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Editorial

Is botulinum toxin the future of orofacial pain management? Evidence and perspectives

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Keywords: botulinum toxin, orofacial pain, trigeminal neuralgia, temporomandibular joint disorder

Botulinum toxin (BTX) demonstrates potential as a complementary therapy in managing chronic orofacial pain (OFP), but challenges in the standardization of protocols and indications remain.

The orofacial region is anatomically intricate, housing a dense network of muscles, nerves and blood vessels. This complexity often makes diagnosing and treating orofacial pain (OFP) a challenging endeavor. While OFP is commonly conflated with intraoral pain caused by dental or periodontal pathology, problems with differential diagnosis emerge when it extends to non-odontogenic pain, such as temporomandibular disorder (TMD) or neuropathic pain. Most of these conditions are associated with a high risk for chronicization and impairment, and they often necessitate multidisciplinary approaches for effective management.[1] Amongst the emerging therapies, botulinum toxin (BTX), a neuromodulator known for its cosmetic applications, has gained attention for its potential to manage several pain conditions. The perception is that in several clinical communities, BTX is even emerging as the one-fits-all solution, so questions and concerns about its potential misuse and abuse arise. For this reason, a short recap of the available information supporting its applications, as well as a look at future perspectives, are needed.

Botulinum toxin exerts its effects primarily by inhibiting the release of acetylcholine at neuromuscular junctions, leading to temporary muscle relaxation. This effect is well-known and is the backbone for all applications in cosmetic medicine and dentistry, as well as for claims about its use within the framework of bruxism and/or musculoskeletal pain management. Beyond the modulation of muscle activity, BTX has demonstrated antinociceptive properties by reducing the release of pain mediators, like glutamate, substance P and calcitonin gene-related peptide (CGRP), in sensory neurons. This dual action makes it a promising candidate for managing chronic pain conditions, including those in the orofacial region.[2] The question is: Has BTX type A (BTX-A) ever done the step from promising to evidence-based treatment?

With regard to the orofacial area, most research has focused on the application of BTX in patients with TMDs and/or bruxism.

Temporomandibular disorders are the most common source of non-odontogenic OFP, and include disorders of the temporomandibular joint (TMJ), masticatory muscles and related structures. Traditional treatment ranges from physical therapy to oral splints and pharmacological interventions.[3] Various studies, including a systematic review by Delcanho et al.,[4] have highlighted the efficacy of BTX in alleviating pain, improving jaw mobility and enhancing the quality of life (QoL). Patients receiving BTX injections showed significantly reduced pain scores on the visual analog scale (VAS) and improved functional outcomes as compared to the placebo group.[5] These effects are particularly evident in patients with myelogenous TMDs, where muscle hyperactivity is the primary pain source.[6] Bruxism is a risk factor for muscle fatigue, TMJ pain[7,8] and dental complications.[9–11] Consequently, BTX has gained traction as effective treatment for bruxism by reducing masseter and temporalis muscle activity. The same review by Delcanho et al. reported significant decreases in muscle hypertrophy among patients treated with BTX, with minimal adverse effects.[4] De la Torre Canales et al. also supported that, despite the limited number of studies on this subject, BTX appears to be a feasible management option for bruxism-related symptoms.[12] However, additional research is essential, particularly regarding the specific treatment indications for the reduction of bruxism itself, a target that is often confused with the management of its potential consequences.

Other potential applications vary from neuropathic to neurovascular pain. Botulinum toxin has shown interesting effects in managing conditions like trigeminal neuralgia (TN) and post-herpetic neuralgia (PHN).[8]

Trigeminal neuralgia is severe, electric shock-like pain affecting the trigeminal nerve. Evidence from studies suggests that BTX injections provide substantial pain relief and reduce the frequency of attacks in refractory TN cases, with the benefits sustained for several months post-injection.[13,14]

Post-herpetic neuralgia is a chronic complication of shingles, and it can cause persistent facial pain. Botulinum toxin has demonstrated efficacy in reducing pain intensity and improving QoL, as highlighted in trials by Xiao et al.[15]

As for headaches and migraines, the approval of BTX by the Food and Drug Administration (FDA) for chronic migraine treatment can be seen as a milestone achievement to pave the track for its application in the orofacial region. Chronic migraine, often associated with referred pain in the jaw and neck, responds well to the neuromodulatory activity of BTX.[11] Research by Lanteri-Minet et al. showed a significant reduction in the number of headache days and headache intensity, with parallel improvement in associated facial pain.[16]

Moreover, preliminary studies suggest that BTX-A significantly reduces referred myofascial pain from the neck muscles, as well as orofacial myofascial pain, at 2–6 months post-treatment, with no major adverse events reported,[17] and that it might alleviate pain and improve oral function in patients with burning mouth syndrome (BMS).[18]

Despite its benefits and encouraging claims, some of the early problems that emerged with BTX therapy are still unchallenged, such as the poor standardization of injection techniques or dosages.[19] Protocols lack uniformity across studies and clinical practices, leading to very different outcomes at the individual level. Also, the high cost and subsequent low accessibility of BTX are impacting its diffusion in the clinical setting and the design of proper investigation in the research setting. The good news is that very few side effects have been reported, although the risk of temporary weakness or facial asymmetry should always be discussed with the candidate patients.

Thus, the role of BTX in OFP management continues to evolve, and future research should prioritize factors such as:

– standardized protocols: Developing evidence-based guidelines for BTX administration in different OFP conditions;
– long-term studies: Assessing the sustainability of therapeutic effects and their impact on patient QoL;
– combined therapies: Exploring BTX as part of multimodal treatment strategies, including physical therapy, pharmacology and cognitive-behavioral interventions[20]; and
– defined diagnosis: Individualizing the use of BTX in different conditions, with the focus on the psychosocial impairment as an outcome variable.

Additionally, advancement in imaging technologies and biomarker studies may refine patient selection, ensuring personalized and effective BTX treatment.

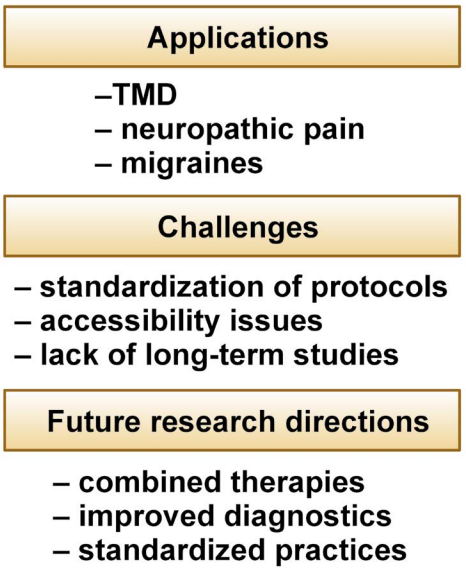

Fig. 1. Key aspects of botulinum toxin (BTX) in orofacial pain (OFP) management, highlighting applications, challenges and future research directions to improve clinical efficacy and standardization

TMD – temporomandibular disorders.

Botulinum toxin represents a transformative approach in OFP management, offering hope to patients with refractory or chronic conditions (Fig. 1). While not a universal solution, its role as an adjunct therapy is partially supported by scientific evidence. The current problem lies in the lack of development as far as the specific indications and protocols are concerned, which may lead to false claims and clinical misuse. Continued research and innovation are essential to overcome the current limitations and fully integrate BTX into mainstream OFP treatment protocols.

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Original paper

Nutritional status, dental caries and parental attitude to junior oral hygiene among children and adolescents with Down syndrome

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Abstract

Background. Children with Down syndrome (DS) frequently experience nutritional issues. Some of them are attributed to gastrointestinal tract abnormalities, while others are due to feeding behaviors. Previous studies have reported inconclusive results regarding the prevalence of dental caries among individuals with DS.

Objectives. The study aimed to assess the nutritional status, prevalence of dental caries and oral hygiene level among children and adolescents with DS aged 8–15 years in comparison to healthy peers.

Material and methods. A case–control observational study was carried out in Damascus, Syria, and its surrounding countryside. The study included 135 participants between the ages of 8 and 15 years who were divided into 2 groups based on the presence of DS. The data was processed using the IBM SPSS Statistics for Windows software, v. 26. The Kruskal–Wallis H test, Mann–Whitney U test and χ^2 test, as well as Spearman's correlation were implemented.

Results. Almost half of the participants in the DS group were classified as overweight or obese (33.9% and 19.1%, respectively), while the percentages of overweight and obese children in the control group were 14.9% and 10.4%, respectively. No statistically significant results were noted between oral hygiene levels and the prevalence of dental caries among the study groups ($p > 0.05$). Only 50% of parents thought that their children needed help during daily oral hygiene practices; however, only 39.4% of parents actually provided such assistance.

Conclusions. Children and adolescents with DS exhibited a tendency toward shorter stature in comparison to their peers. In the DS group, the economic status and paternal education level exhibited an inverse correlation with body mass index (BMI)-for-age. Oral hygiene practices and dietary habits are the most influential factors in the development of dental caries. The DS group followed an unhealthy dietary pattern, characterized by elevated levels of sugar consumption. There is an urgent need for effective promotion programs within the educational plans of schools and centers of special needs. These programs must educate parents and caregivers about effective oral hygiene practices and make them aware of their children's need for help during the oral hygiene process.

Keywords: nutritional status, oral hygiene, Down syndrome, BMI-for-age, parental attitude

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Highlights

- Oral hygiene practices and dietary habits are key determinants in the development of dental caries.
- Syrian children with Down syndrome frequently demonstrate high sugar intake and limited awareness of dental hygiene.
- Tailored dietary guidance and oral health education are essential to improve their oral health outcomes.

Introduction

Down syndrome (DS) is a prevalent genetic disorder that occurs due to trisomy of chromosome 21. The syndrome manifests in 3 different chromosomal forms: simple trisomy 21; translocation trisomy; and mosaic trisomy.[1] Down syndrome is associated with cardiovascular disorders, digestive system abnormalities and metabolic disorders.[2] It is also accompanied by intellectual disabilities, which are measured by the intelligence quotient (IQ) level. The IQ level of individuals with DS ranges from average to limited, and decreases with age.[3]

The following oral manifestations have been observed in individuals with DS: mouth breathing; macroglossia; missing teeth; developmental dental defects; microdontia; crowding; and a high prevalence of periodontal disease that progresses rapidly due to local factors, such as tooth morphology, bruxism, malocclusion, and poor oral hygiene.[4]

Children with DS frequently experience nutritional problems, either due to gastrointestinal tract abnormalities[3] or feeding behaviors.[5] However, many defects of the digestive system are associated with a slow rate of development, including delayed primary tooth eruption.[3] Children with DS also suffer from difficulties in chewing and swallowing. The aforementioned factors have been demonstrated to result in nutritional deficiencies within this group. Moreover, it was found that children with DS exhibited deficiencies in protein, fat, vitamin, and mineral levels (sodium, potassium, calcium, phosphorus, and iron) when compared to their siblings.[6]

Furthermore, individuals with DS have an increased risk of overweight and obesity, which can be attributed to abnormal lipid metabolism and type II diabetes, as well as decreased physical activity due to defects of the body.[3,6] In addition, a significant increase in carbohydrate intake was observed in these children.[5]

Inconclusive results were found when studying the prevalence of dental caries among individuals with DS. The majority of studies have reported a decrease in the incidence of caries among patients with DS, while several studies found an increase in the rate of dental caries when compared to healthy individuals.[7]

The prevalence of dental caries varies across different populations. In general, the majority of adults develop caries at some point during their lives.[8] Previous studies have identified the factors that contribute to dental caries. Diet plays an important role in the occurrence of dental caries. A study has indicated that eating added sugars more than 4 times per day was associated with an increased risk of dental caries.[9] In addition, some researchers have suggested that, in addition to genetic predisposition, the diet and oral hygiene habits of the family remain the main factors contributing to susceptibility to caries.[10] The family structure has a great influence on the development of dental caries, particularly with regard to the parental attitude toward oral hygiene practices and dietary habits of children.[11]

Unfortunately, there is a dearth of available data regarding the oral health of children with DS in Syria. This paper is the first to describe the nutritional status, prevalence of dental caries, and parental attitude to junior oral hygiene among children and adolescents with DS in Syria.

The aim of this study was to assess the nutritional status of children and adolescents with DS, aged 8–15 years, as well as to evaluate the prevalence of dental caries and oral hygiene level among the DS group compared to their healthy peers. A questionnaire was submitted to parents and caregivers of children with DS to evaluate the frequency of sugar intake and daily oral hygiene practices.

Material and methods

Study design

An observational case–control study was carried out in Damascus, Syria, and its surrounding countryside. The data collection was conducted from January to March of 2019, during at least 2 visits to each center. The purpose of the first visit was to explain the study objectives and procedures to the center's officials, as well as disseminating questionnaires and informed consents to parents and caregivers. Subsequent visits were focused on conducting examination procedures and collecting completed questionnaires.

