar polip tespit edilmiştir. *H. pylori* pozitifliği hiperplastik poliplerde diğer poliplere göre anlamlı şekilde daha fazla iken (%40,8'e %23,4) (p=0,04), fundik gland poliplerinde diğer poliplere göre anlamlı şekilde daha az olarak tespit edilmiştir (%15,8'e %41,8) (p=0,03).

Sonuç: Gastroskopik incelemede en sık rastlanan polip tipi hiperplastik poliptir. Hiperplastik poliplerde *H. pylori* pozitifliğinin anlamlı şekilde yüksekken, fundik gland poliplerinde anlamlı şekilde daha az olduğu saptanmıştır. Hastaların uzun süre proton pompa inhibitörü kullanımı durumlarında fundik gland polipinin daha çok görüldüğü tespit edilmiştir. Literatüre bakıldığında poliplerin histopatolojileri ve *H. pylori* enfeksiyonu ilişkisi benzerlik göstermektedir.

Introduction

The term polyp in the gastrointestinal system is used to describe proliferative or neoplastic lesions originating from the mucosal epithelium (1). Gastric polyps are usually detected incidentally during upper gastrointestinal system endoscopy performed for different reasons. They present less commonly as iron deficiency anaemia, bleeding, pyloric outlet obstruction and abdominal pain (2-3).

The incidence of gastric polyps during endoscopic interventions has been reported up to 6% (4). The polyps found most common in the stomach are hyperplastic and fundic gland polyps. However, localization and histopathological types of polyps may differ according to their geographical distribution. While hyperplastic and adenomatous polyps are prominent in areas with *Helicobacter pylori* (*H. pylori*) density, fundic gland polyps can be encountered more frequently in areas where proton pump inhibitors (PPI) are used extensively (5,6).

The aim of this study is to determine the frequency, locations, histopathological features of polyps detected in gastroscopy procedures and their relations with *H. pylori*.

Materials and Methods

The data of 129 patients, who were found to have polyps, during the 8,787 gastroscopy procedures performed between July 2016 and January 2020 at the Gastroenterology Department were retrospectively analysed. The ethics committee approval for the study was obtained from the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 05, date: 03.09.2020). The study was conducted in accordance with the principles of the Declaration of Helsinki. The size, location, histopathological features, *H. pylori* positivity, presence of intestinal metaplasia (IM) and gastric atrophy of the polyps removed from these 129 patients and the patients' usage status of PPI were recorded. Endoscopic procedures were performed with local anaesthesia (10% lidocaine). Polyp removal procedure was performed with biopsy forceps for polyps <5 mm and sclerotherapy needle for polyps larger than 5 mm, with the help of cautery after 1/10,000 adrenaline injection applied to the submucosal area.

Statistical Analysis

Statistical analysis was performed by using the SPSS 26.0 (SPSS Inc., Chicago, IL, USA) program. The conformity of the variables to normal distribution was examined by visual (histogram and probability graphics) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were performed by giving mean ± standard deviation for normally distributed variables. It is given by using the median and interquartile for variables that are not normally distributed. Gender according to polyp types, most common complaints, long PPI use, presence of *H. pylori*, presence of IM and atrophy were evaluated

with chi-square or Fisher Exact tests and the age was evaluated with Wilcoxon test. In cases where the p-value was less than 0.05, they were considered as statistically significant results.

Results

One hundred and twenty nine patients [85 women (65.9%), 44 men (34.1%)], who were found to have polyps after gastroscopy, were included in our study. During this period, polyp was detected in 129 patients (1.46%) during 8,787 gastroscopy procedures. The mean age of the patients with polyp was 59.8±14.4, and their ages ranged from 25 to 89. Localization of polyps was detected as corpus in 43 patients (33.3%), as antrum in 36 patients (27.9%), as fundus in 20 patients (15.5%), as cardia in 14 patients (10.9%), as esophagus in 10 patients (7.8%), as duodenum in 3 patients (2.3%), as hernia pouch in 2 patients (1.6%), as the anastomosis line in 1 patient (0.8%) (Table 1).

The histopathology of polypectomy materials were most frequently listed as hyperplastic polyp, fundic gland polyp and adenomatous polyp (Table 2).

Table 1. Locations of polyps

Polyp location	n	%
Corpus	43	33.3
Antrum	36	27.9
Fundus	20	15.5
Cardia	14	10.9
Esophagus	10	7.8
Duodenum	3	2.3
Hernia pouch	2	1.6
Anastomosis line	1	0.8

When the reasons for the endoscopy request are examined, it was seen that 92 (71.3%) of the cases were dyspepsia, 27 (20.9%) of them were anaemia, 6 (4.7%) of them were weight loss, and 4 (3.1%) of them were bleeding (Table 3).

When patients with hyperplastic polyp type were compared with other patients, *H. pylori* presence was significantly higher in hyperplastic polyp type (40.8% & 23.4%, p=0.04) (Table 4).

When patients with fundic gland polyps were compared with other patients, while long PPI use was significantly higher in patients with fundic gland polyp (89.5% & 46.4%, p=0.001), the presence of *H. pylori*

Table 2. Histopathological results of polyps

Histopathology	n	%
Hyperplastic polyp	84	65.4
Fundic gland polyp	19	14.7
Adenomatous polyp	11	8.5
Squamous papilloma	6	4.7
Inflammatory polyp	5	3.9
Neuroendocrine tumour	1	0.8
Hamartematous cystic polyp	1	0.8
Auxintic gland poly	1	0.8
Gastritis cystica profunda	1	0.8

Table 3. Reasons for requesting endoscopy

Reason for request	n	%
Dyspepsia	92	71.3
Anemia	27	20.9
Weight loss	6	4.7
Bleeding	4	3.1

Table 4. Characteristics of patients according to hyperplastic polyp type

	Hyperplastic polyp	Other patients	p
Gender (F/M)	61/23	24/21	0.367
Age (mean ± SD)	61.4±14.2	57.7±14.4	0.118
Most common application complaint (%)	67.6 dyspepsia	76.4 dyspepsia	0.488
Long PPI use (%)	47.3	60	0.105
H. pylori presence (%)	40.8	23.4	0.04
Polyp size (mean ± SD, mm)	4.9±1.97	5.07±2.57	0.883
IM presence (%)	28.4	16.45	0.082
Presence of atrophy (%)	4.1	7.3	0.339

SD: Standard deviation, F: Female, M: Male, PPI: Proton pump inhibitors, *H. pylori*: *Helicobacter pylori*, IM: Intestinal metaplasia

and IM was significantly higher in the other patient group (15.8% & 41.8%, respectively, p=0.03; 5.3% & 26.4%, p=0.03) (Table 5).

When patients with adenomatous polyp type were compared with other patients, no significant variable was found (Table 6).

Discussion

Structures that protrude from the mucosa to the lumen in the gastrointestinal system are called polyps. These lesions can be neoplastic or non-neoplastic and may arise from the mucosa or submucosa. The diagnosis of polyp can only be made after histopathological examination (7).

Gastric polyps can be encountered in up to 6% of upper gastrointestinal system endoscopic examinations (4). In our study, 129 patients (1.46%) were diagnosed with polyps after 8787 upper endoscopy procedures. Although the most common polyps are hyperplastic polyps, the incidence and histopathological types of polyps may differ geographically. The most common type of polyp in our study was hyperplastic polyp (65.4%). Fundic gland polyp (14.7%) and adenomatous polyp (8.5%) followed this. In a study in which 26,000 patients were included, hyperplastic polyp was found at a rate of 71.3%, fundic gland polyp at a rate of 16.3%, and adenomatous polyp at a rate of 12.4% (5). The rates of our study were consistent with the literature.

It has been reported that hyperplastic and adenomatous polyps are more common than fundic gland polyps in geographical areas where *H. pylori* infection is intense (5,8). In our study, *H. pylori* positivity was significantly higher in patients with hyperplastic polyp compared to patients with other polyp types (40.8% & 23.4%, p=0.04). However, in our study, the relationship between *H. pylori* positivity for adenomatous polyp was not found similarly. This situation can be explained by the low number of patients with adenomatous polyp.

It has been reported in previous studies that fundic gland polyps are more common in areas where PPI

Table 5. Characteristics of patients according to fundic gland polyp type

	Fundic gland polyp	Other diseases	p
Gender (F/M)	(14/5)	(76/34)	0.458
Age (mean ± SD)	55.6±12.5	60.5±14.6	0.527
Most frequent application complaint (%)	89.5 dyspepsia	68.2 dyspepsia	0.05
Long PPI use (%)	89.5	46.4	0.001
H. pylori presence (%)	15.8	41.8	0.03
Polyp size (mean ± SD, mm)	4.1±1.01	5.1±2.3	0.06
IM presence (%)	5.3	26.4	0.03
Presence of atrophy (%)	5.3	5.5	0.726

SD: Standard deviation, F: Female, M: Male, PPI: Proton pump inhibitors, *H. pylori: Helicobacter pylori*, IM: Intestinal metaplasia

Table 6. Characteristics of patients according to the type of adenomatous polyp

	Adenomatous polyp	Other patients	p
Gender (F/M)	7/4	78/40	0.419
Age (mean ± SD)	61.5±14.3	59.5±14.4	0.584
Most frequent application complaint (%)	78.3 dyspepsia	69.8 dyspepsia	0.505
Long PPI use (%)	47.8	53.8	0.386
H. pylori presence (%)	52.2	34.9	0.96
Polyp size (mean ± SD, mm)	5.08±1.8	4.9±2.3	0.492
IM presence (%)	30.4	21.7	0.259
Presence of atrophy (%)	13	3.8	0.107

SD: Standard deviation, F: Female, M: Male, PPI: Proton pump inhibitors, *H. pylori: Helicobacter pylori*, IM: Intestinal metaplasia

are used extensively (6). At the same time, it is known that *H. pylori* positivity is lower in those with fundic gland polyp and the presence of *H. pylori* is protective against the formation of fundic gland polyp (4). In our study, *H. pylori* positivity was significantly lower in patients with fundic gland polyp (15.8% & 41.8%, p=0.03) and the incidence of fundic gland polyp was significantly higher in patients using long PPI (89.5% & 46.4%, p=0.001).

In our study, the presence of IM was found to be significantly lower in patients with fundic gland polyp. This can be explained by the low presence of *H. pylori* in patients with fundic gland polyps. In a study conducted in our country, the rates of *H. pylori* and IM were found to be significantly lower in patients with fundic gland polyp (9).

Neuroendocrine tumours are nodular lesions with smooth surfaces, most commonly found in the corpus. Although there is evidence that its frequency has increased in recent years (10), neuroendocrine tumours are observed at around 1% (11). In our study, neuroendocrim tumour was detected in the corpus location in 1 case (0.8%). The low rate in our study indicates that a larger cohort may be needed.

Conclusion

Hyperplastic polyps were found at most, as similar to the literature. *H. pylori* positivity was found to be significantly associated with hyperplastic polyp. Fundic gland polyps were at the 2nd frequency, and *H. pylori* positivity and IM frequency were found to be significantly less in this polyp type.

Ethics

Ethics Committee Approval: The ethics committee approval for the study was obtained from the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 05, date: 03.09.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: İ.T., Design: E.K., Supervision: İ.T., Fundings: İ.T., Materials: İ.T., Analysis or Interpretation: İ.T., Literature Search: E.K., Writing: E.K., Critical Review: E.K.

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Original Article / Özgün Araştırma

Effects of Melatonin at Different Doses on Experimental Epilepsy Model Induced By Pentylenetetrazole

Pentilenetetrazol ile Oluşturulan Deneysel Epilepsi Modelinde Farklı Dozlardaki Melatoninin Etkilerinin İncelenmesi

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Keywords
Antioxidant, experimental epilepsy, melatonin, oxidative stress, PTZ, rat

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Abstract

Objective: This study aimed to evaluate the effect of melatonin (MEL) treatment on rats with experimental epilepsy induced by pentylenetetrazole (PTZ). Changes in the control, epilepsy and two treatment groups (25 mg/kg and 100 mg/kg) were monitored as intragroup and intergroup changes.

Materials and Methods: Forty male Wistar albino rats (12-14 weeks old) were divided into control, PTZ, MEL25 and MEL100 groups, with 10 rats in each group. Only solvent was injected in the control group, and PTZ at a dose of 35 mg/kg was administered intraperitoneal 12 times in a total of 23 days in the PTZ group. MEL25 and 100 mg/kg were administered in the MEL25 and MEL100 groups, respectively. Parameters tested during and after the experiment were behavioural tests (elevated plus maze), biochemical tests in brain tissue [after decapitation; malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), total antioxidant status (TAS) and total oxidant status (TOS)] and epileptic seizure severity scale scores (every injection day).

Results: Significant differences were observed among the epilepsy, control and treatment groups, except for the hiding times, seizure scores and biochemical tests ($p<0.05$). As a result of biochemical tests applied to the homogenised brain tissue after decapitation, dose-dependent results were found to be related to the different doses of MEL applied in MDA, CAT, SOD, TAS and TOS levels ($p<0.05$).

Conclusion: In this study, MEL showed a protective and healing role against physiological changes caused by experimental epilepsy, through its capacity to reduce oxidative damage and increase antioxidant potency.

Öz

Amaç: Bu çalışmada, pentilenetetrazol (PTZ) ile indüklenen deneysel epilepsi oluşturulmuş sıçanlarda melatonin (MEL) tedavisinin etkisinin değerlendirilmesi amaçlanmıştır. Kontrol grubu, epilepsi grubu ve iki tedavi grubundaki değişiklikler (25 mg/kg ile 100 mg/kg) grup içi ve gruplar arası olarak değerlendirilmiştir.

Gereç ve Yöntemler: Kırk adet erkek Wistar albino sıçan (12-14 haftalık) 4 farklı gruba (kontrol, PTZ, MEL25, MEL100) ayrıldı. Kontrol grubuna sadece çözgen enjekte edildi ve PTZ grubuna 35 mg/kg dozunda PTZ intraperitoneal toplam 23 gün süresince 12 kez günaşırı uygulandı. Melatonin, MEL25 grubu için 25 mg/kg ve MEL100 grubu için 100 mg/kg uygulandı. Deney sırasında ve sonrasında test edilen

parametreler davranış testi (yükseltilmiş artı labirent), beyin dokusunda biyokimyasal testler [dekapitasyon sonrası; malondialdehit (MDA), katalaz (CAT), süperoksit dismutaz (SOD), toplam antioksidan durumu (TAS) ve toplam oksidan durumu (TOS)] ve epileptik nöbet şiddeti ölçek skorları (her enjeksiyon günü) idi.
Bulgular: Epilepsi grubu ile kontrol ve tedavi grupları arasında kaçış ve saklanma süreleri, nöbet skorları ve biyokimyasal testler açısından anlamlı farklılıklar bulundu ($p<0,05$). Dekapitasyon sonrası homojenize beyin dokusuna uygulanan biyokimyasal testler sonucunda MDA, CAT, SOD, TAS ve TOS düzeylerinde farklı dozlarda uygulanan MEL ile ilgili doza bağlı iyileşmeler saptandı ($p<0,05$).
Sonuç: Çalışma sonucunda MEL'nin deneysel epilepsinin neden olduğu fizyolojik değişikliklere karşı koruyucu ve iyileştirici rolü olduğu ve oksidatif hasarı azaltıcı ve antioksidan potansiyeli artırıcı etki gösterdiği tespit edilmiştir.

Introduction

Epilepsy is a neurological disorder, characterized by unpredictable seizures occurring repeatedly. Epileptic seizures affect nearly 50 million people, approximately %1 of the world's population (1). These seizures are associated with cognitive and behavioral disorders that have a significant impact on patients' quality of life. Epileptic seizures may cause cognitive function defects. Abnormal electrical activities of neurons cause changes on behavioral and cognitive processes (2). Patients affected by epileptic seizures often show impaired spatial memory and emotional learning. These changes may be also associated with different comorbidities such as anxiety, sleep, disorders and depression (3,4).

Oxidative stress has an important role at epileptic stage. Reactive oxygen derivatives are mainly responsible for oxidative stress. These can be oxygen-centered radicals that have unpaired electrons or covalent molecules (such as hydrogen peroxide) (5). Reactive oxygen species; H_2O_2, O_2^-, OH^-; are produced during and after epileptic seizures, may contribute brain damage. The brain is vulnerable to free radical damage due to its high oxygen consumption, high lipid content, and also less antioxidant enzymes compared to other tissues. There are several experimental models that show the relation between epileptic seizures and oxidative damages. Moreover, excessive free radical production is associated with injury to cell structures, including lipid structure disruption of cells, enzyme inactivation, and DNA damage (6).

There are various experimental models to find out the mechanisms of acute and chronic epilepsy. For these models of epilepsy are induced by administration of convulsive drugs or electrical stimulation. It is possible to initiate partial and generalized seizures by using various chemicals, such as pentylenetetrazole (PTZ), penicillin, kainic acid, etc. (7). One of the most common experimental model is a chemical inducing method, the PTZ-induced seizures (8). PTZ is a tetrazole compound that is an agent for generalized tonic clonic epileptic seizure induction. It was demonstrated that PTZ affects the disruption of gamma amino butyric acid (GABA) (9). The formation of free radicals has been shown during and after seizures in PTZ-induced models. Especially in chronic PTZ exposure, formation of free radicals significantly increased and oxidative stress damage was found to occur. The PTZ model is a useful model for detecting post-seizure dysfunctions that serve as a screen for possible treatments, offering the possibility to study animal models for cognitive, physical and emotional deficits in human epilepsy (10).

Melatonin (MEL) is produced by the pineal gland and is an indolamine derivative of serotonin. It has long been described as a reproductively active hormone that regulates the sexual physiology seasonally (11). MEL has been shown to have various antioxidant and anticonvulsive effects on the central nervous system of mammals (12,13). Experiments reported that MEL exert proconvulsant effects in humans especially via protecting cortical GABA levels (13). As an antioxidant, MEL is effective in protecting nuclear DNA, membrane lipids, and cytosolic proteins from oxidative damage. It is also shown that MEL has role to suppress brain excitability and prevent seizures. MEL blocks glutamatergic-dependent brain excitability and thus functions as an anti-excitotoxic compound (5,13).

The objective of the present study was to evaluate the effects of different doses of MEL application on the seizure levels and oxidative status of the brain tissues in a rat model of PTZ-induced epilepsy. In addition, together with biochemical and histological evaluations, it was aimed to determine the behavioral changes caused by PTZ with the elevated plus maze test.

Materials and Methods

Drugs and chemicals: PTZ and MEL were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dose of PTZ and MEL were calculated from the corresponding experimental doses (13-15). All injections and experiments were performed between July to August, at the time from 02.00 pm to 07.00 pm.

Animals: Forty male Wistar Albino rats (220±20 g in weight) were maintained under controlled conditions, including 12-hour (h) light/dark cycle, 22-24 °C temperature, and appropriate humidity, with laboratory chow and water provided ad libitum. All animal experiments were carried out in accordance with the approval of the Animal Use Adnan Menderes University Ethical Committee (decision no: 64583101/2014/022, date: 27.02.2014).

Groups: The animals were acclimatized for 15 days before starting experiments. Forty rats were randomly divided in four groups with ten animals in each group: Control: Sham-control, PTZ: PTZ-epileptic, 35 mg/kg PTZ administrated PTZ+MEL25: 25 mg/kg MEL treatment group before the induction of epileptic seizures by the injection of 35 mg/kg PTZ, PTZ+MEL100: 100 mg/kg MEL treatment group before the induction of epileptic seizures by the injection of 35 mg/kg PTZ.

Experimental procedures: After acclimation, experiments started with the first injection of PTZ and finished after the 12th injection of PTZ on the 23rd day. MEL, purchased in powder form, was prepared to be dissolved in 10% dimethyl sulfoxide (DMSO) and given at the appropriate dose (16,17). Control group (Sham-control group) received an intraperitoneal (i.p.) injection of 10% DMSO solution prepared in distilled water every other day (3.5 mL/kg, 12 injections total). PTZ group (PTZ-epileptic group) was administered with PTZ (35 mg/kg, i.p., 12 injections total) every other day. MEL25 group (PTZ+MEL25 group) was injected by 25 mg/kg MEL half an h before the administration of PTZ (35 mg/kg PTZ, 25 mg/kg MEL, i.p., 12 injections total). MEL100 group (PTZ+MEL100 group) was injected by 100 mg/kg MEL half an h before the administration of PTZ (35 mg/kg PTZ, 100 mg/kg MEL, i.p., 12 injections total). Groups were monitored for 1 h after PTZ injection by 2 blind-researchers. At the end of experiments rats were anesthezied by ketamine (80 mg/kg) and xylazine (4 mg/kg) intraperitoneally and sacrificed by decapitation. The brains were collected for biochemical measurements.

Epileptic seizures monitoring: Animals were continuously monitored for 12 h/day at light period for 7 days before experiments and 23 days during experiments. Seizure severity was scored using the Racine scale (18) by direct observation and indirect observation based on the video records. Recordings were analyzed and scored by two independent, blind investigators. The scores of Racine scale are; 0 for no convulsive behavior; 1 for myoclonic jerks; 2 for bilateral forelimb clonus lasting less than 3 s; 3 for bilateral forelimb clonus lasting more than 3 s; 4 for tonic-clonic seizure under 10 minutes (min); 5 for tonic-clonic seizure over 10 min; 6 for death. At the end of the experiments, the scores from both investigators were collected by another researcher and the medians of these scores were used as the final scores.

Elevated plus maze test: The elevated T maze was made of transparent glass and consisted of two open arms, (50x10 cm) two enclosed arms (50x10x40 cm) and a central platform (10x10 cm). The plus-maze was 50 cm above the floor. The researcher observed elevated plus-maze and recorded the data by chronometer behind the closed arms. Pellow's (19) method was used as experimental procedure. Rats were placed individually in a new cage that had same conditions as home cage for 5 min before the test. Every rat was then placed in the center of the plus-maze one by one, that was facing one of the closed arms. After familiarization repeats to test platform, for experiment, measurements were taken by a researcher. Main object was the time spent on open arm enclosed arms. A rat was taken to have entered an arm when all four legs were on the arm. To measure time spent on enclosed arms, open arm time period was recorded and difference to total time was calculated. At the end of the test, the total measurement time for every rat was recorded. The duration of analysis time was kept constant as 5 min-300 seconds in accordance with the protocol in the literature (20).

Tissue samples: On the last day of experiment, 90 min after last PTZ administration, the animals were killed by decapitation and their brains were dissected. Ten milliliters of 140 mM KCl solution/gram of tissue were added and all tissue were then homogenized

in a motor-driven homogenizer (Ultra Turrax, IKA-WERKE, Germany) (21). Then samples centrifuged at 12,000 rpm for 10 min. The supernatant was used for biochemical measurements.

Determination of brain lipid peroxidation: Lipid peroxidation was determined in tissue samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS) (22). Trichloroacetic acid and TBARS reagent were added to the tissue homogenates, then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve using tetraethoxypropane and expressed as nmol/gr tissue.

Determination of brain superoxide dismutase (SOD) activity: SOD activity was determined according to the method of Sun et al. (23). Tissue should be perfused with 150 mM KCl to remove any red blood cells. The tissue samples homogenized in ice-cold 0.1 M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5 mM β-ME, 0.1 mg/mL phenylmethylsulfonyl fluoride. After centrifugation the supernatant contains total SOD activity from cytosolic and mitochondria. The absorbance of the supernatant was read at 450 nm. SOD activity was given as miliunits per milliliter homogenate (mU/mL).

Determination of brain catalase (CAT) activity: CAT activity was assayed according to the method described by Maehly and Chance (24), based on the disappearance of H_2O_2 at 240 nm. 10 µL of homogenate was added to 180 µL of 20 mM potassium phosphate buffer pH 7.2. Subsequently, 10 µL of 5 mM H_2O_2 was added and absorbance was immediately recorded every 30 s for 10 min, using a ELx800™ Microplate Reader (Biotek Instruments Inc. Winooski, USA). One CAT unit was defined as one µmol of hydrogen peroxide consumed per min and the specific activity was calculated as CAT mU/mL homogenate.

Total antioxidant status (TAS) and total oxidant status (TOS) levels: TAS measurement is based on the principle that the color formed by the 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical changes with the antioxidants in the samples added to the medium, and the measurement was performed using the biochemical kit. Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. In order to perform TOS measurement, after decapitation, the brain tissue was homogenized as indicated. Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2Equiv./L).

Statistical Analysis

All data were analyzed by GraphPad 7 statistical program (GraphPad Software, Inc., CA, USA). Elevated Plus Maze durations and Epileptic Seizure scores were compared by the Kruskal-Wallis non-parametric test-Dunn's multiple comparison test and other parameters were compared by One-way ANOVA-Dunnet's multiple comparison test. D'agostino pearson normality test was applied to determine normality. Values represent the mean ± standard deviation of n=10 animals per group. ns= non-significant, $p>0.05$, *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$. The control, MEL25 and MEL100 groups were compared with the PTZ group, and the treatment groups were compared among themselves. In the graphs, the arrows at the top show this comparison. In group comparisons, PTZ group was considered as the focus of comparison. The deteriorations in the control group and changes in the treatment groups were examined in this way.

Results

Epileptic seizure scores: To estimate the severity of seizures, Racine scale was used. Seizure severity was grouped in 3 ways: 1. No effect, 2. Minimal clonic seizure (MCS), 3. Generalized tonic-clonic seizure (GTCS). For 23 days, 12 injections, animals from the groups were monitored and scored based on Racine scale by two blind researchers. 0 severity for Racine scale means no effect, from 1 to 3 severity for Racine scale means MCS and 4th and 5th severity for Racine scale means GTCS. There was no rat to die as a result of PTZ injection, so no animal was grouped 6 severity for Racine scale. For the first injection of PTZ there is no difference significantly between the groups since

35 mg/kg PTZ is not acute dosage for rats to show difference. PTZ injections were associated with both minimal and generalized seizure incidences in all PTZ-exposed groups. For the first MEL injection, there was no significantly different alteration in between the groups (Figure 1). However after first time, MEL injections for both doses showed a protective effect in PTZ-induced seizures. It was showed that, without treatment, PTZ-induced rats had severe GTCSs. However, the animals that were treated by MEL showed MCS with low severity. 100 mg/kg MEL treatment had a protective effect on rats, to increase the MCS and GTCS both for severity and latency compared to the PTZ group and MEL25 group. 25 mg/kg MEL treatment had also positive effects to protect from seizures; however compared to MEL100 group, MEL25 had lower significance against PTZ group. Total results of epileptic seizure scores are shown in Table 1 and summarized in Figure 1 and Figure 2a and 2b.

Biochemical results: In order to investigate whether the antioxidant properties of MEL against PTZ were mediated by an increase in antioxidant enzymes, SOD and catalase activities were measured. Biochemical results; MDA levels, SOD activities and CAT activities are shown in Table 2.

MDA levels: There were significantly increased MDA levels the animals that were grouped as PTZ compared to control group animals. Administration of 25 mg/kg MEL did make a significant difference in brain MDA concentrations between the MEL25 and PTZ groups ($p<0.05$). MEL100 group, that was administrated 100 mg/kg MEL, had more significant difference compared MEL25 group against PTZ group. The results are summarized in Figure 3. The MDA levels in the brains of PTZ group were observed to

Table 1. Seizure score medians between groups

	Seizure scores (Median)
Control	0.0
PTZ	4.036±0.062
MEL25	2.600±0.121
MEL100	1.618±0.121

PTZ: Pentylenetetrazole, MEL: Melatonin

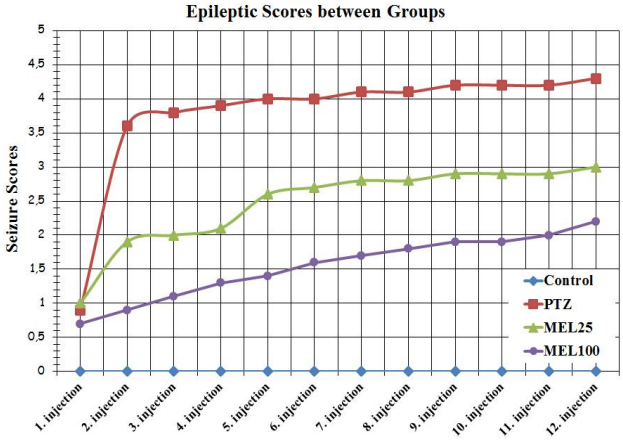

Figure 1. Effects of different doses of melatonin on total epileptic seizures

PTZ: Pentylenetetrazole, MEL: Melatonin

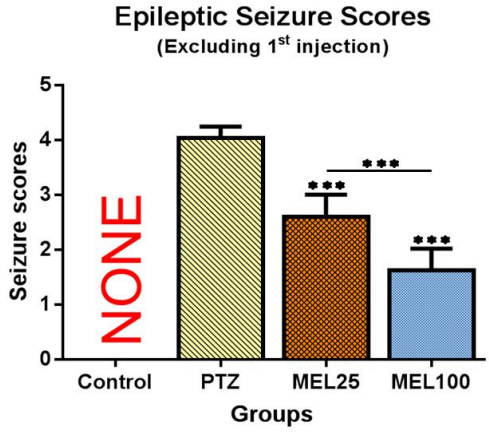

Figure 2a. Effects of different doses of melatonin on total epileptic seizures except 1st day. Group comparisons are: PTZ/MEL25; ***, PTZ/MEL100; ***, MEL25/MEL100; ***

PTZ: Pentylenetetrazole, MEL: Melatonin

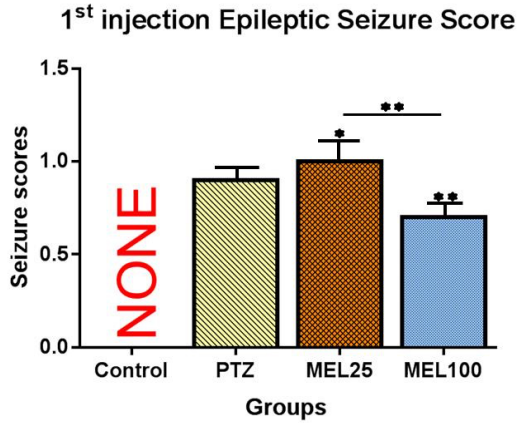

Figure 2b. Effects of different doses of melatonin on first day epileptic seizures. Group comparisons are: MEL25/PTZ; *, PTZ/MEL100; **, MEL25/MEL100; **

PTZ: Pentylenetetrazole, MEL: Melatonin

be significantly increased in comparison to that of control group. It was also observed that, a significant decrease of the MDA levels in comparison to that of PTZ group were more prominently in MEL100 group.