Ethics approval

The study was approved by the Ethics Committee and the Board of Scientific Research at the Faculty

of Dentistry, Damascus University, Syria (approval No. DUUS-2797-12072018). In addition, official permissions were obtained from the Ministry of Health, the Ministry of Social Affairs and Labor, the Ministry of Education, and the Ministry of Higher Education and Research of Syria. Written informed consent to participate in the study was attained from parents or caregivers of all study subjects.

Study sample

The study included 135 participants aged 8–15 years, with a mean age of 11.77 ±2.51 years. The study sample was classified into 2 following groups based on the presence of DS: case group (DS group); and control group.

Case group

The case group included a total of 68 children and adolescents with DS who were registered, at the time of study, in special needs centers affiliated with the Ministry of Social Affairs and Labor in Syria. The diagnosis of DS was made by a medical specialist, and the child's medical record was attached to the center's registration file.

Control group

The control group, which comprised a total of 67 children and adolescents without syndromes or chronic diseases, was selected for the study. Controls were randomly selected from schools located in close proximity to the centers for special needs, and they matched the case group in terms of sex, age and the socioeconomic status.

The subjects were divided into 2 age groups based on their education level, as follows: children attending primary school (aged 8–11 years); and adolescents attending middle school (aged 12–15 years).

Eligibility criteria

The inclusion criteria encompassed children and adolescents aged 8–15 years with no personal reasons that would prevent them from participating in the study.

Children undergoing orthodontic treatment that prevents the dental examination, children exposed to radiation or chemotherapy, those with active infectious diseases that require extra precautions, and extremely uncooperative children were excluded from the study.

Study tools

The following instruments were used in the study: infection control tools; personal protective equipment; a portable (light-emitting diode (LED)) headlight; a World Health Organization (WHO) probe; a disposable dental explorer; a disposable dental mirror; cotton rollers; a stadiometer for height measurement; and a digital scale for weight measurement.

Examination procedure

The examination procedure and data collection were conducted in accordance with the WHO guidelines published in 2013.[12] The oral examinations were performed by a single examiner (REM) in a designated area prepared by a center or a school. During the oral examination, the participant was seated on a chair facing the examiner. The oral examination was conducted using a portable headlight, a WHO probe and a disposable dental mirror. The DMFT/dmft (decayed, missing, and filled permanent teeth/decayed, missing, and filled primary teeth) indices were used to assess dental caries,[12] and the plaque index (Silness & Löe)[13] was employed to evaluate oral hygiene level. Teeth that were supernumerary, third permanent molars, congenitally missing teeth, and teeth that were extracted or filled for reasons other than caries were excluded from the analysis.

Height and weight measurement and BMI calculation

The weight of the participants was measured with the use of a digital scale and recorded in kilograms [kg]. During the measurement process, the subjects wore light clothes and were asked to remove their bags and hats. Weight was measured with a precision of 100 g. Height was determined using a stadiometer and recorded in centimeters [cm]. During the measurement, the participants were barefoot and their feet were in full contact with the ground while in a standing position. The body mass index (BMI) [kg/m^2] was calculated using the patient's weight and height measurements. The LMS (skewness (L), median (M), and coefficient of variation (S)) method was applied to calculate BMI-for-age (*Z*-scores), which is based on the WHO growth reference data for individuals aged 5–19 years.[14] Subsequently, the children were classified into the following 4 categories based on the obtained *Z*-scores of BMI-for-age: underweight; normal weight; overweight; and obese.[15]

Questionnaire

The DS group received a questionnaire that was to be completed by parents or caregivers (available on request from the corresponding author). The questionnaire was developed for the current study and contained demographic data (full name, age in years and months, sex, address, and telephone number), socioeconomic status data (father/mother education level, father/mother job, and family monthly income), medical history (systemic diseases, medications and presence of any medical conditions), dietary information, and daily oral hygiene

practices. The dietary information was related to the frequency of sugar intake by identifying the foods consumed by the majority of Syrian children, based on the findings of the study conducted by Al Bitar and Kouchaji.[16] The frequency of food intake was classified into 6 categories (never, several times a month, once a week, several times a week, once a day, and several times a day). The oral hygiene practices included toothbrushing frequency (less than once a day, once a day, and more than once a day), the use of fluoridated toothpaste, and additional methods incorporated into daily hygiene practices, such as the use of dental floss and mouthwash. The parents' attitude toward their children's oral hygiene was assessed by exploring their opinions on whether children require assistance during daily oral hygiene practices and the extent to which they actually provide it. The reliability of the questionnaire was assessed by requesting that 20 parents complete and discuss questions in person. Cronbach's alpha was used to measure the reliability ($\alpha = 0.80$).

The economic status of the population was classified into 3 categories: good; moderate; and poor. Educational levels of both parents were also divided into 3 categories, namely high (university), moderate (secondary, high school), or low (primary school).

Statistical analysis

The data was processed using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). Descriptive statistics were calculated for all independent and outcome variables. The Kruskal–Wallis H test, Mann–Whitney U test and χ^2 test were implemented. Spearman's correlation was used to study the relationships between variables. A value of $p < 0.05$ was considered statistically significant at the 95% confidence level.

Results

The present study encompassed a total of 135 participants, including 68 subjects (50.4%) with DS and 67 (49.6%) matched healthy children. According to the WHO criteria for survey design, the participants were classified into 2 age groups: children (8–11 years old); and adolescents (12–15 years old). No statistically significant differences were identified between the 2 groups with regard to sex, age, the economic status, and parents' education levels ($p > 0.05$) (Table 1). The prevalence of caries was 82.4% and 88.1% in the DS group and the control group, respectively ($p > 0.05$). Similarly, no statistically significant differences were observed in oral hygiene level ($p > 0.05$). However, the percentage of individuals with poor oral hygiene was 26.5% in the DS group and 17.9% in the control group. A statistically significant discrepancy was identified between the groups for BMI-for-age ($p = 0.004$). Underweight children were found only in the control group, at a rate of 6%, and there were no underweight children in the DS group. Nearly half of the subjects in the DS group were overweight and obese (33.9% and 19.1%, respectively), compared to 14.9% and 10.4%, respectively, in the control group (Table 1).

Table 1. Characteristics of the study sample

Variable		DS group		Control group		*p*-value
		n	%	*n*	%	
Sex	male	37	54.4	37	55.2	0.924
	female	31	45.6	30	44.8	
Age [years]	8–11	37	54.4	37	55.2	0.924
	12–15	31	45.6	30	44.8	
Economic status	good	6	8.8	14	20.9	0.113
	moderate	55	80.9	49	73.1	
	poor	7	10.3	4	6.0	
Mother's education level	high	26	38.2	23	34.3	0.893
	moderate	15	22.1	16	23.9	
	low	27	39.7	28	41.8	
Father's education level	high	25	36.8	26	38.8	0.834
	moderate	15	22.1	12	17.9	
	low	28	41.2	29	43.3	
Caries prevalence rate	caries-free	12	17.6	8	11.9	0.351
	with caries	56	82.4	59	88.1	
Oral hygiene level	good	12	17.6	11	16.4	0.433
	fair	38	55.9	44	65.7	
	poor	18	26.5	12	17.9	
BMI-for-age	underweight	0	0.0	4	6.0	0.004*
	normal weight	32	47.1	46	68.7	
	overweight	23	33.8	10	14.9	
	obese	13	19.1	7	10.4	
Total		68	100.0	67	100.0	–

* statistically significant ($p < 0.05$, Pearson's χ^2 test); DS – Down syndrome; BMI – body mass index.

Moreover, a statistically significant difference was identified when studying BMI differences between the DS and control groups ($p = 0.004$). This index necessitated the evaluation of its components, including height and weight. No statistically significant difference was found for weight between the groups ($p > 0.05$); however, a significant difference was identified for height ($p = 0.000$). The mean height in the DS group was lower (137.07 ±14.38 cm) compared to the control group (148.34 ±15.83 cm) (Table 2).

In the 8–11 age group, no statistically significant differences were observed between the groups in terms of the DMFT index or its components ($p > 0.05$). A lack of statistical significance was also identified between the 2 groups in terms of carious primary teeth, missing primary teeth and the dmft index ($p > 0.05$). However, a statistically significant difference was observed between the 2 groups in terms of filled primary teeth ($p = 0.009$). The mean

Table 2. Results of body mass index (BMI) measurements in the study groups

Variable	DS group		Control group		*p*-value
	M	*SD*	*M*	*SD*	
Height [cm]	137.07	14.38	148.34	15.83	0.000*
Weight [kg]	42.18	15.89	44.23	15.31	0.435
BMI [kg/m^2]	21.66	4.87	19.45	3.30	0.004*

* statistically significant ($p < 0.05$, Mann–Whitney *U* test); *M* – mean; *SD* – standard deviation.

Table 4. Correlation between the prevalence of caries and selected demographic and dental variables in both study groups

Variable	Caries prevalence			
	DS group		control group	
	rho	*p*-value	rho	*p*-value
BMI-for-age	–0.065	0.598	–0.012	0.926
Oral hygiene level	–0.347	0.004*	–0.478	0.000*
Economic status	–0.103	0.402	–0.168	0.175
Mother's education level	–0.053	0.670	–0.187	0.129
Father's education level	0.062	0.615	–0.170	0.169

* statistically significant ($p < 0.01$, Spearman's rho).

number of filled primary teeth in the DS group was lower (0.09 ±0.38) compared to the control group (0.38 ±0.60) (Table 3).

In the 12–15 age group, none of the adolescents in the control group had primary teeth, while 10 adolescents in the DS group had primary teeth. No statistically significant differences were identified between the DS group and the control group with regard to the number of decayed permanent teeth, filled permanent teeth and the DMFT index ($p > 0.05$). However, a statistical significance was observed between the groups when comparing missing permanent teeth ($p = 0.012$) (Table 3).

Upon examining the correlations between the prevalence of caries and the variables mentioned in Table 4, no statistically significant relationships were found with regard to BMI-for-age, the economic status and parents' education levels within the 2 groups ($p > 0.05$). However, a significant negative correlation was identified between the prevalence of caries and oral hygiene level in the DS group ($p = 0.004$) and the control group ($p = 0.000$).

In the DS group, a significant negative correlation was identified between BMI-for-age and the economic status ($p = 0.024$). Additionally, a negative correlation was observed between BMI-for-age and father's education level. However, in the control group, there were no statistically significant correlations between BMI-for-age and other variables (Table 5).

When studying the differences between the DMFT/dmft indices and the variables related to the questionnaire administered to the DS group, there were no statistically significant differences between the DMFT/dmft indices and the following factors: medication intake; the use of fluoridated toothpaste; the use of dental floss or

Table 3. Results of the DMFT/dmft (decayed, missing, and filled permanent teeth/decayed, missing, and filled primary teeth) index measurements in the study groups

Variable			DS group			Control group			*p*-value
			patients, *n*	*M*	*SD*	patients, *n*	*M*	*SD*	
Age [years]	8–11	decayed primary teeth, *n*	33	3.00	3.28	34	2.94	2.87	0.694
		missing primary teeth, *n*	33	0.67	1.99	34	0.59	1.15	0.139
		filled primary teeth, *n*	33	0.09	0.38	34	0.38	0.60	0.009*
		dmft	33	3.76	4.18	34	3.91	3.18	0.365
		decayed permanent teeth, *n*	37	1.24	1.64	37	0.92	1.18	0.688
		missing permanent teeth, *n*	37	0.08	0.49	37	0.00	0.00	0.317
		filled permanent teeth, *n*	37	0.16	0.72	37	0.24	0.76	0.264
		DMFT	37	1.49	1.83	37	1.16	1.40	0.740
	12–15	decayed primary teeth, *n*	10	1.20	1.31	0	–	–	–
		missing primary teeth, *n*	10	0.10	0.31	0	–	–	–
		filled primary teeth, *n*	10	0.10	0.31	0	–	–	–
		dmft	10	1.40	1.35	0	–	–	–
		decayed permanent teeth, *n*	31	3.32	3.83	30	2.20	1.64	0.506
		missing permanent teeth, *n*	31	0.45	1.06	30	0.00	0.00	0.012*
		filled permanent teeth, *n*	31	0.74	1.94	30	0.40	0.968	0.917
		DMFT	31	4.52	4.44	30	2.60	2.06	0.072

* statistically significant ($p < 0.05$, Mann–Whitney *U* test).

Table 5. Correlation between body mass index (BMI)-for-age and selected demographic and dental variables in both study groups

Variable	BMI-for-age			
	DS group		control group	
	rho	*p*-value	rho	*p*-value
Oral hygiene level	0.016	0.899	–0.203	0.099
Economic status	–0.274	0.024*	0.108	0.386
Mother's education level	–0.202	0.098	0.017	0.893
Father's education level	–0.305	0.011*	–0.157	0.205

* statistically significant ($p < 0.05$, Spearman's rho).

mouthwash; and parental assistance during oral hygiene practices ($p > 0.05$). A statistically significant relationship was identified between the DMFT index and brushing frequency ($p = 0.037$) (Table 6).