SOD activity: SOD activity was found significantly low at PTZ group compared to control, MEL25 and MEL100 groups ($p<0.05$). As a result of treatment with 25 and 100 mg/kg MEL, SOD activities are found significantly higher compared to PTZ group ($p<0.05$). There is also dose dependent difference between MEL25 and MEL100 groups, demonstrating that MEL administration supports antioxidant activity in a dose-dependent manner. The results are summarized in Figure 4.

CAT activity: The results revealed that CAT activity was observed to be decreased in the PTZ group in comparison to that of control group. The results of MEL25 and MEL100 groups demonstrated that MEL has supported the CAT activity in the brain in a dose-dependent manner. The highest CAT activity was observed to be in the MEL100 group, while the CAT activity of MEL25 group were observed to be higher than that of PTZ group, although not as higher as MEL100 group. The results are summarized in Figure 5.

TAS and TOS levels: The highest antioxidant content was observed in the control group, where there was a decreased antioxidant content in the PTZ group and the antioxidant power was increased in the MEL treatment groups (Figure 6a). The highest oxidative stress content was observed in the PTZ group, and the oxidant content of the MEL treatment groups decreased in dose-dependent manner, where the oxidant content was the least in the control group (Figure 6b).

Behavioral results: To estimate behavioral results, epileptic seizure monitoring and elevated plus maze tests were done. Elevated plus maze test: Animals that were only exposed to PTZ, had shown significantly

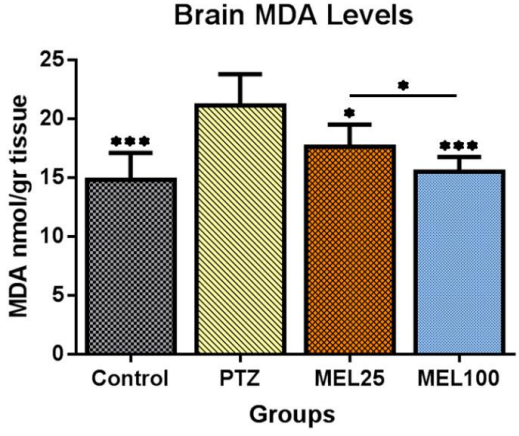

Figure 3. The effect of melatonin administration on the MDA level, as a marker of lipid peroxidation in the rat brain. Group comparisons are: Control/PTZ; ***, PTZ/MEL25; ***, PTZ/MEL100 ***, MEL25/MEL100; ***

PTZ: Pentylenetetrazole, MEL: Melatonin, MDA: Malondialdehyde

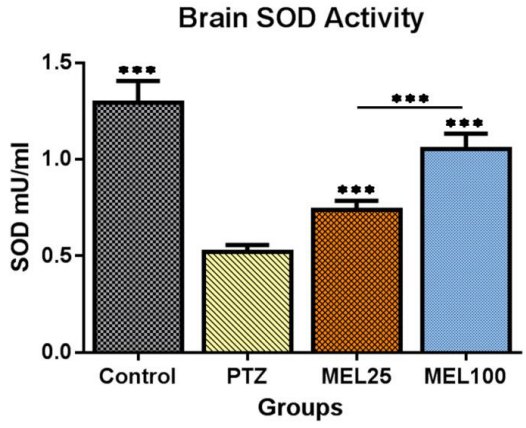

Figure 4. The effect of melatonin administration on the activity of the antioxidant enzyme SOD in the rat brain. Group comparisons are: Control/PTZ; ***, PTZ/MEL25; ***, PTZ/MEL100; ***, MEL25/MEL100; ***

PTZ: Pentylenetetrazole, MEL: Melatonin, SOD: Superoxide dismutase

Table 2. Cerebral MDA, SOD, CAT, TAS and TOS levels

	MDA (nmol/gr tissue)	SOD (mU/mL)	CAT (mU/mL)	TAS (mM)	TOS (μmol H_2O_2)
Control	14.81±1.145	1.292±0.040	0.718±0.035	1.73±0.085	19.42±0.32
PTZ	21.10±0.948	0.520±0.013	0.516±0.018	1.31±0.045	29.84±0.03
MEL25	17.62±0.665	0.736±0.017	0.630±0.014	1.53±0.016	25.19±0.18
MEL100	15.49±0.441	1.053±0.028	0.686±0.014	1.70±0.098	20.86±0.16

PTZ: Pentylenetetrazole, MEL: Melatonin, SOD: Superoxide dismutase, CAT: Catalase, TAS: Total antioxidant status, TOS: Total oxidant status, MDA: Malondialdehyde

low time spending at open arms and group escape time was lower against MEL groups. Animals that were injected MEL had spent more time at open arms compared to PTZ group and dose depended MEL effect had shown. Total escape time scores for control, PTZ, MEL25 and MEL100 groups are shown in Table 3 and summarized Figure 7 and Figure 8, and MEL100 group score is much more similar to control group compared to the other groups.

Discussion

In the present study, we investigated the anticonvulsant activity of dose dependent MEL administration against to oxidative epileptic seizure severity that induced to PTZ. Many academic researches showed that there are different experimental models to create similar seizures that like human epilepsy (25-27). Our study also

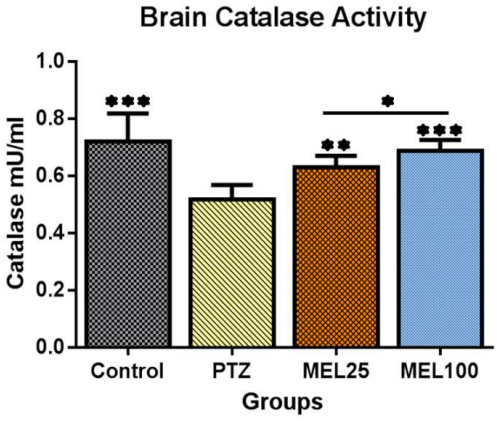

Figure 5. The effect of melatonin administration on the activity of the antioxidant enzyme Catalase in the rat brain. Group comparisons are: Control/PTZ; ***, PTZ/MEL25; **, PTZ/MEL100; ***, MEL25/MEL100; *

PTZ: Pentylenetetrazole, MEL: Melatonin

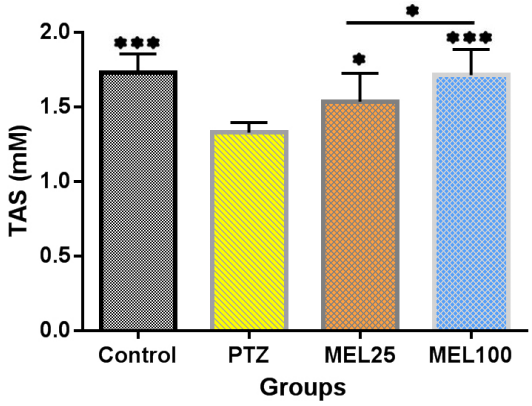

Figure 6a. Comparison of "Cerebral Total Antioxidant Concentration" between groups. Group comparisons are: Control/PTZ; ***, PTZ/MEL25; *, PTZ/MEL100 ***, MEL25/MEL100; *

PTZ: Pentylenetetrazole, MEL: Melatonin, TAS: Total antioxidant status

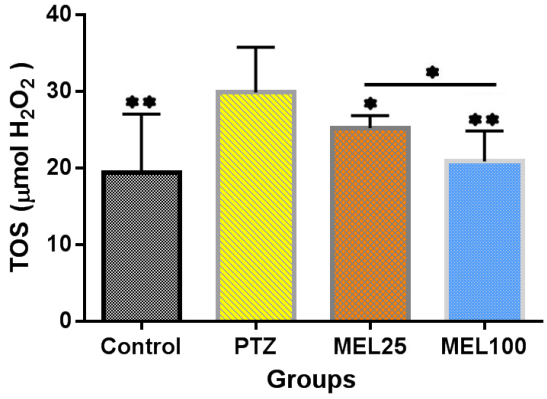

Figure 6b. Comparison of "Cerebral Total Oxidant Content" between groups. Group comparisons are: Control/PTZ; **, PTZ/MEL25; *, PTZ/MEL100 **, MEL25/MEL100; *

PTZ: Pentylenetetrazole, MEL: Melatonin, TOS: Total oxidant status

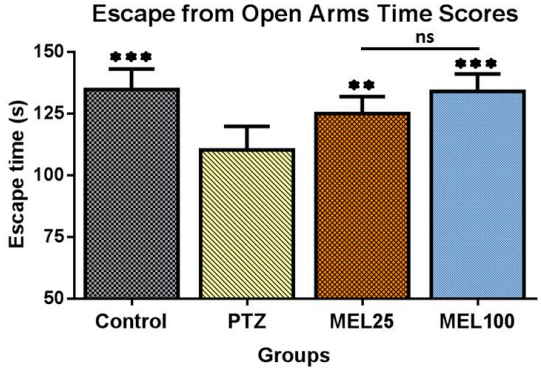

Figure 7. Effects of different doses of melatonin on exploratory and anti-hiding behavior in rats given a 5-minutes test in the elevated plus-maze. These results represent total time spent of open arms. Group comparisons are: Control/PTZ; ***, PTZ/MEL25; **, PTZ/MEL100; ***, MEL25/MEL100; ns

PTZ: Pentylenetetrazole, MEL: Melatonin, ns: Not significant

Table 3. Escape times of rats between groups

	Escape time (s)
Control	134.7±2.633
PTZ	110.3±3.008
MEL25	124.9±2.218
MEL100	133.9±2.258

PTZ: Pentylenetetrazole, MEL: Melatonin

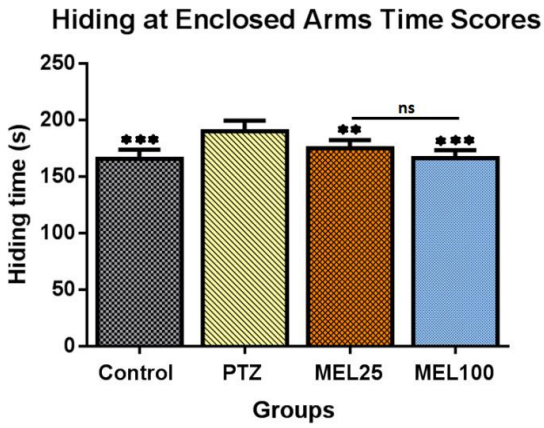

Figure 8. Effects of different doses of melatonin on hiding behavior at enclosed arms in rats given a 5-minutes test in the elevated plus-maze. These results represent total time spent of enclosed arms. Group comparisons are: Control/PTZ; ***, PTZ/MEL25; **, PTZ/MEL100 ***, MEL25/MEL100; ns

PTZ: Pentylenetetrazole, MEL: Melatonin

showed that, 35 mg/kg PTZ injection created tonic-clonic seizures and this created different conditions; like oxidative damage, prevent normal activity of antioxidant system and depressive behaviors of animals. Uyanikgil et al. (15), Wada and Fukuda (28) and Frantz et al. (6) demonstrated these effects on animals that are manipulated by injected PTZ.

Novel studies demonstrated that, MEL has anticonvulsive activity on the experimental animals that were manipulated and showed epileptic seizures induced by penicillin, pilocapine, kainite or PTZ (29,30). However, most studies demonstrated positive effects of MEL on acute epileptic attacks, some studies showed positive results on chronic epileptic seizures. Different from the other researches, our study focused on both chronic period and different doses of MEL (10,12,13,26). As a result of that, we uncovered antiepileptic activity of MEL, during ongoing PTZ injections and after decapitation biochemical results supported these results. Our results showed that, without any protective agents, PTZ group seizure scores are higher than dos dependent MEL administrated groups. Choopankareh et al. (31) also recorded similar results that supported our study.

Previous studies showed the results of increased free radical levels during the seizures (5,31). Similarly, we demonstrated an increase in MDA levels at PTZ administrated group. On the other hand, both low and high dose MEL groups showed decrease in MDA levels, especially high dose MEL had more effective results. Reactive oxygen species, like superoxide anions, hydroxyl radicals, and hydrogen peroxide cause oxidative damage (32) and during the seizures increased oxidative damage create different neurological and psychiatric problems, like neuronal cell death, depression, anxiety etc. (33).

In this study, animals from PTZ group, without administrated MEL, resulted in a significantly decrease in time spent to open arms at the test of elevated plus maze. On the other hand, with MEL treatment, time spent to open arms increased. Pellow et al. (19) who validated plus-maze as a measure of anxiety in the rat, demonstrated that, anxiety significantly reduces exploratory behaviors in open arms, since decreased time spent in open arms and increased time spent in enclosed arms are defined as high anxiety for rats. Wada and Fukuda (28) also found significantly decreasing of spent time open arms the animals that didn't take any drugs except PTZ.

It is known that, ROS both play role to disrupt physiological stages of organism and prevent to produce antioxidant system enzymes (34,35). In a result of this condition, higher MDA levels are estimated to reduce antioxidant activity. Eun et al. (36) demonstrated that, in epileptic cerebral cortices, down regulated SOD transcription occurred and protein level of SOD was lower than the samples that were not from epileptic cerebrums. Previous studies showed that, CAT activity is an essential parameter to identify effective function of antioxidant systems (37,38). In this study both SOD and CAT activity were found higher in MEL administrated groups. In addition, high MEL administrated group had significantly higher results than low MEL administrated group.