The percentage of parents who thought that their children require help in daily oral hygiene practices was 50%. However, only 39.4% of them provided such assistance. Moreover, 50% of parents believed that juniors did not require assistance, yet 7.6% of them offered help (Table 7).

The most frequently consumed food item among the DS group (consumed several times a day) was bread (54.4%). The products most often eaten once a day were biscuits or chips (42.6%), followed by tea with sugar 39.7% (Table 8).

A significant positive correlation was identified between the prevalence of caries and the consumption of biscuits or chips ($p = 0.041$). Additionally, there was a significant negative relationship between oral hygiene level and the consumption of tea with sugar ($p = 0.038$) (Table 9).

Table 7. Parental attitude toward their children's oral hygiene in the DS group based on the questionnaire

Parental attitude			Do you help your child brush their teeth?		Total
			no	yes	
Do you think your child needs help brushing his/her teeth?	no	*n*	28	5	33
		%	42.4	7.6	50.0
	yes	*n*	7	26	33
		%	10.6	39.4	50.0
Total		*n*	35	31	66
		%	53.0	47.0	100.0
p-value			0.000*		–

* statistically significant ($p < 0.05$, Pearson's χ^2 test).

Table 6. DMFT/dmft (decayed, missing, and filled permanent teeth/decayed, missing, and filled primary teeth) indices based on the questionnaire administered in the DS group

Questionnaire		dmft			DMFT		
		n	*M*	*SD*	*n*	*M*	*SD*
Does your child take medications continually?	yes	11	2.73	3.43	16	4.00	5.83
	no	29	3.41	4.15	46	2.50	2.64
p-value			0.889[a]			0.888[a]	
How often does your child brush his/her teeth?	less than once a day	16	4.31	4.51	28	4.14	4.46
	once a day	25	2.64	3.41	35	1.89	2.54
	more than once a day	2	1.50	0.70	5	2.60	2.79
p-value			0.458[b]			0.037[b]*	
Does your child use fluoridated toothpaste?	yes	22	3.41	4.29	28	2.18	2.66
	no	4	2.50	2.88	5	1.55	2.23
	I do not know	14	3.14	3.82	28	4.00	4.56
p-value			0.995[b]			0.071[b]	
Does your child use mouthwash or dental floss for oral hygiene?	yes	2	3.00	1.41	3	4.67	5.03
	no	38	3.24	4.03	59	2.86	3.67
p-value			0.568[a]			0.512[a]	
Do you think your child needs help brushing his/her teeth?	yes	21	3.81	4.25	34	2.79	2.64
	no	21	2.52	3.44	33	2.88	4.44
p-value			0.390[a]			0.584[a]	
Do you help your child brush their teeth?	yes	21	3.90	4.21	32	2.44	2.56
	no	21	2.57	3.48	35	3.34	3.34
p-value			0.311[a]			0.533[a]	

* statistically significant ($p < 0.05$); [a] Mann–Whitney *U* test; [b] Kruskall–Wallis *H* test.

Table 8. Frequency of specific food consumption in the Down syndrome (DS) group

Product	Never		Several times a month		Once a week		Several times a week		Once a day		Several times a day	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Bread	1	1.5	4	5.9	4	5.9	12	17.6	10	14.7	37	54.4
Pasta/rice	5	7.4	8	11.8	15	22.1	27	39.7	8	11.8	5	7.4
Fresh fruit	12	17.6	5	7.4	7	10.3	20	29.4	12	17.6	12	17.6
Ice cream	23	33.8	12	17.6	9	13.2	5	7.4	15	22.1	4	5.9
Biscuits/chips	6	8.8	7	10.3	4	5.9	17	25.0	29	42.6	5	7.4
Honey/jam	29	42.6	11	16.2	6	8.8	11	16.2	11	16.2	0	0.0
Arabic sweets	38	55.9	18	26.5	4	5.9	3	4.4	2	2.9	3	4.4
Chocolate/candies	18	26.5	11	16.2	9	13.2	20	29.4	9	13.2	1	1.5
Dried fruit	56	82.4	7	10.3	1	1.5	2	2.9	2	2.9	0	0
Gum	50	73.5	6	8.8	4	5.9	5	7.4	2	2.9	1	1.5
Soft drink/fruit juice	14	20.6	22	32.4	10	14.7	14	20.6	6	8.8	2	2.9
Milk with sugar	25	36.8	9	13.2	11	16.2	11	16.2	5	7.4	7	10.3
Tea with sugar	3	4.4	10	14.7	15	22.1	9	13.2	27	39.7	4	5.9

Table 9. Correlation between the type of food consumed in the Down syndrome (DS) group and body mass index (BMI)-for-age, caries prevalence rate and oral hygiene level

Product	BMI-for-age		Caries prevalence rate		Oral hygiene level	
	rho	*p*-value	rho	*p*-value	rho	*p*-value
Bread	–0.200	0.102	0.055	0.656	0.201	0.100
Pasta/rice	0.081	0.514	–0.145	0.237	–0.059	0.632
Fresh fruit	–0.072	0.561	0.044	0.720	0.133	0.279
Ice cream	–0.200	0.102	0.151	0.220	–0.041	0.739
Biscuits/chips	–0.063	0.608	0.248*	0.041*	–0.091	0.463
Honey/jam	–0.150	0.223	0.108	0.380	–0.177	0.148
Arabic sweets	–0.042	0.736	0.210	0.086	–0.039	0.755
Chocolate/candies	–0.019	0.876	0.203	0.097	–0.134	0.277
Dried fruit	–0.082	0.507	0.004	0.971	0.075	0.542
Gum	–0.172	0.161	0.119	0.333	0.002	0.985
Soft drink/fruit juice	0.046	0.712	–0.028	0.819	–0.036	0.771
Milk with sugar	–0.051	0.682	–0.032	0.793	0.011	0.929
Tea with sugar	–0.079	0.524	0.189	0.122	–0.252*	0.038*

* statistically significant ($p < 0.05$, Spearman's rho).

Discussion

It is important to eliminate the interfering factors, such as the economic status and parental education level, when comparing the nutritional status and dental caries between the DS group and the control group. Hence, upon examining the differences in sex, age, the economic status, and both parents' education level between the 2 groups, no statistical significance was found (Table 1). The nutritional status was represented by BMI-for-age, as recommended by the WHO.[17] The American Academy of Pediatrics (AAP) has suggested using the growth charts developed by the Centers for Disease Control and Prevention (CDC) or the WHO when following children with DS, until the growth charts specific to DS become available.[18] The current study used the growth charts provided by the WHO in 2007 due to their universality and a large number of measurements, making them representative of all races and countries. Many studies have used these charts, including a study by El Harake et al. on the nutritional status of Syrian children residing in Lebanon.[19]

Almost half of the subjects in the DS group were classified as overweight or obese, while underweight children were only observed in the control group (Table 1). An investigation into the BMI components between the 2 groups revealed that there was no statistically significant difference in weight ($p = 0.435$), while a statistically significant difference was noted in height ($p = 0.000$), resulting in a significant difference in BMI ($p = 0.004$) (Table 2). The mean height in the DS group (137.07 ±14.38 cm) was lower than that in the control group (148.34 ±15.83 cm) (Table 2). The previously mentioned result has been reported in numerous studies.[20–22] In general, measurements may also be influenced by familial and environmental factors, such as diet and physical activity.[23] Nasreddine et al. reported that the prevalence of overweight and obese adolescents in Syria was 18.95% and 8.6%, respectively,[24] which was similar to the prevalence rate observed in the current study for the control group. Obesity was influenced by sex, the consumption of carbohydrates and fats, as well as parents' education level.[24] The findings of this study stood in contrast to those of a study conducted by Sharav and Bowman, which did not observe any significant differences in obesity between children with DS and their siblings.[23] The authors attributed overweight and obesity to the influence of familial and environmental factors more than to DS.[23] In addition, children with DS tend to become

obese with age due to a diet rich in carbohydrates, laziness associated with mental and cognitive disorders, short stature, and metabolic and digestive problems.[3]

Furthermore, the investigation into the relationships between BMI-for-age and variables such as oral hygiene level, economic status and parental education levels revealed no statistically significant correlation in the control group. However, in the DS group, a significant negative correlation was observed between BMI-for-age and father's education level (p = 0.011). A significant negative correlation was identified between BMI-for-age and the economic status (p = 0.024) (Table 5). In general, father's education level was correlated with the economic status of the family. The economic status had a significant influence on the quality of the food consumed.

In the context of Syrian culture, the father traditionally provides food for his family. As the level of education among a given population is low, it affects the quality of food consumed by the family. Moreover, children with DS sometimes follow a different diet compared to their families.[6] Parents may permit their DS children, specifically, to indulge in nutritional excesses and satisfy their preferences.[5]

Unfortunately, the children in both study groups had poor oral health, and there was no preference for any group over the other. Dental caries reached high rates in the DS group and the control group (82.4% and 88.1%, respectively) (Table 1). This finding emphasizes the problem of dental caries in children and stands in contrast to previous studies that reported a lower incidence of caries among individuals with DS compared to healthy controls.[7,25] This discrepancy could be attributed to the differences in the studied population, particularly given the recent rise in the prevalence of dental caries. In 2017, the prevalence of caries in Syrian children aged 8–12 years was 79.1%.[26] The absence of health promotion programs, limited knowledge of effective oral hygiene practices, and neglecting regular visits to the dental clinic contribute to this problem. Syrian children, even those in good health, rarely receive dental care unless they are experiencing dental pain. A study by Al Habashneh et al. reported that one of the reasons for not attending dental care among children with DS is maternal unawareness of the dental needs of their children.[27] Typically, children with DS present with poor oral health due to the numerous DS health concerns, experienced by their parents, who consequently neglect oral health and give it a low priority in comparison to general health.[28] Moreover, medication has been identified as a risk factor for caries in children with DS due to its high sugar content and the necessity of regular administration.[7]

The sample was divided into 2 age groups, children attending primary school (aged 8–11 years) and adolescents attending middle school (aged 12–15 years). The members of each age group shared a set of characteristics, such as educational stage, diet and hormonal changes.

In the group of children, the decayed primary teeth values in the DS group and the control group were 3.00 ±3.28 and 2.94 ±2.87, respectively (Table 3). The mean values for decayed permanent teeth in the DS group and controls were 1.24 ±1.64 and 0.92 ±1.18, respectively (Table 3). As mentioned earlier, the high incidence of caries was observed to be consistent across all subjects, irrespective of the group. The filled primary teeth values were higher in the control group (0.38 ±0.60) compared to the DS group (0.09 ±0.38), with a statistically significant difference between them (p = 0.009) (Table 3). The children in the control group were more likely to undergo restorative dental procedures, while such interventions were usually absent in the DS group.

In the group of adolescents, none of the subjects in the control group had primary teeth, while 10 individuals in the DS group were observed to have them. This observation may suggest a potential delay in the eruption of permanent teeth in the DS group compared to the control group, and/or the long-term survival of primary teeth. In the DS group and the control group, the number of missing permanent teeth was 0.45 ±1.06 and 0.00 ±0.00, respectively (p = 0.012) (Table 3). This finding indicates that the predominant dental treatment provided to individuals with DS is extraction. These results align with the outcomes of previous studies, which reported that the primary dental treatment administered to the healthy group was restoration, followed by extraction, while the primary dental treatment administered to the DS group was extraction.[27,29]

Makieh et al. found a statistically significant relationship between DS and diffuse opacities.[30] Usually, diffuse opacities are related to a low incidence of caries.[31] Despite the high prevalence of diffuse opacities in Syrian children with DS,[30] the prevalence rate of dental caries did not decrease compared to the healthy controls. The high prevalence of both diffuse opacities and dental caries among children with DS prompted us to consider the role of diet and oral hygiene practices as potential contributing factors.

Previous studies did not examine the relationship between medication intake and dental caries in children with DS. Consequently, the current study offers novel insights into this condition. When studying the influence of medications on caries among children with DS, no statistically significant difference was found in the DMFT and dmft scores (p = 0.888 and p = 0.889, respectively) (Table 6). The impact of brushing frequency on DMFT was statistically significant (p = 0.037). The mean DMFT was 1.89 ±2.54 for brushing once a day, 4.14 ±4.46 for brushing less than once a day, and 2.60 ±2.79 for brushing more than once a day (Table 6). A study by Rothen et al. corroborated previous findings.[32] The investigation revealed that the most significant factors associated with a decrease in dental caries are toothbrushing with fluoridated toothpaste once or more per day.[32]

Individuals with DS suffer from degenerative changes in the brain that affect overall body development, resulting in inconsistency and delay in motor development, as well as a lack of coordinated movements.[3] In such patients, the following oral manifestations were identified: mouth breathing; macroglossia; cleft tongue and lips; morphological disorders; inflammation of the corners of the mouth; small teeth; malocclusion; and crowding.[4] Based on the above factors, individuals with DS may not be able to perform oral hygiene practices on their own, necessitating parental or caregiver assistance during daily oral hygiene routines. Parents could help by teaching them effective brushing techniques, reminding them to brush teeth, and monitoring the process. It is important for parents and caregivers to recognize their children's need for help. Only 50% of parents were aware of their children's need for assistance during toothbrushing. However, only 39.4% of those parents provided help (Table 7). The decision of whether or not to provide assistance had no influence on the DMFT and dmft scores. One potential explanation for this finding is that parents may have limited knowledge regarding effective brushing techniques. Therefore, it is necessary to educate parents on the correct techniques of toothbrushing and inform them of the importance of assisting their children during the oral hygiene process.