Oruc et al. (39) compared the TAS and TOS results with some other parameters such as HIF 1alpha after crokin treatment in cerebral ischemia, and found that TOS levels increased and TAS levels decreased in the brain tissue of cerebral ischemic rats, while crocin treatment was shown opposite effect. Koksal et al. (40) found that the increased TOS level as a result of ischemia decreased with the MEL treatment and increased TAS levels were found in rats treated with MEL. In our study, MEL caused increased TOS levels in the brain tissues of rats group in the PTZ-

induced epileptic seizures, and decreased TAS levels to increase. Statistically significant dose dependent effect was evaluated.

Conclusion

In conclusion, our study showed that, MEL has dose depended therapeutic effects on epileptic seizure determination. We found that, different doses of MEL support antioxidant activities capacities and decrease lipid peroxidation. The antioxidant power of MEL was shown at this study. It has been determined that dose-dependent MEL administration decreases the total oxidant content by increasing the antioxidant power, and normalizes the epileptic seizures and the metabolic changes that occur. This has revealed that MEL use is an effective substance that can be used to eliminate direct or indirect negative effects that may arise due to epileptic seizure processes in the long term.

Ethics

Ethics Committee Approval: All animal experiments were carried out in accordance with the approval of the Animal Use Adnan Menderes University Ethical Committee (decision no: 64583101/2014/022, date: 27.02.2014).

Informed Consent: An animal experiment.

Peer-review: Externally peer-reviwed.

Authorship Contributions

Concept: F.Ş., R.O.E., Design: F.Ş., R.O.E., Supervision: F.Ş., R.O.E., Fundings: F.Ş., R.O.E., Materials: F.Ş., R.O.E., Data Collection or Processing: F.Ş., R.O.E., Analysis or Interpretation: F.Ş., R.O.E., Literature Search: F.Ş., R.O.E., Writing: F.Ş., R.O.E., Critical Review: F.Ş., R.O.E.

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

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Original Article / Özgün Araştırma

Differentiating Mild Forms of Cognitive Impairment and Dementia: Where Other Tests Fail, Verbal Memory Assessment May Prove Critical

Bilişsel Bozulmanın Hafif Formları ve Demans Ayrımı: Sözel Bellek Değerlendirmesi Diğer Testler Başarısız Olduğunda Geçerli Olabilir

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Abstract

Objective: Mild cognitive impairment (MCI) and mild dementia are common. In both cases, objective symptoms of cognitive impairment are present; as such, diagnosis is based on patient history and cognitive examination. This study aimed to investigate the role of neurocognitive tests in the discrimination of these disorders, by comparing mild dementia with MCI.

Materials and Methods: A battery of neurocognitive tests were applied to patients admitted to the neurology and psychiatry clinics with diagnoses of MCI (n=30) or Alzheimer-type dementia (mild, n=23; moderate, n=19).

Results: The three groups demonstrated significant differences in the neurocognitive tests, but the mild dementia and MCI groups almost always had similar results. Only the Öktem verbal memory processes test showed significantly different results in the comparison of MCI and mild dementia, with better scores in the MCI group than in the mild dementia group in terms of total learning and delayed free recall subtests (p=0.023 and p=0.001).

Conclusion: The short-term memory recall (total learning) and long-term memory recall (delayed free recall) subtests of the verbal memory processes test can guide in the discrimination of MCI from mild dementia.

Öz

Amaç: Hafif bilişsel bozulma (MCI) ve hafif demans yaygın olarak görülmektedir. Her iki durumda da, bilişsel bozulmanın nesnel semptomları bulunur ve tanı hastanın öyküsü ve bilişsel muayene ile konur. Bu çalışmada, biz nörobilişsel testlerin bu iki durumu ayırt etmedeki rolünü araştırmayı amaçladık. Bu iki durumu saptamak zor olduğu için MCI ve hafif demans üzerine odaklandık.

Gereç ve Yöntemler: Nörobilişsel testlerden oluşan bir batarya MCI (n=30) ve Alzheimer tipi demans (hafif, n=23; orta, n=19) hastalarına uygulanmıştır.

Bulgular: Üç grup nörobilişsel testler açısından anlamlı farklılıklar göstermiştir. Ancak, hafif demans ve MCI'da genellikle benzer sonuçlar olduğu görülmüştür. Sadece Öktem sözel bellek süreçleri testi hafif demans ve MCI arasında anlamlı

farklılık göstermiştir ve MCI'da hafif demansa total öğrenme ve gecikmiş kendiliğinden hatırlama skorları açısından daha iyi sonuçlar görülmüştür (p=0,023 and p=0,001).
Sonuç: Sözel bellek süreçlerinin total öğrenme ve gecikmiş kendiliğinden hatırlama alt testleri hafif demans ve MCI tanılarının ayırımında bize yol gösterici olabilir.

Introduction

Mild cognitive impairment (MCI) is a term used to describe a clinically-relevant cognitive deterioration that lies between natural ageing-related cognitive changes and mild dementia (1). While such individuals demonstrate objective evidence of cognitive impairment, their functionalities are either normal or close to normal, generally indistinguishable from their past lives (1,2). Although MCI is often associated with Alzheimer's dementia, it can develop due to many causes (1). Some patients with MCI develop dementia, while some remain stable or return to normal cognitive function. The frequency of regaining normal cognitive function among patients with MCI has been reported to be 4-15% in clinical studies (3,4) and around 29-55% in community-based studies (5,6). Efforts to recognize and treat mild dementia has made it critical to identify which types of MCI may result in cognitive worsening. The results of studies focused on this topic have demonstrated that the presence of non-amnestic type MCI, having high Mini-Mental State Exam (MMSE) scores, being young, and absence of the APOE 4 allele are factors associated with reduced risk of progression to dementia (7).

Early or mild dementia is a condition in which cognitive impairment and reduced cognitive functionality are shown in many areas (e.g. attention, learning, memory and language). Early cases manifest as difficulties in performing complex tasks and progress into problems in simple daily activities. In contrast to MCI, individuals with dementia almost always demonstrate worsening cognitive capabilities, progressing into moderate to severe dementia with time.

In relation with the increasing age of the population, MCI and mild/moderate dementia have become common disorders today. Early diagnosis and treatment are crucial to reduce cognitive worsening and the subsequent loss of ability, especially in MCI. Our aim in this study was to assess whether neurocognitive tests could be effective in distinguishing MCI from Alzheimer's disease-related mild or moderate dementia.

Materials and Methods

Study Group

In our study, we evaluated an extensive set of neurocognitive tests in 72 patients who applied to Adnan Menderes University Hospital's neurology or psychiatry clinics and had been diagnosed with MCI (n=30) or mild (n=23) or moderate (n=19) Alzheimer-type dementia between June 2017 and May 2018. Patients with early-onset dementia or cognitive loss, individuals with a history of head trauma or non-Alzheimer's dementia, those with severe systemic diseases (including cancer, advanced organ failure that could affect cognition and metabolic diseases), and subjects that were receiving medical treatment for any neurological or psychiatric disease were excluded.

Ethics

The study plan was approved by the Ethics Committee of the Aydın Adnan Menderes University Faculty of Medicine on 07.06.2018, with the protocol number 2018/1417. Since the test results and diagnostic evaluations of patients were retrospectively evaluated and no interviews were conducted with the patients or their relatives during the study, written informed consent was not obtained from the patients.

Measurements

The severity of dementia was determined by using the Clinical Dementia Rating (CDR) scale (8). Stage 0 is normal, stage 0.5 is questionable dementia, stage 1 is mild dementia, stage 2 is moderate dementia, stage 3 is severe dementia. All neurocognitive assessments were conducted by a psychologist who was trained for the tests. Participants were applied the Wechsler Memory Scale-Revised (WMS-R) digit span (forward and backward digit span) subtest (9), -III mental control subtest (10), verbal fluency test (11), WMS-IV Logical memory-immediate recall subtest (12), Wechsler Adult Intelligence Scale-Revised Form (WAIS-R) Binary Similarities Subtest (13), Öktem Verbal Memory Processes Test (14), Judgement of

Line Orientation Test, Benton Facial Recognition Test (15), Boston Naming Test (16), clock-drawing test (17) and MMSE (18). Patient groups were compared with regard to results obtained from each test.

In the WMS-R digit span test, the patient is asked to repeat numbers in the same order they were spoken, while in the backward digit span, the patient is asked to repeat numbers in the reverse order. The WMS-III mental control subtest involves counting in series. Depending on the patient's education level, tests include counting backward from 20, counting by threes from 1 to 40, counting the days of the week or months in reverse order. The Verbal Fluency Test evaluates patients' semantic fluency via articulation of animal and fruit-name pairs, and their lexical fluency which is checked by the articulation of the letters, K, A and S. In the WMS-IV logical memory-immediate recall subtest, patients' capability in following a storyline and recall is evaluated. Finally, in the WAIS-R Binary Similarities subtest, the patient is told the name of two objects that do not resemble each other and the their task is to identify and explain the common aspects of these two objects.

Öktem Verbal Memory Processes Test: Consists of two steps. In the first, 15 words are read 10 times to the patient. After each set, the patient is asked to repeat what they can remember (total learning). In the second step, patients are asked to repeat the 15 words after 45 minutes (delayed free recall). In the Judgement of Line Orientation test, the patient is asked to identify which two lines among 11 lines from the bottom page match the angles of the two lines in the top of the page. The Benton Facial Recognition Test consists of face-matching (one target face, six options to choose from) tasks applied consecutively in two phases. In the initial phase, the target image has an exact match among the given options; whereas, in the second phase the faces in the options section are altered in terms of orientation or lighting characteristics and the patient is asked to select the single target to three of these images. The aim of the Boston Naming Test is to evaluate patients' skill in naming pictured objects. As the name clearly states, the clock-drawing test is applied by asking the patient to draw an analogue clock according to pre-determined conditions.

The MMSE consists of 8 "categories" or sub-dimensions that assess time/place orientation, recall/repeating ability, arithmetic, language and complex commands (the latter is often addressed by drawing a standardized shape). The target score indicating normal cognition is 24 points (out of a possible 30). Scores equal to or lower than 9 are associated with severe cognitive impairment, 10-18 points suggest moderate impairment and 19-23 suggests mild impairment.

Statistical Analysis

Statistical evaluation of data was conducted using the IBM SPSS Statistics (version 18.0) package program. The Kolmogorov-Smirnov test was used to investigate the suitability of the data for normal distribution. Because the data is not normally distributed, Kruskal-Wallis test is used for group comparisons for continuous variables and shown with mean ± standard deviation and comparisons between groups were performed via Welch ANOVA. Games Howell is used for post-hoc corrections. For statistical significance, $p<0.05$ value was accepted.

Results

The age range of the study group was 50-92 years, 41 of the patients were females and 31 were males. The distributions of sex, marital status and education levels were similar in the three groups. Mean age was significantly lower in the MCI group compared to the mild dementia ($p=0.017$) and moderate dementia ($p=0.001$) groups. The clock-drawing test also demonstrated a significant difference when the three groups were compared ($p=0.038$) (Table 1).

The overall comparison of scores (3 groups) obtained from tests showed significance in all scores except for the MMSE. When post-hoc corrections were performed, the moderate dementia group was found to have significantly lower scores compared to the mild dementia and MCI groups (respectively) in terms of the following tests: forward digit span ($p=0.125$ and $p=0.002$), backwards digit span ($p=0.013$ and $p<0.001$), WMS III ($p=0.036$ and $p<0.001$), semantic verbal fluency ($p=0.056$ and $p<0.0001$), lexical verbal fluency ($p=0.013$ and $p=0.001$). When looking at the WAIS-R scores, a significant difference was determined in the comparison of moderate dementia and MCI ($p<0.001$), with lower scores in the moderate dementia group. Compared to the mild and moderate dementia groups, the Öktem Verbal Memory Processes Test yielded significantly higher

Table 1. Comparison of sociodemographic characteristics of groups

	Mild dementia (n=21)		Moderate dementia (n=20)		MCI (n=29)		Statistical analysis		
	n	%	n	%	n	%	F	df	p
Sex									
Female	13	61.9	12	60.0	16	55.2	0.251	2	0.882
Male	8	38.1	8	40.0	13	44.8			
Marital status									
Married	9	42.9	10	50.0	17	58.6	3.015	4	0.555
Single	0	0.0	0	0	1	3.4			
Widowed/divorced/separated	12	57.1	10	50.0	11	37.9			
Clock-drawing test									
0	6	60.0	12	80.0	5	21.7	16.305	8	0.038
I	2	20.0	2	13.3	6	26.1			
II	0	0.0	0	0.0	3	13.0			
III	2	20.0	0	0.0	5	21.7			
IV	0	0.0	1	6.7	4	17.4			
Educational status									
Illiterate	6	28.6	8	40.0	7	24.1	10.164	10	0.426
Literate	6	28.6	5	25.0	4	13.8			
Primary school	7	33.3	7	35.0	11	37.9			
Secondary school	0	0.0	0	0.0	3	10.3			
High school	2	9.5	0	0.0	3	10.3			
University	0	0.0	0	0.0	1	3.4			

MCI: Mild cognitive impairment, df: Degree of freedom, F: Wlch's F ratio

scores for patients with MCI, both in terms of total learning (p=0.018 and p<0.0001) and also delayed free recall (p=0.001 and p<0.001). In the Benton Line Orientation Test, scores of the moderate dementia group were significantly lower compared to the MCI group (p<0.0001). In the Benton Facial Recognition and the Boston Naming Tests, the scores of those with moderate dementia were significantly lower compared to the MCI group (p=0.002 and p=0.010). Group comparisons are shown in Table 2 and Table 3.

Discussion

The assessment of our data comparing patients with MCI and Alzheimer's disease-related mild/moderate dementia indicate that individuals with moderate dementia negatively differentiate from the other subjects, while those with MCI differentiate positively, in terms of neurocognitive tests. Meaning that it was often possible to differentiate moderate dementia (but not mild dementia) from MCI with the majority of tests applied; however, remarkably, the Öktem Verbal Memory Processes Test appears to be the only test that may have a role in distinguishing mild dementia from MCI. This feature of the test, which is particularly evident in the Total Learning score, may be critical in the clinical setting when differential diagnosis is necessary.

It is important to diagnose mild dementia before significant changes occur in brain regions due to neuro-degeneration. Early and accurate diagnosis of dementia is important for risk assessment and care management. Objective findings of cognitive impairment have been shown to be present in both MCI and mild dementia (1,19-21). The differential diagnosis of MCI and mild dementia is based mainly on story and cognitive examination. One of the

Table 2. Comparison of groups' neurocognitive test scores according to Kruskal-Wallis

	Mild dementia (n=23)		Moderate dementia (n=19)		MCI (n=30)				
	Mean	SD	Mean	SD	Mean	SD	X^2	df	p
Age (years)	75.81	8.87	78.05	7.89	68.21	9.74	13.857	2	0.001
Forward digit span	2.95	1.81	1.78	1.73	3.60	1.15	11.722	2	0.003
Backward digit span	1.79	1.54	0.50	0.98	2.60	1.22	19.294	2	<0.001
WMS III	2.00	2.00	0.50	1.09	2.92	1.44	18.646	2	<0.001
WMS IV	3.60	4.60	1.23	4.43	8.32	7.11	11.914	2	0.003
Verbal fluency semantic	7.36	4.95	3.24	4.38	10.13	5.48	13.530	2	0.001
Verbal fluency lexical	10.36	7.68	1.65	5.65	12.00	9.51	12.390	2	0.002
WAIS-R	2.00	2.39	0.38	0.88	3.23	2.40	15.083	2	0.001
Öktem Total Learning	33.14	26.64	10.00	21.66	62.39	34.44	20.584	2	<0.001
Öktem Delayed Free Recall	1.50	2.76	0.50	1.41	5.87	4.01	21.619	2	<0.001
Judgement of Line Orientation	7.00	8.28	0.0	0.0	7.89	9.02	9.731	2	0.032
Benton Facial Recognition	26.53	15.95	16.47	16.41	34.04	14.55	14.187	2	0.001
Boston Naming Test	17.78	9.84	12.16	8.68	20.32	9.26	8.995	2	0.008
Mini-Mental State Exam	14.50	4.10	10.42	6.02	17.17	6.56	6.512	2	0.039

MCI: Mild cognitive impairment, WMS: Wechsler Memory Scale, WAIS-R: Wechsler Adult Intelligence Scale-Revised Form, SD: Standard deviation, df: Degree of freedom

major problems is the absence of a standard test to distinguish between Alzheimer's dementia, MCI and changes that naturally occur with aging. Despite ongoing efforts, the effectiveness of neurocognitive tests used in the assessment and distinction of normal aging, MCI and Alzheimer-type dementia are subject to debate (22-24). The MMSE is the most commonly used screening test in the clinic to detect patients with cognitive impairment (25). However, MMSE has limitations in detecting MCI and is unable to detect slight changes in cognitive impairment (26). In our study, when evaluated among 3 groups, it was found that MMSE did not demonstrate a significant difference between. However, due to the low p-value (0.068) we performed a secondary analysis (omitted from the results due to the initial insignificance) to determine whether it was effective for the identification of mild dementia or MCI. This analysis showed that MMSE was similar to the majority of tests. That is, MMSE scores were significantly different between moderate dementia and MCI, but were similar in the mild dementia versus MCI and the moderate dementia versus mild dementia comparisons. These results support the idea that the Öktem Verbal Memory Processes Test may provide an important advantage for differential diagnosis. It must be noted that a significant difference was present between the mild dementia and MCI groups in terms of age; however, the same was true for the moderate dementia versus MCI comparison, while the mild and moderate dementia groups were similar -suggesting that the effect of age may not be significant in this context.

In a study by Jia et al. (27), the mean age of the patients with mild dementia was higher than those with MCI (even though statistically insignificant), and it was found that the MCI and mild dementia groups were both at risk of worsening with time. As mentioned previously, while almost all patients with mild dementia cognitively worsen over the years, some of those with MCI may return to cognitive normality after some time. It has been shown that the age of patients with MCI is an important risk factor for the progression of cognitive worsening (7,27-30).

Table 3. Group comparisons of variables with Welch ANOVA

		df	SD	MS	F	p
Age (years)	Between groups	2	1335.753	667.877	8.258	0.001
	Within groups	67	5418.947	80.880		
	Total	69	6754.700	-		
Forward digit span	Between groups	2	34.909	17.455	7.249	0.002
	Within groups	59	142.058	2.408		
	Total	61	176.968	-		
Backward digit span	Between groups	2	46.213	23.107	14.252	<0.0001
	Within groups	59	95.658	1.621		
	Total	61	141.871	-		
WMS III	Between groups	2	60.236	30.118	12.908	<0.0001
	Within groups	55	128.333	2.333		
	Total	57	188.569	-		
WMS IV	Between groups	2	413.187	206.594	6.018	0.005
	Within groups	39	1338.813	34.329		
	Total	41	1752.000	-		
Verbal fluency semantic	Between groups	2	464.989	232.494	9.200	<0.0001
	Within groups	51	1288.812	25.272		
	Total	53	1753.870	-		
Verbal fluency lexical	Between groups	2	899.922	449.961	7.077	0.002
	Within groups	50	3179.097	63.582		
	Total	52	4079.019	-		
WAIS-R	Between groups	2	75.365	37.683	9.231	<0.0001
	Within groups	44	179.614	4.082		
	Total	46	254.979			
Öktem Total Learning	Between groups	2	26519.600	13259.800	15.646	<0.0001
	Within groups	50	42373.193	847.464		
	Total	52	68892.792	-		
Öktem Delayed Free Recall	Between groups	2	320.420	160.210	16.547	0.0001
	Within groups	50	484.109	9.682		
	Total	52	804.528	-		
Judgement of Line Orientation	Between groups	2	540.127	270.063	5.262	0.009
	Within groups	39	2001.778	51.328		
	Total	41	2541.905	-		
Benton Facial Recognition	Between groups	2	3273.720	1636.860	6.766	0.002
	Within groups	57	13789.930	241.929		
	Total	59	17063.650	-		
Boston Naming Test	Between groups	2	761.794	380.897	4.438	0.016
	Within groups	62	5321.745	85.835		
	Total	64	6083.538	-		
Mini-Mental State Exam	Between groups	2	276.385	138.193	4.046	0.028
	Within groups	29	990.583	34.158		
	Total	31	1266.969	-		

SD: Standard deviation, MS: Mean square, F: Wlch's F ratio, df: Degree of freedom, WMS: Wechsler Memory Scale, WAIS-R: Wechsler Adult Intelligence Scale-Revised Form

The Öktem Verbal Memory Processes Test can distinguish many parameters related to memory (14). The first of these parameters is immediate memory, the second is the process of learning or acquiring knowledge and the third is the process of memory and recall. Recall is evaluated in two ways: delayed free recall and delayed cued recall. In our study, the two sub-parameters identified to have a possible role in distinguishing patients with MCI and mild dementia were the total learning and delayed free recall scores.

When assessing total learning, the interviewer reads 15 words 10 times and asks the patient to recall the words each time. The score is calculated by taking the sum of the words at the end of each repeat. It evaluates the processes of maintaining attention and short-term memory. Patients with MCI were found to be able to remember significantly more words in this area compared to patients with mild dementia (and also those with moderate dementia). This suggests that attention is maintained in MCI patients and that they have little difficulty in learning compared to individuals with dementia. The delayed free recall section evaluates long-term memory. It depends on recalling the initial 15 words, that were spoken in the prior step, after a duration of 45 minutes. Patients with MCI were found to recall a significantly higher number of words than those with mild dementia, which suggests that long-term memory functions are preserved in MCI patients.

These tests evaluate frontal lobe and temporal lobe functions. Earlier studies have reported significant differences between MCI and Alzheimer's disease in terms of frontal lobe functions (31-33). Results of the present study support these conclusions. However, contrastingly Yagi et al. (34) reported that they found no significant difference between these two groups in terms of frontal lobe function.

Quantitative analysis of clock drawing is a very widely used method to assess cognitive functions. It is considered to be an ideal screening test that is independent of culture, language and education. The results of this test pertain to various cognitive skills; therefore, it is utilized to evaluate cognitive skills in a comprehensive manner. Many areas, such as planning, visual, memory, visual-spatial skills, motor planning and application, digital memory, abstract thinking and concentration, are evaluated (35-37). Umidi et al. (38) evaluated MMSE and clock-drawing test in MCI and dementia in comparison to healthy subjects. Both parameters were normal in healthy controls; MMSE was normal and clock drawing was disrupted in MCI; and both parameters were abnormal in cases with dementia. In another study, Beber et al. (39) applied clock-drawing test in two ways (free drawing and the incomplete copy method) for patients with MCI and dementia and healthy controls. They reported a significant difference between all groups with free drawing. In our study, we found that clock-drawing test was distinctive between moderate dementia and MCI patients -similar to Umidi et al. (38). The contradictory results in Beber's study may be due to the inclusion of patients at different stages of cognitive decline.

Apart from the significant difference in age (lower in the MCI group), there are also some other limitations to discuss. Firstly, this work was conducted in a retrospective manner with a single point of measurement. Therefore, the absence of patients' longitudinal analyses (with regard to time since the development of symptoms triggering hospital application) and lack of cognitive progression data are important limitations. Secondly, although all patients that underwent the analyzed tests were included in the study, there is probability of baseline differences (or biases) related to the identification of tests that were applied to each patient. Besides, other types of dementia (vascular, frontotemporal etc.) could not be included in the study due to very low patient numbers. In addition, subjects were not separated with regard to amnestic or non-amnestic MCI. Finally, the absence of a geriatric depression scale to assess whether depression was effective on neurocognitive tests was also another significant limitation.

Conclusion

To conclude, we believe that the total learning and delayed free recall scores of the Öktem Verbal Memory Processes Test can guide in the discrimination of MCI from mild dementia. After appropriate training, these tests can be administered by all clinicians and the early examination and diagnosis of MCI and mild dementia may be possible. The results of this study must be supported by future research with increased patient numbers. It is also critical to note that future studies on this topic should strive to assess whether linguistic, cultural and education-related differences among subjects can affect the results of cognitive

assessment via the Öktem Verbal Memory Processes Test.

Ethics

Ethics Committee Approval: The study plan was approved by the Ethics Committee of the Aydın Adnan Menderes University Faculty of Medicine on 07.06.2018, with the protocol number 2018/1417.

Informed Consent: Written informed consent was not obtained from the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.Ş., Design: Y.B.Ş., Supervision: Y.B.Ş., Fundings: A.Ş., Materials: A.Ş., Y.B.Ş., Data Collection or Processing: A.Ş., Y.B.Ş., S.Ö., Analysis or Interpretation: A.Ş., Literature Search: Y.B.Ş., Writing: A.Ş., Critical Review: Y.B.Ş.

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Original Article / Özgün Araştırma

The Effects of Vitamin D Level on Bone Lesions and Prognostic Factors in Multiple Myeloma

Multipl Miyelomda D Vitamini Düzeyinin Kemik Lezyonları ve Prognostik Faktörler Üzerine Etkileri

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Keywords
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Abstract

Objective: Several studies have found that low vitamin D levels are associated with an increased risk of hematologic malignancies and a poor prognosis. In this study, we investigated the relationship between 25-hydroxyvitamin D levels and bone lesions as well as prognostic factors in patients with multiple myeloma. **Materials and Methods:** We evaluated 184 people who had just been diagnosed with multiple myeloma. Complete blood count, biochemical parameters, serum 25-hydroxyvitamin D [25(OH)D] level, presence of lytic bone lesion and fracture, and disease stage were all recorded. The patients were divided into three groups based on their 25(OH)D levels: deficient (<20 ng/mL), insufficient (20-29 ng/mL), and normal (≥30 ng/mL). SPSS-21 was used to perform statistical analyses. **Results:** The 25(OH)D was deficient in 121 patients (65.8%), insufficient in 28 patients (15.2%), and normal in 35 patients (19%). Age, presence of lytic bone lesion and bone fracture, plasma cell rate in bone marrow, and stage of disease (p=0.02; p=0.01; p=0.007; p=0.02, respectively) all differed significantly between these groups. Patients with normal 25(OH)D levels had lower risk of lytic bone lesion and fracture. Furthermore, deficiency and insufficiency of 25(OH)D had a negative impact on disease stage, and advanced disease stage is a poor prognostic parameter for multiple myeloma. **Conclusion:** Patients with normal 25(OH)D levels have a lower risk of lytic bone lesion and vertebral fracture. The stage of the disease is influenced by 25(OH)D deficiency and insufficiency. Therefore, early detection and treatment of 25(OH)D deficiency and insufficiency in patients with multiple myeloma may reduce mortality and morbidity rates.

Öz

Amaç: Birkaç çalışma, düşük D vitamini düzeyinin, hematolojik malignitelerin artmış insidansı ve kötü prognozu ile ilişkili olduğunu bildirmiştir. Biz bu çalışmada multipl miyelomlu hastalarda, 25-hidroksivitamin D [25(OH)D] düzeyi ile kemik lezyonları ve prognostik faktörler arasındaki ilişkiyi araştırdık. **Gereç ve Yöntemler:** Yeni tanı almış 184 multipl miyelom hastası değerlendirildi. Tam kan sayımı, biyokimyasal parametreler, serum 25(OH)D düzeyi, litik kemik lezyonu ve kırık varlığı, hastalık evresi kaydedildi. Hastalar 25(OH)D düzeyine göre 3 gruba ayrıldı; eksik (<20 ng/mL), yetersiz (20-29 ng/mL) ve normal (≥30 ng/mL). İstatistiksel değerlendirmeler SPSS-21 programı ile yapıldı.

Bulgular: 25(OH)D düzeyi 121 hastada (%65,8) eksik, 28 hastada yetersiz (%15,2) ve 35 hastada (%19) normaldi. Bu gruplar arasında yaş, litik kemik lezyonu ve kırık varlığı, kemik iliğinde plazma hücre oranı ve hastalık evresi açısından anlamlı fark vardı (sırasıyla p=0,02, p=0,01, p=0,007, p=0,02). 25(OH)D düzeyi normal olan hastalarda litik kemik lezyonu ve kırık daha azdı. Ek olarak; 25(OH)D eksikliği ve yetersizliği hastalığın evresini olumsuz etkiledi ve hastalığın ileri evresi multipl miyelom için kötü prognostik parametredir. **Sonuç:** 25(OH)D düzeyi normal olan hastalarda litik kemik lezyonu ve kemik kırığı daha az görülür. 25(OH)D eksikliği ve yetersizliği hastalığın evresini olumsuz etkiler. Bu nedenle; 25(OH)D eksikliği ve yetersizliğinin erken tespiti ve tedavisi multipl miyelomlu hastalarda mortaliteyi ve morbidite oranını azaltabilir.