A study by Jaghasi et al. found that bread was the most consumed food daily by 58.8% of schoolchildren in Damascus.[33] This outcome is consistent with the current study, which revealed that 54.4% of children with DS consumed bread several times a day. From all common products enumerated in Table 9, biscuits/chips had a significant positive correlation with caries prevalence rate. Furthermore, 42.6% of children with DS consumed biscuits or chips once a day. Tea with sugar, consumed by 39.7% of children with DS once a day, had a negative correlation with oral hygiene level ($p = 0.038$) (Table 8,9). The abovementioned results highlight an inappropriate consumption of sugars and a lack of control in food intake among children with DS. Furthermore, the consumption of sugars on more than 3 occasions daily increases the incidence of dental caries.[33,34]

It is important to note that the present study did not take into account the quantity of sugar intake or its placement between meals, and it was limited to analyzing sugar intake only in terms of frequency.

Conclusions

Children and adolescents with DS exhibited a tendency toward shorter stature in comparison to their peers, which resulted in BMI-for-age values indicating overweight or obesity. The consumption of sugars had no influence on BMI-for-age. In the DS group, the economic status and father's education level correlated inversely with BMI-for-age. Oral hygiene practices and dietary habits remain the most significant factors contributing to the development of dental caries. The current study found that the economic status and parental education levels have no impact on dental caries. Additionally, the analysis revealed that medication intake did not have an influence on the DMFT and dmft indices. The common type of dental treatment provided to the control group was restoration, while the predominant dental intervention administered to the DS group was extraction. The children with DS followed inappropriate diet, characterized by a high intake of sugars. There is an urgent need to incorporate effective promotional programs within the educational plans of schools and centers of special needs, which would include appropriate dietary and oral health care instructions. In addition, it is imperative to educate parents and caregivers on effective oral hygiene practices and make them aware of their children's need for assistance during the oral hygiene process. Additionally, an emphasis should be placed on the importance of treating dental caries at its earliest stages.

Ethics approval and consent to participate

The study was approved by the Ethics Committee and the Board of Scientific Research at the Faculty of Dentistry, Damascus University, Syria (approval No. DUUS-7972-12072018). In addition, official permissions were obtained from the Ministry of Health, the Ministry of Social Affairs and Labor, the Ministry of Education, and the Ministry of Higher Education and Research of Syria. Written informed consent to participate in the study was attained from parents or caregivers of all study subjects.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Use of transcutaneous electrical nerve stimulation (TENS) to reduce anxiety in pediatric dentistry

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Abstract

Background. Dental anxiety presents an ever-present challenge in pediatric dentistry. With the development of dental medicine, new discoveries make undergoing dental therapy easier for patients and reduce their anxiety during dental procedures. Transcutaneous electrical nerve stimulation (TENS) is a therapeutic approach applied to make medical procedures more bearable to patients.

Objectives. This research aimed to examine the possibilities of reducing dental anxiety in children by using TENS.

Material and methods. A total of 125 children aged 9–14 years were included in the study. The respondents were divided into 3 groups. Group A was treated using no anesthesia for the procedure, group B using TENS, and group C with local anesthesia. All respondents underwent a similar dental procedure. The same protocol was used while treating their first permanent molar.

Results. The results show that TENS serves as a distraction to patients, and therefore reduces their anxiety during dental procedures. Anxiety after the procedure was significantly lower in the TENS group as compared to the group that did not receive local anesthesia, and even in comparison with the group that received local anesthesia.

Conclusions. The TENS device proved to be a useful tool for pediatric dentists to distract their younger patients and make them less anxious, and therefore more cooperative during dental treatment.

Keywords: TENS, dental anxiety, pediatric dentistry

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Highlights

- Transcutaneous electrical nerve stimulation (TENS) is an effective therapeutic method used to reduce patient discomfort during medical and dental procedures.
- The TENS device helps manage pain by stimulating the release of endogenous opioids in the central nervous system, either through spinal cord circuitry or the activation of descending pain inhibition pathways.
- Patients reported feeling calmer and more relaxed after undergoing dental treatment enhanced with TENS therapy.
- In pediatric dentistry, the use of the TENS device proved valuable for reducing anxiety and improving cooperation among younger patients, making it a helpful tool for enhancing the overall dental experience.

Introduction

Examples of anxiety disorders include generalized anxiety disorder, social anxiety disorder (a social phobia), specific phobias, and separation anxiety disorder.[1] Traumatic events seem to trigger anxiety disorders in people who are already prone to anxiety.[2] Inherited traits can constitute a risk factor.[3,4] For some people, anxiety may be related to a physical health problem.[5,6] At the same time, people may suffer from different anxiety disorders. Common signs and symptoms of anxiety include feeling nervous, restless or tense, a sense of impending danger, panic or doom, a rapid heart rate, rapid breathing (hyperventilation), sweating, shaking, feeling weak or tired, trouble concentrating and an inability to think about anything but current worries, insomnia, gastrointestinal problems, difficulty controlling worry, and the need to avoid things that cause anxiety.[7]

A fear of dental procedures is a special form of fear, with which the child tries to adapt to the new situation with the medical staff and the medical environment. Given the specificity of the situation, we can expect different reactions from the child than usual. By knowing the stages of child development, and including family, cultural, genetic, interpersonal, and intrapsychic factors, we can understand the change in the child's behavior. Some fears are completely justified. Pathological forms of fear can be explained as a fear of unknown persons, unknown surroundings, potential pain, or injury. Also, the child may be afraid of being separated from their parents, and react with anxiety in the circumstances unknown so far.[8] Sometimes, patients have a negative image of a visit to the dentist, as a punishment for disobedient behavior. In the environment of a dental office, talking about the immediate cause of the child's bad or good behavior is impossible. However, it is possible to state that pain and negative experiences are potential causes of fear. Dental anxiety is of multifactorial etiology; there is not one or several exclusive factors, but only variables that influence the final picture.[8–11] The final model of behavior is the mutual interaction of the child's psychological constitution and objective factors in their environment. The child's ability to cope with stress is influenced by the richness of the affective life and the characteristics of the child's temperament,[12] as well as the potential separation from the mother and the ability to adapt to new situations.[13,14] When successfully establishing contact with the child, intelligence has a great effect. According to some studies, children with a higher IQ quotient feel a lower level of anxiety when visiting the dentist.[15] A fear of an unknown situation, the very thought of the dentist, as well as previous experiences during procedures in a dental office, are also responsible for increased anxiety in the child. Conversations with people who have previously had negative experiences also significantly impact the level of anxiety in children. Age, gender and character traits play an important role with regard to the occurrence and manifestation of anxiety. According to some authors, girls are more prone to anxious behavior, and this difference between girls and boys increases proportionally with the child's age.[16,17] The potentially provoking factors for anxiety are the expected pain, actual pain, the feeling and smell of certain instruments, the sensory sensation when working with a drill, the uncertainty of the overall situation, a feeling of helplessness, the unkindness of the staff, and a feeling of being trapped in the dental unit.[18]

In dental medicine, treating children and young patients requires a different approach than treating adults. Children still need to be shaped into cooperative patients in the future and are more psychologically liable when undergoing dental treatment. Doctors of dental medicine need to take into account not exposing children to unnecessary trauma, which could lead to developing dental anxiety or a dental phobia in the future. The dental approach when treating children and young adults should be as comfortable and enjoyable as possible to make every visit to the dentist a positive experience to look forward to. Adaptation to the dentist helps in building trust between the child and the members of the dental team. Children with dental anxiety see receiving dental care as a mutual effort between them and the dental team.

Developing a sense of control by taking an active role during treatment and communicating their wishes to the dentist helps ease their anxiety. Positive reinforcement is another commonly used behavioral technique in pediatric dentistry. It is believed that small tangible rewards or verbal acknowledgment might provide better cooperation or appropriate behavior from the patient.[19] Biofeedback is a technique of providing physiological information in real time. This feedback is referred to as augmented or extrinsic feedback that provides additional information, other than the information from intrinsic sensory receptors. Biofeedback has been proven to have therapeutic effectiveness in reducing dental anxiety and decreasing the negative emotions caused by stress.[20] Where dental treatment is urgent, and is combined with a high level of anxiety, sedation is a possibility in the case of younger patients. Sedation is a drug-induced condition in which the patient is rendered free of anxiety. It increases patient cooperation while maintaining the consciousness of the patient.[19,21]

This study evaluates the use of transcutaneous electrical nerve stimulation (TENS) in easing dental anxiety in pediatric patients. The TENS device stimulates the nervous system, and serves as an aid to alleviate pain and anxiety in patients undergoing dental treatment. It can also serve as a distraction. It makes children feel in control when treated, as well as more comfortable and cooperative in a dental office.

Transcutaneous electrical nerve stimulation consists in the use of the electrical current produced in a device to stimulate nerves for therapeutic purposes. It covers the full range of currents used to stimulate nerves, although it is often used with a more restrictive intent, i.e., to describe the type of impulses produced by portable stimulators used to treat pain.[22,23] Scientific research shows that high and low frequencies of TENS stimulate the release of endogenous opioids in the central nervous system (CNS) by activating local circuits within the spinal cord or due to the activation of descending pathways of pain inhibition.[24,25]

The equipment of the TENS device consists of the main unit, electrodes and lead wires. The TENS unit is a generator of electrical impulses, the lead wires establish an electrical connection by linking the electrodes to the TENS unit, and the electrodes convert the electrical current from the TENS unit into the ionic current in the tissue. The electrodes may be applied for intraoral and extraoral use.

Transcutaneous electrical nerve stimulation is successfully used for analgesia during various dental procedures, such as placing a rubber dam, in restorative dental medicine and endodontics, prosthodontics, oral prophylaxis, and tooth extraction. It is also used to reduce discomfort when administering local anesthesia injections and to relieve periodontal pain associated with orthodontic separation.[25]

Material and methods

The study included children who had a Class I cavity in the first permanent molar. The minimum age for inclusion was 9 years, and the maximum age was 14 years. The excluding factor was the patient's cognitive uncooperativeness, i.e., the lack of verbal and non-verbal communication on the child's part.

The research was approved by the Ethics Committee at the School of Medicine of the University of Zagreb, Croatia, prior to the commencement of the research (No. 05-PA-15-11/2017). All collected data was anonymous, and parents (or guardians) had to sign an informed consent form after being acquainted with the procedure. It was also noted on the forms that all collected data would be strictly confidential. The identities of the research participants were known only to the dentist who conducted the research and the appropriate institution. All children received instructions on how to complete the stress tests immediately before the examination and after the informative interview.

The respondents were divided into 3 groups according to the nature of their procedure and a feeling of pain during dental treatment. Group A included children who were treated using no anesthesia for the procedure. Children from group B had the TENS device applied on their facial skin, and group C included children who received local anesthesia. The distribution of respondents per group is shown in Fig. 1.

The prepared cavities were of equal depth in all 3 groups. Dental treatment started with opening the cavity with a round diamond grinder on a pipe with abundant water cooling. The carious contents were removed with a round steel drill with a low number of revolutions of the micromotor. Maximum attention was paid to working intermittently so that the cavity would not get too hot. A feeling of pain in the patients was closely monitored to make sure the children were comfortable during the procedure.

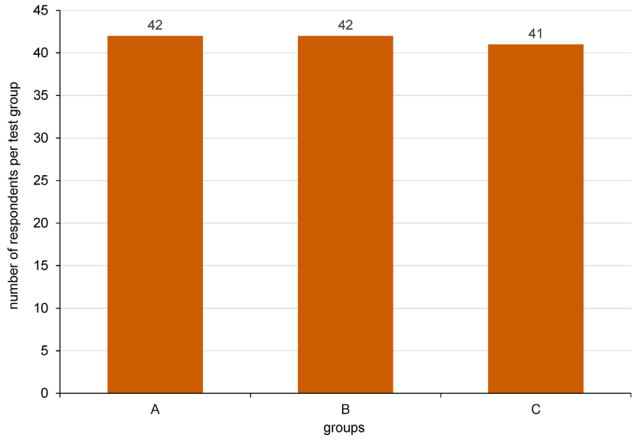

Fig. 1. Division of respondents into test groups

Groups: A –treated without local anesthesia; B – treated using transcutaneous electrical nerve stimulation (TENS); C – treated with local anesthesia.