Introduction

Vitamin D promotes calcium absorption from the intestine and provides sufficient serum calcium and phosphate concentrations for mineralization of the bones. Vitamin D deficiency mainly causes loss of bone density and it can contribute to osteoporosis and bone fractures. In addition; low vitamin D level is associated with cardiovascular diseases, solid organ and hematological cancers, metabolic syndrome, infectious and autoimmune diseases (1,2). Vitamin D receptors are present in hematopoietic cells and variety of tissues in the body (3). Vitamin D regulates various genes that responsible for cell proliferation by binding to the active vitamin D receptor and inhibits the growth of cancer cells (4-6). Vitamin D deficiency may contribute to carcinogenesis by impairing these normal regulatory processes. In some studies reported that vitamin D deficiency was associated with inferior event free survival and overall survival in patients with diffuse large B cell and T cell non-Hodgkin lymphoma and a worse outcome in patients with acute myeloid leukemia (7,8).

Multiple myeloma (MM) is a non-curative hemalogical malignant disease and it originates from plasma cells. The patients with MM have some prognostic marker e.g; plasma cell labeling index, the presence of some cytogenetic abnormalities, gene expression profile, the stage of disease (9). Bone lesions occur in 80-90% of all cases with MM at the time of diagnosis and are among the most important causes of morbidity (10). Osteoclast-mediated bone destruction increases and osteoblastic activity reduces significantly because of cytokines and chemokines that released from tumor cells and stroma cells in bone marrow (11). In addition, vitamin D deficiency causes hyperparathyroidism that increases osteoclastic activity and decreases osteoblastic activity. Therefore vitamin D deficiency can contribute to the formation of bone lesions, risk of fall in MM. Bone lesions are often seen in the vertebral column, skull, ribs, pelvis and long bones and it may cause impairment in quality of life, neurological deficit, pathological fracture and hypercalcemia. Conventional radiography, computerized tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) can be used to detect bone lesion. The aim of the present study was to verify the potential association between the level of 25 hydroxyvitamin D [25(OH)D] and lytic bone lesion, vertebral fracture, prognostic factors in MM.

Materials and Methods

In this study, we studied 184 newly diagnosed and treated MM patients at the Department of Hematology of the Atatürk University. Approval of the Atatürk University Ethics Committee was obtained for the study and informed consent was received from all participants (decision no: 31, date: 27.02.2020). The patients who measured the level of serum 25(OH)D at the time of admission were received in this study. The patients with chronic kidney failure and receving regular vitamin D supplements were excluded of this study.

We retrospectively examined the medical files of all cases and recorded sex, age, the levels of hemoglobin, serum creatine, blood urea nitrogen, total protein, albumin, calcium, globulin, serum immunoglobulin (G,A,M), 25(OH)D, the presence of lytic bone lesion and fracture, plasma cell rate in bone marrow, stage of disease. The presence of lytic bone lesion and fracture were evaluated by using conventional radiography and CT/MRI/PET-CT findings. We used International Staging System (ISS) as the prognostic score. In this scoring system, if the albumin level ≥3.5 mg/dL and β2 microglobulin level <3.5 mg/dL is stage 1, β2 microglobulin level ≥5.5 mg/dL is stage 3 and patients who don't fulfill the stage 1 or 3 criteria are evaluated as stage 2.

Vitamin D level is often measured by automated immunoassays techniques (12). The serum level of 25(OH)D was measured using the CLIA method in the Siemens ADVIA Centaur XP device in our study. Vitamin D sufficiency, insufficiency and deficiency were defined as 25(OH)D level of 30-100 ng/mL, 20-29 ng/mL, and less than 20 ng/mL, respectively in Clinical Practice Guideline that it was published by the endocrine society's (13). In this study, the patients were divided into 3 groups according to this guideline; deficient [25(OH)D<20 ng/mL], insufficient [25(OH)D= 20-29 ng/mL], and normal [25(OH)D≥30 ng/mL].

Statistical Analysis

Statistical evaluations were made by SPSS-21 windows software (Armonk, NY: IBM Corp). Demographic variables were evaluated with simple descriptive analysis. We used independent t-test for determine difference between two goups if normal distribution was present, Mann-Whitney U test was used if the group distribution was abnormal. One-way ANOVA was applied to test the mean differences between multiple groups. For all analyses, p-value <0.05 was considered to indicate statistical significance.

Results

The mean age of our cases was 68.74±10.47 years and 55 (29.9%) patients were female and 129 (70.1%) patients were male. The median 25(OH)D level at the time of diagnosis, was 19.6±18.2 ng/mL (range =3-71 ng/mL) in 184 patients. There were 121 cases (65.8%) with the 25(OH)D deficiency, 28 (15.2%) cases with 25(OH)D insufficiency, and 35 (19%) cases with 25(OH)D normal. Vitamin D deficiency increased with increasing age (Table 1).

Fifty seven (31%) patients had stage 1, 53 (28.8%) patients had stage 2, 74 patients (40.2%) had stage 3. The level of 25(OH)D was 22.95±21.22 ng/mL in patients with stage 1, 20.72±16.21 ng/mL in cases with stage 2, and 13.72±14.18 ng/mL in cases with stage 3. As the disease stage increased, the 25(OH)D level decreased statistically significantly (p=0.01).

Eighteen (9.8%) of all patients had bone fracture and 105 of all patients (57.1%) had lytic bone lesion. Bone fracture and the presence of lytic bone lesion were associated with 25(OH)D sufficiency and insufficiency (Table 2).

Laboratory findings of the patients were presented in Table 3. We didn't find a relationship between

Table 1. The association with 25-hydroxyvitamin D level and age

Vitamin D level	Age			
	Mean ± SD	Minumum	Maximum	p-value
25(OH)D deficiency (n=121)	73.14±8.9	50 years	88 years	0.02
25(OH)D insufficiency (n=28)	68.39±9.04	47 years	78 years	
Normal 25(OH)D level (n=35)	67.55±10.92	24 years	92 years	

25(OH)D: 25-hydroxyvitamin D, SD: Standard deviation

Table 2. The association with 25-hydroxyvitamin D level and bone fracture, lytic bone lesion

Bone fracture		
Vitamin D level	**Present (number patient) (%)**	**p-value**
25(OH)D deficiency (n=121)	15 (12.39%)	0.007
25(OH)D insufficiency (n=28)	2 (7.14%)	
Normal 25(OH)D level (n=35)	1 (2.85%)	
Lytic bone lesion		
Vitamin D level	**Present (number patient) (%)**	**p-value**
25(OH)D deficiency (n=121)	90 (74.38%)	0.01
25(OH)D insufficiency (n=28)	8 (28.57%)	
Normal 25(OH)D level (n=35)	7 (20%)	

25(OH)D: 25-hydroxyvitamin D

the levels of 25(OH)D and hemoglobin, calcium, sedimentation rate, albumin, globulin, creatine, β2 microglobuline (Table 4). The statistically significant relationship was between the level of 25(OH)D and plasma cell count in bone marrow (Table 5).

Discussion

Vitamin D mainly provides the protection of calcium, phosphorus homeostasis and bone mineralization and its deficiency is a common public health problem around the world (14). Vitamin D deficiency causes rickets in children and osteomalacia in adults. But the consequences of its deficiency are not limited to rickets and osteomalacia. Several studies reported vitamin D deficiency was associated with diabetes mellitus, hypertension, obesity, metabolic syndrome, cardiovascular disease, infectious and autoimmune diseases (15,16). In addition, it is known that one of the causes of colon, breast and ovarian cancers is vitamin D deficiency (17,18). In the northern states of the United States, prostate, breast

Table 3. Laboratory findings of all the patients

Variable	Minimum	Maximum	Mean
Plasma cell in bone marrow (%)	10	65	30.8±11.76
Hemoglobin (g/dL)	4.6	13.2	10.91±2.37
Sedimentation rate	2	140	65.37±37.73
Total protein (g/dL)	6.1	15.5	8.45±2.14
Albumin (g/dL)	1.1	5.1	3.41±0.72
Globulin (g/dL)	1.8	10.7	5±2.42
Calcium (mg/dL)	8.5	15.2	9.27±1.51
Creatine (mg/dL)	0.3	8.1	1.45±1.32
β2 microglobuline	1.6	13.2	5.98±4.48
Serum immunoglobulin G	1.31	65.92	26.53±13.97
Serum immunoglobulin A	0.1	37	3.16±3.97
Serum immunoglobulin M	0.01	5.9	0.81±0.74

Table 4. The relationship with 25(OH)D level and laboratory findings

Variable	25(OH)D deficiency	25(OH)D insufficiency	Normal 25(OH)D level	p-value
Hemoglobin (g/dL)	10.83±2.37	11.28±2.01	10.91±2.37	0.66
Sedimentation rate	65.09±36.44	67.75±42.64	64.40±39.10	0.93
Albumin (g/dL)	3.38±0.71	3.52±0.83	3.38±0.68	0.64
Globulin (g/dL)	4.98±2.46	4.89±2.30	5.19±2.42	0.86
Calcium (mg/dL)	9.34±1.48	9.28±2.01	9.03±1.08	0.57
Creatine (mg/dL)	1.32±1.21	1.75±1.53	1.67±1.46	0.16
β2 microglobuline	5.90±4.38	5.66±5.40	6.52±4.12	0.71

25(OH)D: 25-hydroxyvitamin D

Table 5. The relationship with 25(level and the rate marrow OH)D of plasma cell in bone (%)

	Plasma cell rate in bone marrow (%)			
25(OH)D level	**Mean ± SD**	**Minimum**	**Maximum**	**p-value**
25(OH)D deficiency (n=121)	68.39±9.04	47	88	0.02
25(OH)D insufficiency (n=28)	67.55±10.92	24	92	
Normal 25(OH)D level (n=35)	35.75±13.53	18	65	

25(OH)D: 25-hydroxyvitamin D, SD: Standard deviation

and colon cancer were more frequently than the sunnier states. Therefore, the relationship between vitamin D deficiency and incidence of cancer has been investigated in some studies (19-21). The altitude of Erzurum province, where the study is conducted, is 1,900 meters and the number of sunny days is low. Therefore, only 19% of our patients had normal vitamin D level.

Vitamin D level is determined by measuring 25(OH)D. The half-life of 1.25(OH)D_2 is only 4 hours while the half-life of 25(OH)D is 2-3 weeks and 25(OH)D circulates at a 1,000-fold higher concentration than 1,25(OH)D_2. Therefore, we determined the patient's vitamin D status by using the 25(OH)D level. The vitamin D receptors are found in almost all cells in the human body. 25(OH)D affects angiogenesis by reducing the expression of vascular endothelial growth factor and interleukin 8. Vitamin D makes the antitumor effect by regulating proliferation, differentiation and apoptosis. We did not measure vitamin D receptor level in this study.

Vitamin D deficiency is common in patients with MM (22,23). Hudzik et al. (24) evaluated 675 MM patients. They reported that 25(OH)D level was <10 ng/mL in 52 (7.7%) patients and it was 10-30 ng/mL in 394 (51%) patients. Vitamin D deficiency was reported 16-37% of all patients with MM by Alvin C et al. (25). Graklanov et al. (26) evaluated 37 patients with newly diagnosed MM. They reported that 1 patient (2.7%) had vitamin D insufficiency (serum levels between 20-30 ng/mL) and 36 patients (97.3%) had vitamin D deficiency (levels below 20 ng/mL). Severe vitamin D deficiency (<10 ng/mL) was observed in 81% of all patients. Vitamin D deficiency was detected in 80.9% of all patients in our study. This may be explained by the lower exposure to sunlight due to high altitude and climate in the Erzurum. In addition, the fact that the people of Erzurum prefer the style of clothing that prevents the use of the sun can also contribute to this situation.

Vitamin D level was found significantly low in the patients with acute myeloid and lymphoblastic leukemias. It has been reported that vitamin D deficiency to be associated with poor prognosis and worse response to treatment in patients with hematological malignancies (27,28). Lauter et al. (29) determined that 25(OH)D insufficiency (<10 ng/mL) was associated with elevated plasma cells count in the bone marrow. Gedik et al. (30) and Graklonav et al. (26) did not find a relationship between 25(OH)D level and ISS staging of MM. In addition Nath et al. (31) 41 patients with MM and they reported that there was no association between vitamin D status and stage of myeloma. But, it was reported that the prevalence of vitamin D deficiency increased in parallel with ISS in another study (25). We detected that the patients with vitamin D deficiency and insufficiency had elevated plasma cell count in bone marrow and advanced stage disease. This two paramethers are negative prognostic factors in MM. Increased β2 microglobuline level is also a poor prognostic marker. We didn't define a relationship between β2 microglobuline level and vitamin D status. In addition, we could not evaluate the survival of patients due to lack of data.

Vitamin D deficiency was related to high C-reactive protein (CRP) and creatine levels and advanced stage disease in MM (32,33). But we didn't find any significant correlation between vitamin D status and serum creatine level. Alvin C et al. (25) examineted 148 patients with MM. They detected that the patients with vitamin D deficiency [25(OH)D<20 ng/mL] had higher serum CRP, creatine levels and lower serum albumin level than patients without vitamin D deficiency (25). But we didn't find any correlation between vitamin D status and serum albumin, creatine levels.

Vitamin D deficiency can cause musculoskeletal pain, proximal muscle weakness, increased risk of falls. Low levels of vitamin 25(OH)D can cause secondary hyperparathyroidism and bone resorption via osteoclasts, which may accelerate osteopenia and osteoporosis in adults. Skeletal complications, such as lytic bone lesion, hypercalcemia, compression fracture are the main causes of morbidity in MM. Therefore, early diagnosis and treatment of vitamin D deficiency may reduce skeletal complications. Alvin didn't find any correlation between low vitamin D level and skeletal morbidity. Badros et al. (33) evaluated 100 MM patients and they reported that there was no significant correlation between vitamin D status and presence of lytic bone disease and fracture. Nath et al. (31) reported that MM cases with vitamin D deficiency have higher skeletal morbidity, but this is not statistically significant (73% vs 50%, p=0.19). But we defined that the lytic bone lesion and bone fracture were more common in patients with vitamin D deficiency and insufficiency.

Conclusion

Low vitamin D level is important public health problem. Because it is associated with increased malignancy incidence and worse prognosis in patients with hematological and solid organ malignancies. Therefore treatment of vitamin D deficiency may be reduce cancer devolopment and worse prognosis in patients with cancer. This hypothesis should be supported by studies involving more cases.

Ethics

Ethics Committee Approval: Approval of the Atatürk University Ethics Committee was obtained for the study (decision no: 31, date: 27.02.2020).

Informed Consent: Informed consent was received from all participants

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.S., F.E., Design: G.S., F.E., Supervision: G.S., F.E., Fundings: G.S., Materials: G.S., Data Collection or Processing: G.S., F.E., Analysis or Interpretation: F.E., Literature Search: F.E., Writing: G.S., F.E., Critical Review: F.E.

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Original Article / Özgün Araştırma

Neutrophil-lymphocyte Ratio and Serum Ferritin, Folate, Vitamin B12 and 25-hydroxyvitamin D Levels in Children and Adolescents with Primary Headaches

Primer Baş Ağrısı Olan Çocuk ve Adölesanlarda Nötrofil-lenfosit Oranı, Serum Ferritin, Folik Asit, B12 Vitamini ve 25-hidroksivitamin D Düzeyleri

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Headache, complete blood count, 25-hydroxyvitamin D, ferritin, vitamin B12, folic acid

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Abstract

Objective: Headache is one of the most common complaints in general pediatric outpatient clinics. We aimed to investigate the complete blood count (CBC) parameters and serum levels of 25-hydroxyvitamin D [25(OH)D], ferritin, vitamin B12 and folic acid in children and adolescents with primary headaches.

Materials and Methods: In this study, 149 patients with primary headache [migraine, tension-type headaches (TTH) and unclassified headache] and 104 healthy controls, aged 5 to 18 years, were evaluated retrospectively. Age, gender, physical examination findings and laboratory parameters [CBC, 25(OH)D, vitamin B12, folic acid and ferritin] were also recorded for both groups.

Results: The headache types in the primary headache group were TTH (48.1%), migraine (28.2%) and unclassified headache (23.7%). The primary headache group had lower median levels of ferritin and serum vitamin B12 than healthy controls (p=0.028 and p=0.014, respectively). The neutrophil-lymphocyte ratio (NLR) was significantly higher in the primary headache group (p=0.033). A binary logistic regression analysis model showed low ferritin levels as an independent risk factor for primary headaches (odds ratio =1.018, 95% confidence interval =1.003-1.032).

Conclusion: The study results demonstrated lower serum vitamin B12 and ferritin and a higher NLR in children with primary headaches when compared to healthy children. We suggest that these findings require support from larger prospective studies.

Öz

Amaç: Baş ağrısı, genel çocuk polikliniklerinde en sık görülen şikayetlerden biridir. Bu çalışma ile birincil baş ağrısı olan çocuk ve ergenlerde, tam kan sayımı (TKS) parametreleri, 25-hidroksivitamin D [25(OH)D], ferritin, vitamin B12 ve folik asit düzeylerini araştırmayı amaçladık.

Gereç ve Yöntemler: Bu çalışmada 5-18 yaş arasındaki primer baş ağrısı olan 149 hasta [migren, gerilim tipi baş ağrısı (GTBA), sınıflandırılmamış baş ağrısı] ve 104 sağlıklı kontrol retrospektif olarak değerlendirildi. Her iki grup için de yaş, cinsiyet, fizik muayene bulguları ve laboratuvar parametreleri [TKS, 25(OH)D, vitamin B12, folik asit, ferritin] kaydedildi.

Bulgular: Birincil baş ağrısı grubundaki baş ağrısı tipleri GTBA (%48,1), migren (%28,2) ve sınıflandırılamayan baş ağrısı (%23,7) idi. Birincil baş ağrısı grubunda ortanca ferritin ve serum B12 vitamini seviyeleri sağlıklı kontrollere göre anlamlı olarak daha düşüktü (sırasıyla p=0,028, p=0,014). Nötrofil lenfosit oranı (NLO), birincil baş ağrısı grubunda anlamlı olarak daha yüksekti (p=0,033). Lojistik regresyon analizi, düşük ferritin seviyelerinin birincil baş ağrıları için bağımsız bir risk faktörü olduğunu göstermiştir (olasılık oranı =1,018, %95 güven aralığı =1,003-1,032).

Sonuç: Bu çalışmada birincil baş ağrısı olan çocuklarda sağlıklı çocuklara göre serum vitamin B12 ve ferritin düzeyleri daha düşük, NLO daha yüksek olarak bulunmuştur. Bu sonucun daha fazla sayıda hastayı içeren prospektif çalışmalarla desteklenmesi gerektiğini düşünmekteyiz.

Introduction

Headache is one of the most common neurological symptoms in children and adolescents and affects approximately 88% of this age group (1). Headaches are important health problems due to their effects on academic achievements, physical, and mental condition. Also, headaches constitute the majority of admission to pediatric outpatient clinics and the most prevalent types of primary headaches are migraine and tension-type headaches (TTH). In recent years, the prevalence of childhood migraine and TTH has increased due to the lifestyle changes of children (2).

One of the most widely accepted hypotheses in the pathogenesis of migraine is neurogenic inflammation. It is thought that some external factors (hormones, stress, and diet) initiate attacks in genetically susceptible individuals, stimulate noradrenergic and serotonergic nerve fibers, causing vasodilation of intracranial vessels, the release of inflammatory neuropeptides (substance P, neurokinin, *calcitonin* gene-related protein) by stimulating the trigeminal nerve, and ultimately causing vasodilation, inflammation, and pain (3). Apart from these, there are a few studies indicate that primary headaches may be associated with platelet count, and levels of hemoglobin, vitamin D, ferritin, folate, and vitamin B12 (4-7).

Iron is the most commonly consumed nutrient in the human diet. Studies have shown that iron plays a crucial role in the production of serotonin, dopamine, and norepinephrine (8). Many studies exist investigating the neurological effects of iron deficiency. It has been reported that children with iron deficiency had more anxiety, depression, social and attention deficit problems (9,10). Additionally, an association between serum ferritin level and depression was reported in adults (10). Also, low serotonin levels between the attacks and increased levels during attacks were reported in patients with headaches (11).

The *methylenetetrahydrofolate reductase (MTHFR)* gene allows the remethylation of homocysteine to methionine. It is claimed that decreased MTHFR activity predisposes to migraine (12). There are some studies show that supplementation with folate and vitamin B12 ameliorate the attacks of migraines, especially in patients with certain genetics variants of enzymes involved in homocysteine metabolism (13). There are few studies investigating serum vitamin B12 levels in children with primary headaches (4,6).

Evidence shows that homocysteine decreases noradrenaline and serotonin synthesis (14). Folic acid, vitamin B6, and vitamin B12 have been shown to lower serum homocysteine levels and reduce migraine symptoms (15).

Neutrophils and lymphocytes have important roles in the inflammatory response. Since the number of neutrophils increases and the number of lymphocytes decreases as a response to stress, the neutrophil-lymphocyte ratio (NLR) is used as an indicator of inflammation (16). Several studies exist concerning the relationship between hematological parameters including NLR and headaches. The results of these previous studies are inconsistent (5,17,18).

The relationship between vitamin D and primary headaches is not well elucidated. Some studies reported a possible relationship between vitamin D and migraine. Although the relation between vitamin D and primary headaches is not clear, calcium, which plays a role in the contraction of smooth muscle cells may have a role. Also, the widespread distribution of vitamin D receptors in human brain tissue suggests that vitamin D may have autocrine/paracrine properties in the brain (19).

A limited number of studies have evaluated the relationship between complete blood count (CBC) parameters, serum levels of 25-hydroxyvitamin D [25(OH)D], ferritin, vitamin B12, folic acid levels, and primary headaches in pediatric patients. Thus, we aimed to investigate the CBC, serum levels of 25(OH)

D, ferritin, vitamin B12, and folic acid in children and adolescents with primary headaches.

Materials and Methods

This retrospective study included children and adolescents aged 5-18 years who were admitted to the general pediatric outpatient clinic with headaches for at least 6 months between June 2020 and November 2020. Exclusion criteria were as follows; children with chronic diseases (autoimmune diseases, infections, neuro-metabolic diseases, mental retardation, endocrinological diseases, psychiatric disorders), acute infections, secondary headaches, children who were taking any medication or vitamin supplements. The diagnosis of primary headaches (migraine, TTH, unclassified headache) was made by a pediatric neurologist according to the International Classification of Headache Disorders (ICDH III-beta) criteria (20). The control group included healthy children without a history of headache or any diseases who were admitted to the general pediatric outpatient clinic for evaluation before sportive activities. Age, gender, physical examination findings, and laboratory parameters [CBC, 25(OH)D, vitamin B12, folic acid, ferritin] were also recorded for both groups.

We have obtained the approval of the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 11, date: 25.02.2021) outlined in the Second Declaration of Helsinki. No informed consent has been collected since it is a retrospective study.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics (kurtosis and skewness), visual methods (histogram), and analytical tests (Kolmogorov-Smirnov test) were used to determine the normal distribution of numerical variables. Categorical data were presented with n and %, and numerical data with mean ± standard deviation (SD) if normally distributed, and median (IQR) if non-normally distributed. In the comparison of two groups, student t-test was used if the data was normally distributed, and Mann-Whitney U test was used if the data were non-normally distributed. Chi-square test was used for comparison of categorical data. Possible factors determined by the prior analysis were analyzed by binary logistic regression analysis to determine the risk factors. A p-value <0.05 was accepted as statistically significant.

Results

a) Patient Characteristics

A total of 149 children with a headache and 104 healthy control subjects were included in the study. Fourteen children were excluded due to a secondary headache (eight hypertension, four sinusitis, two refractive errors, one allergic rhinoconjunctivitis). There were no significant differences between the groups in terms of age, SD scores (SDS) for weight, height, and body mass index (p>0.05). The headache types in the study group were TTH (48.1%), migraine (28.2%), and unclassified headache (23.7%). Thirty-eight percent of the patients had a positive family history of a primary headache.

b) Laboratory Characteristics

White blood cell, platelet counts, hemoglobin, hematocrit, serum 25(OH)D, and folic acid levels were not different between the groups. The median levels of ferritin and serum vitamin B12 were significantly lower in the primary headache group than those of healthy controls (p=0.028, p=0.014, respectively). The NLR was significantly higher in the primary headache group (p=0.033) (Table 1). Based on binary logistic regression analysis including NLR, serum ferritin, and B12 levels, every 1-unit decrease in serum ferritin constitutes a 1.018 (95% confidence interval =1.003-1.032) fold risk for primary headache (Table 2).

Discussion

In the present study, serum levels of ferritin and vitamin B12 were lower and NLR was higher in children with a primary headache than those of control subjects. We also found low ferritin levels as an independent risk factor for primary headaches.

Migraine is the most common type of primary headache in young children (21,22). However, there are studies also reporting different results. In a Korean study, the prevalence of TTH was reported to be higher than migraine in children both aged below 7-years and those aged above 7-years (23). According to a study conducted in school-age children in Turkey, the estimated prevalence rates of unclassified headache, migraine, and TTH were 4.6%, 7.2%, and

Table 1. The comparison of demographic features and laboratory results between the groups			
	Primary headache group (n=135)	Control group (n=104)	p
Age (years)†	13 (5.80)	12.95 (6.15)	0.177
Female	77 (57%)	50 (48%)	0.169
Male	58 (43%)	54 (52%)	
Weight-SDS†	-0.13 (2.10)	-0.21 (2.40)	0.611
Height-SDS*	0.05±0.98	0.13±1.35	0.143
BMI-SDS*	-0.01±1.46	0.28±1.57	0.713
Hemoglobin (mg/dL)†	13.20 (1.60)	13.30 (1.58)	0.234
Hematocrit (%)†	39.50 (4.10)	40.60 (4.88)	0.186
Platelet (x10^3)†	288.50 (85.00)	293.00 (113.50)	0.455
WBC†	7650 (2720)	7025 (2417)	0.480
NLR†	1.50 (0.92)	1.24 (0.78)	0.033
Vitamin B12 (pg/mL)†	273.00 (137.00)	303.00 (160.25)	0.028
Folic acid (ng/mL)†	7.70 (3.90)	7.40 (3.33)	0.323
Ferritin (mg/dL)†	21.38 (20.52)	27.28 (22.41)	0.014
25-hydroxyvitamin D (ng/mL)†	20.20 (12.50)	21.05 (11.30)	0.148

SDS: Standard deviation score, BMI: Body mass index, WBC: White blood cell, NLR: Neutrophil-lymphocyte ratio, *mean ± standard deviation, †median (interquartile range)

Table 2. Binary logistic regression analysis for prediction of independent risk factors for primary headaches					
	β	Odds ratio	95% CI for OR		p
			Lower limit	Upper limit	
Ferritin	0.017	1.018	1.003	1.032	0.015
Vitamin B12	0.001	1.001	1.000	1.003	0.129
NLR	-0.170	0.843	0.600	1.185	0.329
Constant	-0.964	0.382	-	-	0.051

CI: Confidence interval, OR: Odds ratio, NLR: Neutrophil-lymphocyte ratio

7.8%, respectively (24). Just et al. (25) suggested that ethnic and geographic differences might have a role in the prevalence of headache type. Methodological differences might also have a contribution to the different results. Consequently, the prevalence of primary headache types can vary from country to country, and also region to region (25). TTH was the most frequent type of primary headache in the present study.

Iron deficiency (with or without anemia) may lead to different symptoms, such as angular cheilitis, koilonychia, hair loss, loss of appetite, fatigue, sleep disorders, restless leg syndrome, breath-holding spell, dry and rough skin (26). Few studies exist investigating the association between headaches and serum ferritin levels. Aydin et al. (4) reported that levels of serum ferritin, folate, vitamin B12, and 25(OH)D were significantly lower in patients with migraines than those of the control group. In a case-control study, the levels of hemoglobin and serum ferritin were significantly lower in females with migraines than those of the control group (7). Similarly, in the present study, serum ferritin levels were significantly lower in the primary headache group. Moreover, we found every 1-unit decrease in serum ferritin constitutes a 1.018 fold risk for primary

headache. There are studies regarding the association between anxiety, depression, and primary headaches. In a study by Rousseau-Salvador et al. (27), children with chronic daily headaches had higher depression scores than the standardized reference population. Romano et al. (28) found a significant relationship between anxiety, depression, and primary headaches. In a case-control study, 67 females with depression were compared with 125 healthy subjects and serum ferritin levels were significantly lower in the depression group. Therefore, it is suggested that there was a probable association between depression and decreased ferritin levels before the occurrence of anemia (10). The association between migraine and serotonin is a controversial issue for decades. Curran et al. (29) showed that the urinary excretion of the 5-hydroxyaindoleacteic acid, which was the main metabolite of serotonin, was increased. Hoyer et al. (11) reported a low serotonin content between migraine attacks and increasing levels during attacks. Therefore, these results gave rise to the thought of migraine was associated with chronically low serotonin levels with transient increases during attacks. Kaladhar and Narasinga Rao (30) performed a study in rats to investigate the effects of iron deficiency on serotonin uptake and showed decreased serotonin uptake. The results of the present study can be explained by the studies which were mentioned above concerning the relationship between iron deficiency and serotonin uptake.