The cavity was rinsed with a jet of water, and gently dried with dry air without drops of water or oil. The etching of enamel surfaces with 37% orthophosphoric acid gel was then carried out for 20 s, followed immediately by dentin etching, which lasted for 10 s. All the acidic contents were completely rinsed out of the cavity with a jet of water and the cavity was slightly dried. All surfaces were coated with a dentin adhesive; it was placed on the cavity walls and left for up to 20 s. Then, the excess adhesive was gently removed from the surface with a jet of air so that a shiny surface was obtained. Depending on the manufacturer's instructions, the dentin adhesive was illuminated and polymerized with a suitable light source. Direct composite materials (Gradia Direct, colors A2 and A3; GC International, Lucern, Switzerland) were used to fill the cavity. The material was placed in layers, and each layer was polymerized for 40 s. The excess material was removed with a suitable diamond bur. Premature contacts were registered with articulation paper. They were removed with a smaller diamond bur. All surfaces of the composite filling were polished with a suitable polishing bur. Interdental floss was used to check the approximate filling surfaces and contact relations. All procedures were performed in the morning, during daylight hours, by one suitably qualified therapist in the pediatric and preventive dental medicine clinic at the Faculty of Dentistry, University of Zagreb. The atmosphere was pleasant. In the waiting room, various cartoons for children were shown on television and the patients were also offered the option to draw.

Local anesthesia was used in the research. The type of anesthesia depended on the jaw in which the tooth was prepared (the maxilla or the mandible). The anesthetic used was Ubistesin™ Forte 4% with adrenaline (40 mg/mL + 0.01 mg/mL) (Pierrel, Capua, Italy). A short needle was used for plexus anesthesia and a long one for mandibular anesthesia. Before the application of anesthesia, the mucous membrane was lightly anesthetized with a topical anesthetic spray. The spray anesthetic used was lidocaine (100 mg/mL). Before applying the anesthetic, the children were asked to close their eyes, so that they would not activate an anxiety reaction by looking at the needle and fearing it.

Before the cavity preparation, the electrodes of the TENS unit were placed extraorally at the place where the mandibular or maxillary nerve is located, depending on which tooth was prepared (in the maxilla or the mandible). The electrodes themselves were attached to the skin using negative-pressure silicone around the electrode. No other adhesive or gel was applied between the skin and the electrode. A conventional TENS mode with a high frequency of 120 Hz was used, producing segmental analgesia with a rapid onset (<30 min) and rapid termination (<30 min). The patients regulated the administration of TENS during treatment by themselves, as shown in Fig. 2.

The self-assessment manikin (SAM) scale was used to assess the respondents' anxiety. The SAM scale measures emotional responses to stimuli in 3 dimensions – pleasure, arousal and dominance (a feeling of control). Each dimension was evaluated on a scale from 1 to 9, determined by 5 corresponding thumbnails and 4 intermediate spaces with described reference points: from "extremely unpleasant" to "extremely pleasant" for pleasure; from "extremely calm" to "extremely agitated" for arousal; and from "extremely submissive" to "extremely dominant" for dominance. The internal consistency for pleasure was 0.63 for the younger group and 0.82 for the older group, 0.98 for arousal –for both groups, and 0.66 for dominance –also for both groups.[26]

Statistical analysis

The statistical methods used were descriptive. They comprised the calculation of arithmetic means and standard deviations ($M \pm SD$), and the recording of the minimum (min) and maximum (max) values, as well as the calculation of the Pearson coefficient of asymmetry of distribution alpha (α). The two-way analysis of variance (ANOVA) was used to test the significance of differences with regard to the groups of participants and the observation time (before or after treatment).

Statistical analysis was conducted using MS Excel (Microsoft Corporation, Redmond, USA) and IBM SPSS Statistics for Windows, v. 20.0 (IBM Corp., Armonk, USA).

Fig. 2. Application of transcutaneous electrical nerve stimulation (TENS) in a young patient

Results

A total of 125 children were included in the research – 40 boys (32%) and 85 girls (68%). The age range of the examined children was from 9 to 14 years, with the average age being 11.53 ±1.78 years.

The results in terms of the pictorial SAM scale scores (unpleasant–pleasant) according to the groups of subjects (TENS, with or without local anesthesia) before and after the procedure are shown graphically in Fig. 3. The TENS group felt the most pleased after treatment in comparison with the other 2 groups.

The results of the *t* test for dependent samples, i.e., the significance of the differences in the SAM scale unpleasant–pleasant scores between the 3 groups of subjects before and after the procedure, are shown in Table 1. Statistically significant differences ($p < 0.01$) with regard to the observation time (before and after treatment) were noted in the TENS group (more pleasant after treatment) and the group with no anesthesia (more unpleasant after the procedure). The young patients described their experiences as more pleasant after dental treatment when using the TENS device.

The results in terms of the pictorial SAM scale scores (calm–agitated) according to the groups of subjects (TENS, with or without local anesthesia) before and after the procedure are shown graphically in Fig. 4. The respondents felt much calmer after dental treatment when using TENS during the procedure.

The results of the *t* test for dependent samples, i.e., the significance of the differences in the SAM scale calm–agitated scores between the 3 groups of subjects before and after the procedure, are shown in Table 2. Statistically significant differences ($p < 0.01$) with regard to the observation time (before and after treatment) were noted in the TENS group (calmer after treatment) and the group with no anesthesia (more agitated after the procedure).

The results as descriptive statistics for the SAM scale scores (submissive–dominant) according to the groups of subjects (TENS, with or without local anesthesia) before and after the procedure are shown graphically in Fig. 5. The graph shows the greatest difference in the group which underwent the dental procedure using local anesthesia.

The results of the *t* test for dependent samples, i.e., the significance of the differences in the SAM scale submissive–dominant scores between the 3 groups of subjects before and after the procedure, are shown in Table 3. A statistically significant difference ($p < 0.05$) with regard to the observation time (before and after treatment) existed only in the group with local anesthesia. The young patients did not experience a remarkable change in the feeling of dominance after the dental procedure using the TENS device, but felt more dominant after the administration of local anesthesia instead.

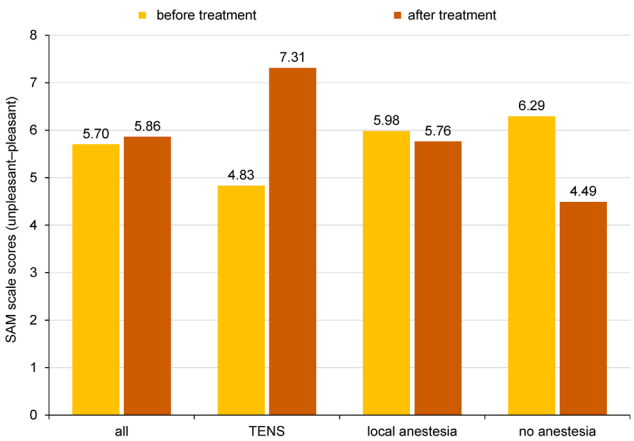

Fig. 3. Mean self-assessment manikin (SAM) scale scores (unpleasant–pleasant) by groups of respondents before and after treatment

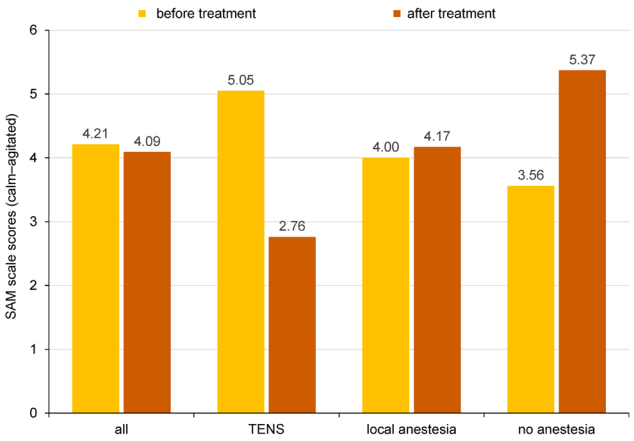

Fig. 4. Mean self-assessment manikin (SAM) scale scores (calm–agitated) by groups of respondents before and after treatment

Table 1. Mean self-assessment manikin (SAM) scale unpleasant–pleasant scores in the 3 groups of subjects before and after treatment (*t* test)

Group	*N*	Before treatment	After treatment	*p*-value
All	125	5.70 ±2.15	5.86 ±2.91	0.532
TENS	42	4.83 ±2.25	7.31 ±2.03	0.000**
Local anesthesia	42	5.98 ±1.96	5.76 ±3.08	0.649
No anesthesia	41	6.29 ±2.00	4.49 ±2.84	0.000**

Data presented as mean ± standard deviation (*M* ±*SD*).
1 – unpleasant; 9 – pleasant; ** statistically significant ($p < 0.01$).

Table 2. Mean self-assessment manikin (SAM) scale calm–agitated scores in the 3 groups of subjects before and after treatment (*t* test)

Group	*N*	Before treatment	After treatment	*p*-value
All	125	4.21 ±2.44	4.09 ±3.03	0.671
TENS	42	5.05 ±2.51	2.76 ±2.29	0.000**
Local anesthesia	42	4.00 ±2.39	4.17 ±3.18	0.727
No anesthesia	41	3.56 ±2.21	5.37 ±3.04	0.001**

Data presented as *M* ±*SD*.
1 – calm; 9 – agitated; ** statistically significant ($p < 0.01$).

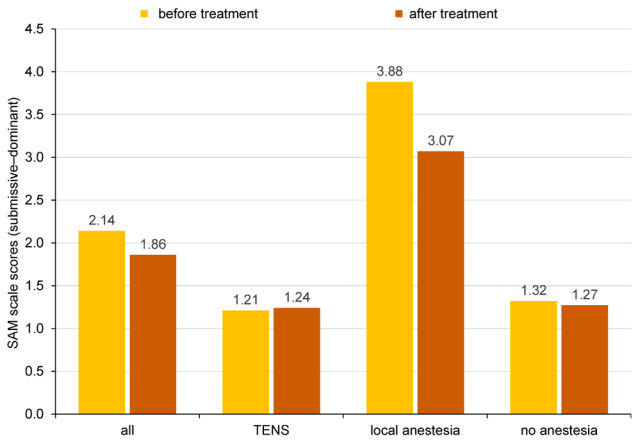

Fig. 5. Mean self-assessment manikin (SAM) scale scores (submissive–dominant) by groups of respondents before and after treatment

Table 3. Mean self-assessment manikin (SAM) scale submissive–dominant scores in the 3 groups of subjects before and after treatment (*t* test)

Group	*N*	Before treatment	After treatment	*p*-value
All	125	2.14 ±2.07	1.86 ±1.89	0.082
TENS	42	1.21 ±0.98	1.24 ±1.25	0.920
Local anesthesia	42	3.88 ±2.45	3.07 ±2.39	0.044*
No anesthesia	41	1.32 ±1.19	1.27 ±1.12	0.534

Data presented as *M* ±*SD*.
1 – submissive; 9 – dominant; * statistically significant ($p < 0.05$).

Discussion

There are various possible applications for TENS in dentistry, from managing pain during dental procedures to treating chronic pain conditions affecting the maxillofacial region, including trigeminal neuralgia (TGN), postherpetic neuralgia (PHN) and temporomandibular joint disorders (TMDs), as well as xerostomia, Sjörgen's syndrome and acute orofacial pain. Saranya et al. compared TENS and microcurrent nerve stimulation (MENS) in the treatment of masticatory muscle pain in 60 patients.[27] Patients were randomly divided into 2 groups (A and B) and each group into 2 subgroups (A1, A2, B3, and B4), based on the visual analog scale (VAS) scores. Group A patients were given TENS for 20 min, and group B patients were given MENS for 20 min. Each patient was invited to treatment for 5 days, and the same intensity and frequency were maintained throughout the treatment period. The study showed that both TENS and MENS were equally effective in improving functional mouth opening, although MENS showed a better and more immediate effect in terms of pain relief.[27] Side effects, such as tingling and paresthesia, that occur in some patients using TENS are not present when using microcurrent. In 1983, Hansson and Ekblom studied the use of TENS for alleviating acute orofacial pain in 62 patients, using high frequency (100 Hz), low frequency (2 Hz) and placebo TENS.[28] The study included patients who suffered from pain for 1–4 days; 38% of patients who received any form of TENS showed a 50% reduction in pain, and 10% of patients who received a placebo showed a pain reduction of more than 50%.[28] Steller et al. attempted to determine whether the battery-operated electrical stimulation applied to the hard palate and tongue could initiate salivary flow stimulation in 29 patients with Sjörgen's syndrome in a double-blinded study.[29] They used the device for 4 weeks, 3 times a day, for 3 min. The conclusion was that using TENS was successful only in patients with some residual salivary flow present.[29] In PHN, the normal presynaptic inhibition of C fibers does not occur due to the destruction of most of the larger myelinated afferent nerve fibers.[30] This causes pain and abnormal skin sensitivity. The use of TENS can increase the activity of the remaining fibers and re-introduce normal inhibition.[31] In 1998, Mittal et al. treated 10 patients suffering from PHN.[32] They used TENS (70 Hz) for 20 min a day for 10 days. The study showed successful results, reducing 50% of pain in 60% of patients. The authors conclude that patients who respond better to the therapy are those with a shorter duration of PHN.[32] The most negative behavior in pediatric patients is a fear of the needles used in local anesthesia.[33,34] Transcutaneous electrical nerve stimulation can help reduce a fear of injections. Studies show that 53–78% of children preferred TENS over local anesthesia.[35]

The prior experiences of the children in dental offices were not relevant to this study, as the results referred to differences in the anxiety levels before and after the dental procedure. The pain levels were also noted during the procedure, but pain is not mentioned in the results of this research, as it is out of the scope of this paper. All procedures were carried out ethically, taking into consideration the patient's comfort during dental treatment, as the comfort of the patient is a top priority in a dental office.