We found lower vitamin B12 levels in children with primary headaches than control subjects. Vitamin B12 deficiency may lead to various neurological disorders such as motor delay, apathy, seizures, and involuntary developmental disorders (31). There are few studies concerning the association between migraine, TTH, and vitamin B12 levels (4,6,32). In a case-control study (6), it is showed that serum vitamin B12 levels were significantly lower in pediatric patients with TTH than those of the control. Togha et al. (32) showed that serum vitamin B12 levels were significantly lower in adults with migraines. Similarly, serum vitamin B12 levels were significantly lower in the primary headache group than those of the control group in the present study. As we browse through the probable explanations of the relationship between serum vitamin B12 levels and pain; animal studies showed that vitamin B12 may have both central and peripheral cyclooxygenase (COX) inhibitory features. The possible relation between the pain and vitamin B12 could be partially explained by the inhibitory action of vitamin B12 on both central and peripheral COX enzymes that demonstrated in animal studies. Additionally, a probable role to control COX levels during the inflammatory process was also proposed (33,34). Furthermore, vitamin B12 has a neurotransmitter-moderated antinociceptive effect by lowering homocysteine levels which decreases serotonin synthesis (14).

Folate is a water-soluble synthetic form of folic acid which is also necessary for homocysteine metabolism (35,36). Studies exist concerning the relationship between folate and headaches (4,37). There are reports suggesting dietary supplementations with folate and B vitamin complexes in patients with primary headaches (38). In the present study, no significant differences were found between the case and control groups, in terms of folate levels.

NLR is associated with peripheral inflammation and oxidative stress in chronic neurological diseases. Additionally, it is used as a predictor of cardiovascular disease, stroke, and cancer prognosis (39). In the present study, the median NLR value was significantly higher in the primary headache group than in the control group. The results of the previous studies regarding the relationship between hematological parameters and headaches are inconsistent. In a case-control study, NLR was significantly higher in adults with acute migraine attacks than those of the control group (5). In another study, there were no significant differences between the patients with migraine, and the control group, in terms of NLR values (40). A case-control study performed in adults with migraine showed no significant differences between the three groups (migraine with aura, migraine without aura, and healthy control), in terms of NLR (17). However, the levels of C-reactive protein, platelet to lymphocyte ratio, and neutrophil to monocyte ratio were higher in the migraine group than those of the healthy subjects. In a study by Turan et al. (18), higher levels of procalcitonin during migraine attacks were found. In a study by Domingues et al. (41), the levels of chemokines were examined in adults with TTH. According to the results, interleukin 8 levels were higher in the TTH group than in the control group. Moreover, the patients with a headache at the time

of blood sampling had higher levels of monocyte chemoattractant protein-1 than the patients without a headache at the time of blood sampling. Based on these previous study results, it was suggested that proinflammatory mechanisms might have a role in the pathogenesis of TTH and migraine (5,17,18,41). The results of the present study were consistent with the most of the previous studies concerning the association between primary headaches and inflammation.

Vitamin D levels are associated with bone mineralization, non-specific pain, and inflammatory skeletal myopathy. Also, there are studies regarding the relationship between vitamin D deficiency and headache. In a cross-sectional study, a significantly low level of serum 25(OH)D in non-migraine headaches was reported (42). Prakash et al. (43) proposed that headache symptoms were also benefitted from calcium and vitamin D therapies in patients with chronic tension headache and osteomalacia. Gungor et al. (44) found that the severity of headache was related to low serum 25(OH)D levels in children with migraines. In the present study, there were no significant differences between primary headache and healthy control groups, in terms of 25(OH)D levels.

There are some limitations in our study. Firstly, the study was performed in a single-center with relatively small sample size. Secondly, it was a retrospective study which might have a risk of bias.

Conclusion

The present study showed lower levels of vitamin B12 and ferritin, and higher NLR in children with primary headaches. Furthermore, serum ferritin levels were found as an independent risk factor for primary headaches. Hence, we suggest that the CBC, iron, and vitamin B12 status of the children and adolescents with primary headaches should be evaluated.

Ethics

Ethics Committee Approval: We have obtained the approval of the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 11, date: 25.02.2021) outlined in the Second Declaration of Helsinki.

Informed Consent: No informed consent has been collected since it is a retrospective study.

Peer-review: Externally and internally peer-reviwed.

Authorship Contributions

Concept: E.Ç., Design: E.Ç., M.A., Supervision: E.Ç., Materials: E.Ç., Data Collection or Processing: A.A., M.A., Analysis or Interpretation: A.A., M.A., Literature Search: A.A., M.A., Writing: A.A., M.A., Critical Review: A.A., M.A., E.Ç.

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Case Report / Olgu Sunumu

Isolated Intraventricular Haemorrhage Following Evacuation of Chronic-subacute Haemorrhage: A Case Report

Kronik-subakut Subdural Hematomun Boşaltılmasını Takiben Gelişen İzole İntraventriküler Kanama: Bir Olgu Sunumu

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Abstract

Chronic subdural haemorrhage is commonly encountered in the elderly. However, the outcomes of surgery for chronic subdural haemorrhage are frequently benign; surgeons may sometimes encounter unexpected complications after evacuation of chronic subdural haemorrhage as isolated intra-ventricular haemorrhage. A few hypotheses have been developed to explain this phenomenon. Here, we present a case report and explain this phenomenon with new a hypothesis.

Öz

Kronik subdural kanama, sıklıkla yaşlılarda karşılaşılır. Kronik subdural kanamanın cerrahi sonuçları sıklıkla iyi olmasına karşın, bazen cerrahlar subdural kanamanın boşaltılmasını takiben izole intraventriküler kanama gibi beklenmeyen komplikasyonlarla da karşılaşabilirler. Bu fenomeni açıklamaya yönelik birkaç hipotez geliştirildi. Bu çalışmada bir olgu sunumu yapılarak yeni bir hipotezle bu fenomen açıklanmıştır.

Keywords
Chronic subdural haemorrhage, complication, intra-ventricular haemorrhage

Anahtar Kelimeler
Kronik subdural kanama, komplikasyon, intraventriküler kanama

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Introduction

Chronic subdural hematoma (CSH) is an abnormal collection of liquid blood between the dura mater and the arachonoid mater which is frequently encountered in neurosurgical conditions, especially in the elderly (1,2). Various ethological factors have been identified but head trauma is the most common (3). Surgeons may encounter unwanted post-operative complications such as cerebral edema, tension pneumocephalus, recurrent hematoma, seizure, failure of the brain to re-expand (3-5). But intra-ventricular hemorrhage (IVH) after evacuation of CSH is a rarely encountered complication. We report a patient who had an isolated IVH which was identified following evacuation of left frontotemporaparietal CSH.

Case Report

A 72-year-old female patient was referred to the emergency room with right hemipharesis and two days of complaints of confusion. Right hemiparesis, lack of orientation and pathological reflexes were identified in her first neurological examination. Also, we learned from her children that she had suffered a head trauma three weeks previously and she did not take an anticoagulant thearpy. Cranial computed tomography (CT) and cranial magnetic resonance imaging (MRI) were performed, and chronic-subacute subdural hemorrhage and septations with 1.2 centimeter midline shift were identified on CT (Figure 1A) and on MRI (Figure 1B). We decided to perform urgent surgery via burr hole exploration. Informed patient consent was obtained from her guardians. The patient's body and head were laid on her right side and then burr hole was made in the left frontal bone with a high-speed drill under local anesthesia and intravenous sedation. The external membrane of the chronic subdural hemorrhage at the left frontal bone was coagulated with bipolar and then opened. Compressive hemorrhage in the subdural space leaked out on its own. Frontal feeding catheter was placed in the subdural space. The subdural space was washed with saline. The frontal part of the internal membrane of the CSH was opened under the operating microscope with a bipolar. The frontal burr hole feeding catheter was left in place. The feeding catheter was connected to a closed drainage system. A cranial CT was performed on the 3rd post-operative day (Figure 2A) and then the feeding catheter was withdrawn. Bilateral IVH was observed in the occipital horns of the lateral ventricular, but there was no midline shift. The second cranial CT was performed on the 6th day of post-operative (Figure 2B). We observed hemorrhage in the occipital horn of the lateral ventricular again, but there was no acute hydrocephalus. The patient was discharged on the 7th day after surgery with complete recovery.

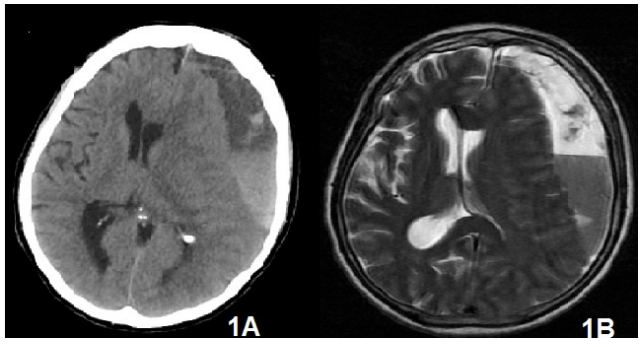

Figure 1. Preoperative axial cranial computed tomography: There was no hemorrhage in the lateral ventricular before surgery (1A), Preoperative T2 weighted axial cranial magnetic resonance imaging: Left frontotemporoparietal subacute-chronic subdural hemorrhage with 1.2 centimeters midline shift (1B)

Discussion

Different bleeding paterns have been definied after evacuation of subdural hemorrage in the literature. Cortical hyperemia beneath the hematoma, subarachnoid hemorrhage (SAH), supratenterial intracerebral, intra-ventricular, and rometo cerebellar hemorrhages are rarely reported (6). In a large series of 1,000 cases CSH, 4 cases of post-operative intracranial bleeding were described (4%) (7). Rusconi et al. (6), have been reported unusual post-operative hemorrhagic events with an incidence of 0.78% (3 patients) (6). Other authors have been reported an incidence range between 0.2-4% (8,9). Overdrainage, rapid brain decompration and shift of the intracranial contents, massive cerebrospinal fluid (CSF) loss, veneous outflow impairment and vascular dysregulation with blood flow increas, are the mechanisms currently debated (6).

Two possible mechanisms have been suggested to explain isolated IVH after evacuation of CSH. The first of these was suggested by Cook et al. (10) Their hypothesis was that elderly patients with physiological aging of the cerebral vascular tree may not tolerate a

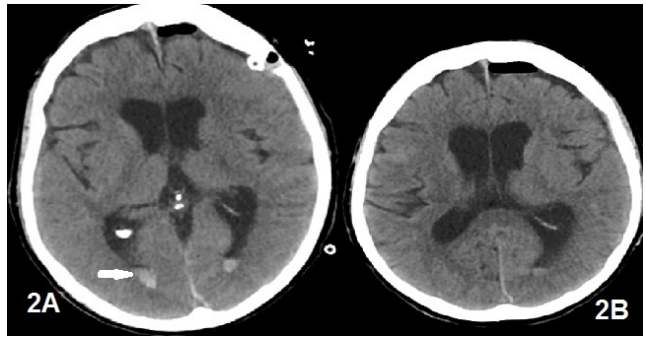

Figure 2. First axial cranial computed tomography (CT) after surgery: Midline shift recovered but a new intraventricular hemorrhage was detected (white arrow) (2A), second axial cranial CT after surgery: Neither increase in intraventricular hemorrhage nor development of hydrocephalus were detected (2B)

sudden restoration of normal perfusion pressure in areas of deranged cerebral vascular autoregulation (5). The second hypothesis was suggested by Savardekar and Salunke (5). They hypothesized that after sudden decompression of the brain, the differential expansile qualities of solid (brain) and liquid (CSF) components of the cranium may result in mechanical stress at the interface. It may then cause rupture of the engorged subependymal veins (2). In addition to these, Muneza et al. (3) suggested that fragile cerebral vessels, direct vascular damage and a shift of the midline structures might be contributory factors for IVH (4).

Our case is similar to the literature. The patient was elderly, and she had a subacute-CSH which included a 1.2 centimeter midline shift. CSH was evacuated via burr holes under local anesthesia and intravenous sedation. We identified an isolated hemorrhage in the lateral ventricular occipital horns on the 3rd day after surgery. Our opinion is that when evacuation of CSH is started suddenly it may cause rapid correction of midline shift. Rapid correction of the midline shift and rapid movement of the brain may lead to vascular shearing owing to mechanical stress especially on coroid plexus vessel. The elderly may be susceptible to this vascular shear due to vascular aging. Our hypothesis seems to be supported by two hypotheses. In addition to these, the other causes of isolated IVH include coagulation disorders, pituitary apoplexy, sickle cell anemia, drug consumption and vasculitides, but none of these was demonstrated. In our opinion is that, isolated IVH is a different event and may not be explained with vascular self-adjustment which was suggested to explain for intracerebral hemorrhage and SAH following removal of the subdural hemorrhage.

Multiple surgical techniques have been defined in tle literature for evacuation of chronic subdural hemorrhage, including twist drill craniostomy, burr-hole craniostomy, and craniotomy. And various complication have been reported related with surgical techniques (11). In our case, we have prefered the burr hole craniostomy for evacuation of subacut-chronic subdural hemorrhage under the local anesthesia and intravenous sedation. Our opinion is that gradual and graded evacuation can be archived by covering of the burr hole with a sponge or a cotonoid immedietly after opening the dura and the outher membrane. And than, A sponge or a cotonoid can be removed intermittent. By that mechanism, evacuation of subdural hemorrhage can be controlled and slowed down in a controlled way and this procedure can be continued till the subdural pressures equalize with the atmospheric pressures. This gradual and graded evacuation of subdural hemorrhage may decrease the mechanical stres on coroid plexus vessels.

In conclusion, although isolated IVH is rarely encountered, surgeons should keep it in mind because IVH may result in lethal complications following evacuation of CSH. Clinical awareness of this complication is essential. We suggest that slow and gradual decompression may protect the patient from this complication. Aging, amount of midline shift and speed of correction of midline shift may be contributory factors. Causative factors have not yet been clearly identified. Therefore, further investigations and large patient series are needed to clarify this phenomenon.

Ethics

Informed Consent: Informed patient consent was obtained from her guardians.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Z.K., Y.S.A., A.Y., Design: Z.K., A.Y., Supervision: Z.K., A.T., Data Collection or Processing: Z.K., O.B., M.Ö.Y., Analysis or Interpretation: Z.K., A.T., A.Y., Literature Search: Z.K., O.B., A.T., Writing: Z.K., Critical Review: Z.K., A.T., A.Y.

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