The SAM scale showed statistically significant differences in the young patients' perception of the dental procedure performed with the use of TENS in comparison with administering local anesthesia or using no anesthesia during the dental treatment of the first permanent molar. The results showed that the dental procedure combined with the application of TENS was perceived as more pleasant to the patients, and they felt calmer after the procedure as well. The pleasure aspect and the calmness of the patient during dental procedures may also be beneficial to the dentist carrying out the procedure. Calmer patients, especially younger children, get a lot of positive outcomes from dental procedures, as it is easier to follow protocols such as isolating the treatment site, and focus more on the treatment itself. Young patients who feel pleased and calm during dental procedures will experience less anxiety in the future as well. The feeling of dominance in the young patients who underwent the dental procedure with the administration of local anesthesia is an interesting finding.

The children who overcame their fear of needles and local anesthesia will be more cooperative and agree to local anesthesia more easily in the future.

Using the SAM scale in this research proved useful, given that the subjects were young children. The scale is easy to understand, since each dimension is determined by 5 corresponding thumbnails. It was easy for young patients to express their feelings of pleasure, arousal and dominance on the SAM scale used in this research.

The positive experience the study participants gained in a dental office will increase the likelihood of their cooperation during the following dental treatment procedures in the future. The anxiety levels may be lower, thus reducing the risk of developing a dental phobia when the patients grow up. Positive experiences in a dental office are of great importance, since they shape a cooperative attitude in patients, encourage them to take care of their oral health and approach regular checkup appointments at the dentist responsibly. Also, such patients may have a positive influence on people in their close environment, such as siblings or – in the future – their own children.

This study confirmed that TENS was a successful distraction method for dental procedures. The results show that the group in which the TENS device was applied felt less anxiety after the procedure, in contrast to the groups with and without local anesthesia, where the patients felt more anxiety after the procedure. This could be explained by the fact that the group without anesthesia felt little or insignificant fear before the procedure, and felt some unexpected pain during treatment, which caused an increase in the level of anxiety after the procedure. A similar situation occurred in the group that received local anesthesia. Regardless of the painlessness of the procedure, the fear of the needle used during anesthesia caused an increase in anxiety among the subjects. The TENS device, used during the entire dental procedure, distracted the examinee's attention from the procedure itself and the sensations connected with it. Given that each child has a different pain tolerance threshold, the same amount of electricity could not be given to all subjects equally, and for this very reason, each subject could control the strength of the current individually. The children were focused on the tingling sensation caused by the mild administration of electricity and on dosing the electricity so that the treatment would be pleasant and not painful. The TENS device also served as a "toy" which the child had fun with during the procedure. Transcutaneous electrical nerve stimulation can be used as a mild analgesic during various dental procedures, although it cannot completely replace local anesthesia. It can also exert the placebo effect, as the self-administration of the electrical current can distract attention from the procedure being performed on the tooth. The assumption is that the amount of electricity that children administer to themselves is not high enough to cause discomfort to the child. The pain was closely monitored throughout the procedure to make sure the patient was comfortable. However, the pain-alleviating effect of the TENS device was not within the scope of this research. This can be pointed out as a limitation of the present study. The recommendation for future studies on the TENS device in pediatric dentistry would be to explore its effectiveness in pain management in comparison with the conventional use of local anesthesia for dental procedures.

Conclusions

Positive experiences in a dental office from a young age are crucial for ensuring patient cooperation in the future. Reducing dental anxiety is one of the many challenges dentists face when treating young patients. If not properly addressed, dental anxiety in children can develop into a dental phobia in adulthood, potentially leading to the neglect of oral health due to fear. The TENS device may serve as a useful tool for dentists to help distract younger patients and make them more comfortable, and even excited about visiting a dental office.

Modern dental medicine emphasizes noninvasive and innovative approaches to treatment, focusing on painless, pleasant procedures that help reduce patient anxiety. Preventing oral diseases has never been more important, and minimizing dental anxiety can play a key role in encouraging patients to attend regular checkups. The TENS device may offer a valuable solution for promoting oral health in both young patients and adults.

Ethics approval and consent to participate

The research was approved by the Ethics Committee at the School of Dental Medicine of the University of Zagreb, Croatia, prior to the commencement of the research (No. 05-PA-15-11/2017). All collected data was anonymous, and parents (or guardians) had to sign an informed consent form after being acquainted with the procedure.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

All collected data was strictly confidential. The identities of the research participants were known only to the dentist who conducted the research and the appropriate institution.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Assessment of the risk of sleep-disordered breathing and its contributing factors in the pediatric orthodontic population: A cross-sectional study

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Abstract

Background. Sleep-disordered breathing (SDB) is a group of disorders that can affect the upper airway by increasing the chances of collapsibility during sleep.

Objectives. The primary objective of the present study was to estimate the prevalence of pediatric SDB in the orthodontic population using the Pediatric Sleep Questionnaire (PSQ). The secondary objective was to assess the factors associated with pediatric SDB in the orthodontic population.

Material and methods. A cross-sectional study was conducted on a sample of 60 children aged 7–12 years who attended the orthodontic clinics at Aga Khan University Hospital, Karachi, Pakistan. The parents/guardians of the patients were asked to complete a validated PSQ and an additional health history questionnaire regarding the factors associated with SDB. The risk of SDB was reported for the pediatric orthodontic population. Logistic regression analysis was applied to assess the factors associated with SDB in the pediatric orthodontic population.

Results. A score suggestive of a high risk for SDB (≥33%) was found in 12 (20%) out of 60 patients. Patients with a history of allergies were 3.96 times more likely to have SDB ($p = 0.049$). In comparison with female patients, male patients had a higher susceptibility to SDB.

Conclusions. The prevalence of SDB was found to be 20% in the pediatric orthodontic population. The frequency of allergies was higher among the patients in the high-risk SDB group. Orthodontic practitioners are advised to incorporate routine SDB screening into their clinical practice, as there could be a specific subgroup of SDB patients that may go undetected in general pediatric clinics.

Keywords: pediatric, sleep, sleepiness, sleep disorders, Pediatric Sleep Questionnaire

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Highlights

- Sleep-disordered breathing (SDB) involves upper airway disorders that increase airway collapsibility during sleep, negatively impacting quality of life in both children and adults.
- Based od the Pediatric Sleep Questionnaire (PSQ), SDB was found in 20% of the pediatric orthodontic population studied.
- A higher frequency of allergies was observed in patients at high risk for SDB.
- Screening for SDB in orthodontic patients enables early intervention and the management of contributing factors, and enhances overall treatment outcomes.

Introduction

Sleep-disordered breathing (SDB) is a group of disorders that can affect the upper airway by increasing the chances of collapsibility during sleep.[1] Sleep-disordered breathing can have numerous adverse effects on the quality of life (QoL), affecting both young individuals and adults.[2] Interestingly, certain differences do exist between the pediatric and adult population when considering this condition.[3] In the adult population, there appears to be a higher prevalence of the disease among males, indicating a gender difference.[4] However, no definitive distinction is observed in the pediatric population.[1] Additionally, there is a notable difference in etiology. In the adult population, obesity is recognized as the primary cause, while in the pediatric population, enlarged tonsils are considered to be the main culprit.[5,6] The evaluation of such features during a routine orthodontic examination emphasizes the crucial role of orthodontists/general practitioners in diagnosing patients and promptly referring them to the appropriate department.[7]

By virtue of physical and cephalometric inspections, multiple risk factors that contribute to the development of SDB have been identified.[8] These factors include a constricted maxilla, a V-shaped arch form, a shortened mandible, macroglossia, and bidental arch constriction.[9] A study conducted by Topaloglu-Ak et al. stated that sleep bruxism, temporomandibular disorders (TMDs) and untreated dental caries also had a negative impact on pediatric sleeping habits and characteristics.[10]

Polysomnography (PSG) takes precedence as the primary diagnostic modality for evaluating SDB.[11] It involves electrooculography, electroencephalography, chin electromyography, the airflow measurement, monitoring the respiratory effort, tracking the oxygen saturation levels, and recording electrocardiography. Performing PSG in young patients requires specialized training in pediatrics to help children and their families adjust to the intensity of the monitoring. While PSG for children shares similarities with the procedure for adults, there are key differences to consider. Notably, these differences are most apparent in the positioning and placement of the monitoring leads due to the size disparity between children and adults. Furthermore, variations regard the identification of the sleep stages, the evaluation of the respiratory patterns and the assessment of the severity of sleep-related issues in adults vs. children.[12] Nevertheless, there are certain drawbacks associated with PSG, including time requirements, high costs of initial installation, and the need for expert sleep physiologists to ensure accurate performance.[13] Certain predictive tools, like the Berlin questionnaire (BQ), the STOP-BANG questionnaire, the Snoring, Trouble Breathing, and Un-Refreshed (STBUR) questionnaire, and the Pediatric Sleep Questionnaire (PSQ), were designed to minimize this burden.[14]

The PSQ is a reliable and validated tool to predict the risk of SDB in the pediatric population. This is a 22-unit questionnaire that covers various aspects, including but not limited to the snoring frequency, the observed apneas, difficulty in breathing, as well as inattentive and hyperactive behavior.[11] This questionnaire serves as a screening tool to identify children who may need further investigation with PSG for a more detailed assessment.[11] Early diagnosis can significantly influence the well-being of patients with SDB. Untreated SDB can potentially contribute to the development of attention-deficit/hyperactivity disorder (ADHD) and cardiovascular diseases (CVDs) characterized by arterial narrowing (atherosclerosis), ultimately leading to a diminished QoL.[15,16] Thus, screening for SDB and evaluating its risk in the orthodontic population can help orthodontists to make timely decisions, work on the factors associated with SDB and improve the overall treatment experience of these patients.

The aim of the present study was to assess the risk of SDB and the contributing factors associated with it in the pediatric orthodontic population using PSQ.

Material and methods

After attaining an approval from the institutional ethical review committee (ERC) (No. 2022-7818-22971), a questionnaire-based cross-sectional study was conducted at Aga Khan University Hospital, Karachi, Pakistan.

The sample size was estimated with the OpenEpi® software, v. 3.01 (https://www.openepi.com/Menu/OE_Menu.htm) by using the findings of Sogut et al., who proclaimed an SDB prevalence of 3.3% in Turkish children.[17] Consequently, keeping the absolute precision at 5% and a confidence interval (*CI*) of 95%, a sample size of 50 (*N*) was required. To address the sample attrition, we inflated the sample size by 20%, reaching the final sample size of 60 subjects in our study.

Data was collected from 60 patients seeking orthodontic consultation followed by orthodontic treatment, aged 7–12 years. The age range was determined by the research team, which consisted of a research assistant, 2 orthodontic residents and an orthodontist. The purpose of establishing this age range was to ensure inclusivity for the pediatric population seeking orthodontic care. The inclusion criteria were as follows: patients aged 7–12 years seeking orthodontic treatment; and patients' parents/guardians signing an informed consent form and observing the sleep pattern of their children. Pediatric patients with the presence of any craniofacial/dental anomaly, syndrome, trauma, or a previous history of orthodontic treatment were excluded to keep the homogeneity of the sample and to avoid any kind of bias in the study.

In this cross-sectional study, during the first visit, all participants' parents/guardians completed the appropriate informed consent forms, and the patients' initial records were taken. After obtaining consent, the parents/guardians were asked to fill out PSQ (Table 1), assisted by an orthodontic team member. The PSQ used in the present study is a reliable questionnaire developed and validated by Chervin et al.[11] It was applied to assess SDB. The PSQ comprises 22 questions with the responses assessed as follows: 'yes' = 1; 'no' = 0; and 'don't know' = 00. It records the frequency of the observed apneas, snoring, daytime sleepiness, difficulty in breathing, and attentiveness in pediatric patients.[11] The final score to confirm the presence of SDB has been recommended to be 0.33 (33% of positive responses), with higher percentages demonstrating a positive indication of SDB. The patients who scored 33% or higher in positive responses were classified as being at high risk for SDB.[1,11]

The parents/guardians were also asked to fill out an additional health history questionnaire to assess the contributing environmental and health factors for SDB in the pediatric patients.[1] This questionnaire consisted of questions confirming the presence or absence of asthma, environmental allergies, indoor pets, pre-term birth, nocturnal enuresis (NE), a family history of sleep apnea, and smoking environment.[1] The treating practitioners ensured that patients identified as being at high risk for SDB received follow-up care and any necessary referrals.

Table 1. Pediatric Sleep Questionnaire (PSQ)[11]

No.	Questions		Responses		
1.	While sleeping does your child:	snore more than half the time?	Y	N	DK
		always snore?	Y	N	DK
		snore loudly?	Y	N	DK
		have heavy or loud breathing?	Y	N	DK
		have trouble breathing, or struggle to breathe?	Y	N	DK
2.	Have you ever seen your child stop breathing during the night?		Y	N	DK
3.	Does your child:	tend to breathe through the mouth during the day?	Y	N	DK
		have a dry mouth on waking up in the morning?	Y	N	DK
		occasionally wet the bed?	Y	N	DK
4.	Does your child:	wake up feeling unrefreshed in the morning?	Y	N	DK
		have a problem with sleepiness during the day?	Y	N	DK
5.	Has a teacher or other supervisor commented that your child appears sleepy during the day?		Y	N	DK
6.	Is it hard to wake your child up in the morning?		Y	N	DK
7.	Does your child wake up with headaches in the morning?		Y	N	DK
8.	Did your child stop growing at a normal rate at any time since birth?		Y	N	DK
9.	Is your child overweight?		Y	N	DK
10.	This child often:	does not seem to listen when spoken to directly?	Y	N	DK
		has difficulty organizing tasks and activities?	Y	N	DK
		is easily distracted by extraneous stimuli?	Y	N	DK
		fidgets with hands or feet, squirms in seat?	Y	N	DK
		is "on the go" or often acts as if "driven by motor"?	Y	N	DK
		interrupts or intrudes on others (e.g., butts into conversation or games)?	Y	N	DK

Y – 'yes'; N – 'no'; DK – 'don't know'.

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA) and the Stata software, v. 12.0 (StataCorp, College Station, USA). The normality of the data was assessed using the Shapiro–Wilk test, revealing a non-normal distribution. Descriptive statistics, such as median (*Me*) and interquartile range (*IQR*), were provided for the age of patients. The PSQ responses were collected from 60 patients and a positive score ≥33% was considered as a high risk for SDB. Frequencies were reported for the patients having a high risk for SDB and the factors associated with it. Furthermore, all individuals in the sample underwent additional health history questionnaire evaluations to assess their conditions. The results obtained from these evaluations were then compared between the patients categorized in the high-risk and low-risk SDB groups. Logistic regression analysis was applied to assess the factors associated with SDB. A statistical significance level of $p \leq 0.05$ was adopted.

Results

The total sample size was 60, with a gender distribution of 30 males and 30 females (Fig. 1). The age of the patients recruited in this study was 10.04 (8.00–11.33) years (Table 2).

A high risk for SDB (≥33%) was found in 12 (20%) out of the 60 patients. Evaluations from the additional health history questionnaire showed that 36.7% of the recruited population had environmental allergies (dust), 13.3% were exposed to smoke and 8.3% had indoor pets (Table 3). Among the patients who were at a high risk for SDB, 8 were males and 4 were females. In this group, 7 individuals had environmental allergies, whereas in the low-risk SDB group, 15 patients had environmental allergies (Table 4).

Logistic regression analysis was applied to assess the factors associated with SDB. It showed statistically significant results for the factor of allergy – the patients with a history of allergies were 3.96 times more likely to have SDB (p = 0.049). Moreover, the patients diagnosed with ADHD were found to have a 5.22 times greater likelihood of experiencing SDB as compared to individuals without ADHD (p = 0.120). In the case of the patients who wet the bed, the probability of having SDB was 4.80 greater in comparison with those who did not (p = 0.281). However, these results were not statistically significant (Table 5).

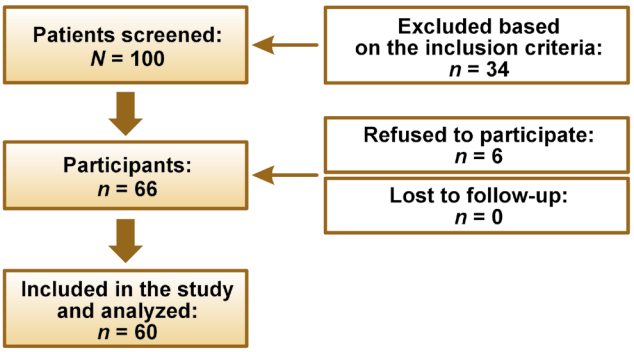

Fig. 1. Flow diagram of the study

Table 2. Demographics of the study group (*N* = 60)

Gender	Age [years]
Male (*n* = 30)	9.33 (8.00–11.08)
Female (*n* = 30)	10.30 (8.00–11.66)
Total (*N* = 60)	10.04 (8.00–11.33)

Data presented as median (interquartile range) (*Me* (*IQR*)).

Table 3. Risk of sleep-disordered breathing (SDB) and its associated factors (*N* = 60)

Variable	*n* (%)
SDB	12 (20.0)
Bed wetting	2 (3.3)
Allergy	22 (36.7)
ADHD	4 (6.7)
Indoor pets	5 (8.3)
Smoke exposure	8 (13.3)

ADHD – attention-deficit/hyperactivity disorder.

Table 4. Associated environmental and health factors in the high-risk vs. low-risk sleep-disordered breathing (SDB) groups (*N* = 60) (Fisher's exact test)

Variable	High-risk SDB group *n* = 12 M: *n* = 8 F: *n* = 4	Low-risk SDB group *n* = 48 M: *n* = 22 F: *n* = 26	*p*-value
Bed wetting	1 (8.3)	1 (2.1)	0.363
Allergy	7 (58.3)	15 (31.3)	0.102
ADHD	2 (16.7)	2 (4.2)	0.175
Indoor pets	1 (8.3)	4 (8.3)	1.000
Smoke exposure	1 (8.3)	7 (14.6)	1.000

Data presented as number (percentage) (*n* (%)).
M – male; F – female.

Table 5. Factors associated with sleep-disordered breathing (SDB) (logistic regression analysis)

Variable	*OR*	95% *CI*	*p*-value
Bed wetting	4.80	0.276–83.340	0.281
Allergy	3.96	1.007–15.618	0.049*
ADHD	5.22	0.649–42.039	0.120
Indoor pets	1.12	0.113–11.175	0.920
Smoke exposure	0.60	0.066–5.447	0.650

OR – odds ratio; *CI* – confidence interval; * statistically significant ($p \leq 0.05$).

Discussion

This research was conducted to provide an insight into the risk of SDB in the Asian pediatric orthodontic population and its associated factors. Our study, based on a tertiary care hospital sample, yielded a risk of 20%. When looking into the literature globally, we found a recently conducted study from Alberta, Canada, reporting a prevalence of SDB of 10.8% in their orthodontic population.[1] Additionally, 2 separate studies that focused on SDB in the orthodontic population reported a prevalence rate of 7.3% in their site-specific sample[13] and 18% in their university-based sample.[18] Utilizing the same objective questionnaire (PSQ), an SDB prevalence of 3.3% was observed in German preschool children.[19] According to the findings of Graf et al., 53% of the children included in the study exhibited snoring, as reported by their parents.[20] According to a meta-analysis conducted by Lumeng and Chervin, the prevalence rates of nocturnal childhood snoring ranged from 1.5% to 12.0%, with an average rate of 7.45% based on parent-reported data.[21] A study conducted by Ancoli-Israel et al. compared the risk of SDB in the Caucasian and African-American populations.[22] The study findings revealed that African-Americans exhibited a significantly higher risk of severe SDB, with a relative risk (RR) approx. twice as high (RR = 2.13) as that observed in Caucasians.[22] Another study also reported a higher risk of SDB in African-Americans as compared to the Asian, Hispanic and European populations.[23]

Considering the prevalence at the regional level, children from Saudi Arabia had a 21% prevalence of SDB in the sample population.[24] Kobayashi et al. conducted their study in Japan and reported the incidence of obstructive sleep apnea (OSA) in children to be 7.9%.[25] Subsequently, authors from India used a modified STOP-BANG questionnaire and reported a 14% prevalence of OSA in Indian adolescents.[26]

One of the key competences of the clinician is the ability to predict the presence or absence of the disease in the general population. Investigators have pointed out that a convex facial profile, mandibular retrusion, tonsillar hypertrophy, and mouth breathing at an early age can lead to SDB.[27] Further predictive factors reported by researchers include body adiposity and distal molar occlusion at the age of 6–8 years.[27] In contrast to SDB in adults, which is predominantly associated with obesity, the pediatric population shows associations with adenotonsillar hypertrophy, allergies, frequent colds, and habitual mouth breathing.[28] Our results are in agreement with the previous studies showing higher chances for SDB, especially in male patients with environmental allergies. Allergic reactions can lead to breathing irregularities through multiple pathways, including (1) inflammatory cytokines, such as histamine, which cause disturbance in the sleep/wake cycle; (2) nasal congestion, which is related to snoring and ultimately affects sleep quality; and (3) the dysfunction of the 2 important autonomic nervous system reflexes, namely the trigeminocardiac reflex and the nasotrigeminal reflex. Both are important in terms of sleep regulation and impairment.[29]

Among the other indicators described above, NE is also considered an important characteristic predictive of SDB. One study conducted in Taiwan concluded a positive association between environmental allergies, SDB and childhood NE.[30] A prospective epidemiological study on 4,318 children reported that 33.1% of the children with SDB had NE.[18] This is in accordance with our results, which showed 4.8 times higher chances for SDB among children with NE. Furthermore, work conducted by Lai et al. validated the findings of previous studies by reporting that children with allergic rhinitis had a higher incidence and risk of NE, especially males.[31] Another critical factor causing OSA is an increased body mass index (BMI). Barone et al. conducted a study to assess the association of overweight and NE with SDB.[32] They reported a non-significant association. However, when viewed separately, these characteristics (overweight and NE) showed a high probability of being present in children with OSA.[32] In our study, we found an insignificant association of these factors with the risk of SDB.

The PSQ used in this study is the most widely used questionnaire to detect the risk of SDB in children and adolescents. The accepted age range to use this tool is 2–18 years. This comprehensive questionnaire has a sensitivity and specificity of 0.87 and 0.85, respectively.[11] The PSQ is considered a time-consuming questionnaire by some researchers, so to rectify this situation, Kadmon et al. developed a more concise questionnaire termed I'M SLEEPY.[33] It consists of 8 questions and has a sensitivity and specificity of 82% and 50%, respectively.[33] A systematic review and meta-analysis published in 2020 found that PSQ had the highest sensitivity (74%) for detecting symptoms of moderate pediatric obstructive sleep apnea syndrome (OSAS).[34] In screening for mild and severe pediatric OSAS, PSQ and pulse oximetry (PO) demonstrated equal sensitivity. Therefore, PO can be an excellent tool, along with PSQ, to detect SDB effectively when PSG is impractical.[34]

A study by Dastan et al. stated that the volume of the upper airway was significantly smaller in individuals with the dolichofacial pattern.[35] Orthodontic interventions, such as functional appliances, in the growing phase and surgical interventions in adults can help in reducing the effect of the abovementioned feature, i.e., the decreased volume of the upper airway, and improve the quality of sleep and the overall QoL of these subjects.[36] Treatment options for SDB vary, depending on the severity of the condition and the age of the patient. Treatment modalities for SDB encompass a spectrum of approaches, including non-surgical options, like mandibular advancement splints and continuous positive airway pressure, as well as surgical options, such as adenoidectomy and maxillomandibular advancement.[37,38] One mode of treatment can be the expansion of the maxillary arch via different methods, i.e., mini-screw-assisted rapid maxillary

expansion (MARPE) and surgically assisted rapid maxillary expansion (SARPE), which are usually advised in adolescent and adult patients, respectively.[38] A relatively new technique called endoscopically assisted surgical expansion (EASE) for the treatment of OSA has been advised.[39] The method is claimed to be less invasive and provides consistently better results as compared to the previously mentioned maxillary expansion modalities. It is a surgical procedure performed under general anesthesia requiring nasal endoscopy for visualizing the midpalatal osteotomy, along with the separation of the pterygomaxillary suture with the use of a piezoelectric blade. After osteotomy, a transpalatal distractor is used to expand the nasal floor until the patient's symptoms are alleviated or 7 mm of expansion has been achieved. The removal of the appliance is advised to be performed under local anesthesia after 2 months of obtaining the desired results.[39]

It is important to refer to the American Association of Orthodontists (AAO) white paper for OSA, which clearly states the responsibility of an orthodontist to screen, diagnose and advise a proper referral to patients.[40] It is recommended that orthodontists who wish to treat SDB patients get equipped with the latest knowledge, skills and training in this field. It is encouraging to know that no orthodontic treatment causes or worsens OSA; rather, some form of orthodontic treatment is regarded as beneficial. However, an interdisciplinary team approach would serve the patient's interests best.

Given the nature of observational studies, including our own, it is important to acknowledge the potential risks of bias and the confounding factors. The limitations of this research are a relatively small sample size and being a single-center study; the results cannot be generalized to the Asian population. An additional limitation is the absence of an objective method, such as PSG, for evaluating SDB in the orthodontic population. However, our study focused on assessing the risk of SDB in this population. As a cost-effective alternative to PSG, we utilized PSQ, which is a practical choice, especially for individuals who are not deemed to be at high risk for SDB and where PSG initial installation costs might be prohibitive. Despite these limitations, diligent attempts were made to minimize these issues. We employed rigorous statistical controls to account for various factors, and utilized a validated and reliable tool to accurately measure symptoms of SDB within the orthodontic population.

The literature suggests that adults with sleep bruxism may experience a poor quality of sleep, potentially leading to TMDs.[41,42] Therefore, studies should be carried out to assess factors such as sleep bruxism, and their association with SDB and TMDs. Additional recommendations would be to have a larger sample size and to include other parameters that can predict SDB in a non-invasive and inexpensive way.

The discrepancies in health conditions observed between patients attending general pediatric clinics and orthodontic clinics suggest that orthodontists may come across a specific subset of individuals with a higher likelihood of SDB that could potentially go unnoticed in other healthcare settings. As a result, the proactive engagement of orthodontists in screening for SDB can play a vital role in identifying the progression of the disorder in a timely manner, enabling a comprehensive and collaborative approach to diagnosis and treatment for patients who might otherwise remain undiagnosed.

Conclusions

The prevalence of SDB was found to be 20% in the pediatric orthodontic population. The frequency of allergies was higher among patients in the high-risk SDB group.

Orthodontic practitioners are advised to incorporate routine SDB screening into their clinical practice, as there could be a specific subgroup of SDB patients that may go undetected in general pediatric clinics.

Ethics approval and consent to participate

The study was approved by the institutional ethical review committee (ERC) (No. 2022-7818-22971) at Aga Khan University Hospital, Karachi, Pakistan. The patients' parents/guardians signed an informed consent form before the commencement of the study.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Effects of rapid maxillary expansion on three-dimensional angular and linear parameters of the Eustachian and auditory tubes in adolescents: A cone-beam computed tomography study

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Abstract

Background. Rapid maxillary expansion (RME) is a common treatment for maxillofacial skeletal abnormalities. Numerous studies have explored the impact of RME on skeletal, dental and nasal structures.

Objectives. The aim of the study was to evaluate the effects of RME on the three-dimensional (3D) Eustachian tube (ET) and auditory tube morphologies of adolescents using cone-beam computed tomography (CBCT).

Material and methods. A total of 97 patients (59 females and 38 males; age: 13.27 ±2.10 years) with maxillary transversal deficiencies who had undergone orthodontic RME were enrolled in the study. The treatment concluded when a cusp-to-cusp relationship was achieved between the palatal cusp of the maxillary first molar and the buccal cusp of the mandibular first molar. Cone-beam computed tomography was performed before maxillary expansion (T0) and after expansion (T1). The measurement of 2 linear and 4 angular, 3D, right and left ET parameters, as well as the ET and auditory tube angles, was conducted. The paired *t*-test was used to compare the data.

Results. There was no significant difference between T0 and T1 in any angular or linear ET parameters or the left auditory tube angle ($p > 0.05$). The T0 right auditory tube angle was significantly greater than the T1 angle ($p < 0.05$). Rapid maxillary expansion did not modify the 3D ET parameters; only the right auditory tube angulation improved after expansion.

Conclusions. Compared to conventional radiography, CBCT provides better morphological, angular and linear data regarding the ET and auditory structures. No significant differences were observed in linear or angular ET parameters based on the status of maxillary expansion treatment. The right auditory tube angle differed significantly between T0 and T1.

Keywords: rapid maxillary expansion, cone-beam computed tomography, Eustachian tube, auditory tube

Highlights

- Rapid maxillary expansion (RME) resulted in no significant changes in three-dimensional linear or angular parameters of the Eustachian tube in adolescents.
- A significant improvement in the right auditory tube angle was observed after RME, with no corresponding change on the left side.
- Cone-beam computed tomography (CBCT) enables accurate 3D evaluation of the Eustachian tube and auditory structures compared to conventional imaging.
- RME may affect auditory tube angulation unilaterally, but its overall impact on Eustachian tube morphology is limited.
- Additional studies are needed to determine the clinical relevance of the observed unilateral auditory tube angle change.

Introduction

Rapid maxillary expansion (RME) is commonly used to treat skeletal transverse maxillary constriction, an upper-jaw skeletal discrepancy in the transverse plane associated with a posterior dentoalveolar crossbite, a narrow deep palate, nasal obstruction, and/or abnormal oral breathing. Numerous studies have explored the impact of RME on skeletal, dental and nasal structures.[1,2] The maxilla is linked to the cranial base and facial bones; therefore, RME may have an effect on these regions. Conductive hearing loss attributable to middle ear and Eustachian tube (ET) issues improved after RME.[3–6] The Eustachian tube is a rigid, epithelium-covered tube extending from the nasopharynx to the middle ear. The Eustachian tube, composed of bone and fibrocartilage, plays a crucial role in the proper functioning of the middle ear. The length and angle of the ET determine the nasopharyngeal content of the middle ear. Tubal disturbance has been observed to be more prevalent in individuals with very deep palatal vaults and malformations of the palate and nasopharynx.[7–9] Studies on cleft palate have emphasized the relationship between the upper palate and the ET. In such subjects, the etiology of ET malfunction is attributable to the abnormal insertion of the tensor veli palatini and levator veli palatini muscles.[7–12] Bluestone et al. discovered a relationship between poor ET function and the incidence of chronic otitis media in patients with cleft lip and palate who had or had not received surgery.[12] Maxillary and nasopharyngeal tissue expansion advances the pharyngeal ostium of the ET. Thus, RME may be useful in the treatment of hearing impairments associated with ET dysfunction.[13,14] The vast majority of angular and linear ET measurements have been derived with the aid of general computed tomography (CT). Cone-beam computed tomography (CBCT) is associated with a low radiation dosage, a short imaging period and better image quality. Additionally, CBCT produces multiplanar three-dimensional (3D) images and models, facilitating the analysis of the craniofacial skeleton.[15] To the best of our knowledge, no prior study has used CBCT to investigate the effect of RME on 3D ET morphology. In this study, we measured angular and linear ET parameters in adolescents.

Material and methods

The clinical research protocol was reviewed and approved by the Clinical Research Ethics Board of Eskisehir Osmangazi University, Turkey (approval No. 2018-58). Informed consent was obtained from all subjects and/or their legal guardian(s). All patients were referred to the Orthodontic Clinic at the Faculty of Dentistry of Eskisehir Osmangazi University for treatment of transverse maxillary constriction between 2016 and 2019. In this retrospective study, a total of 97 patients (59 females and 38 males) with a mean age of 13.27 ±2.10 years were enrolled. Patients with unilateral or bilateral crossbites, maxillary transversal deficiencies and deep palatal vaults were included in the study and received orthopedic therapy using a hybrid RME appliance. No patient exhibited any maxillo- or craniofacial malformations, or a cleft lip or palate. None of the patients had previously received any orthodontic or orthognathic surgery. A hybrid RME device was used to treat all patients. After the induction of local anesthesia, 2 mini screws (Standard 2.0 mm × 7 mm LOMAS mini screws; Mondeal Medical Systems GmbH, Mühlheim an der Donau, Germany) were inserted 2 mm medial to the suture sites in the anterior palatal region. Anterior maxillary expander screws (Palatal-S Split Screws; FORESTADENT, Pforzheim, Germany) were connected to the mini screws using acrylic plates. The posterior arms of the expanders were laser-welded to the first maxillary molars via molar bands. The posterior parts of the hybrid RME appliances were cemented to the first molars using multi-cure glass ionomer cement (3M Unitek, Monrovia, USA). The acrylic anterior part of each RME device was bonded to the palatal mini screws using a flowable composite. Parents were instructed to rotate the RME appliance by 25% twice daily until a cusp-to-cusp relationship was established between the palatal cusp of the maxillary first molar and the buccal cusp of the mandibular first molar. Then, the expansion screw was secured with a flowable composite that is capable of light curing.[16]

Ultra-low-dose CBCT was performed prior to maxillary expansion (T0) and after expansion (T1). Clinical changes in the anatomy and morphology of the maxilla and the ET bony structures were evaluated.

All 3D CBCT images were obtained with patients in a standing position using a CBCT device (ProMax® 3D Mid; Planmeca, Helsinki, Finland). The settings were as follows: 94 kVp; 14 mA; and 27 s. The images were consistent with the field of view (FOV) (20.0 cm × 20.0 cm × 10.2 cm) and voxel size (0.600 mm). The assessment of all CBCT images was conducted in 3D by a single clinician. The SimPlant® O&O image software (Materialise Dental N.V., Leuven, Belgium) was used for 3D image evaluation. The 3D morphological linear and angular parameters are listed in Table 1. The 3D morphological ET traces are displayed in Fig. 1–3.

Table 1. Three-dimensional (3D) morphological linear and angular Eustachian tube (ET) parameters

Location	Description
ET pharyngeal orifice (right–left)	The point nearest the pharynx at which a loop-shaped ET lumen was apparent in the axial slice.
ET tympanic orifice (right–left)	The point situated just before the external auditory canal was manifested in the axial slice.
ET length (right–left)	The distance from the pharyngeal to the tympanic orifice was revealed by 3D CBCT.
ET angle (right–left)	The pharyngeal and tympanic orifices of the ET were apparent in the same slices. The measurement direction was aligned with the line of the ET. The ET angle was defined as the angle between a straight line along the ET and the Frankfort horizontal plane.
Auditory tube angle (right–left)	The angle between a line running through the tympanic orifice of the ET and the center of the longitudinal axis of the center of the bony external ear canal was evident on axial slices.

CBCT – cone-beam computed tomography.

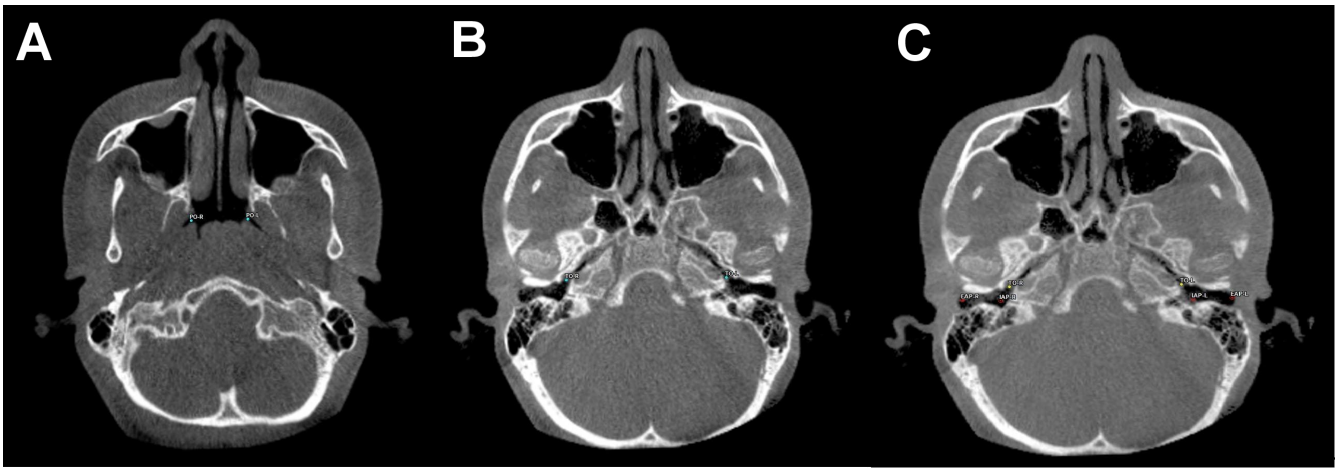

Fig. 1. Axial cone-beam computed tomography (CBCT) slices
A. Pharyngeal orifice (PO); B. Tympanic orifice (TO); C. Internal auditory point (IAP) and external auditory point (EAP).

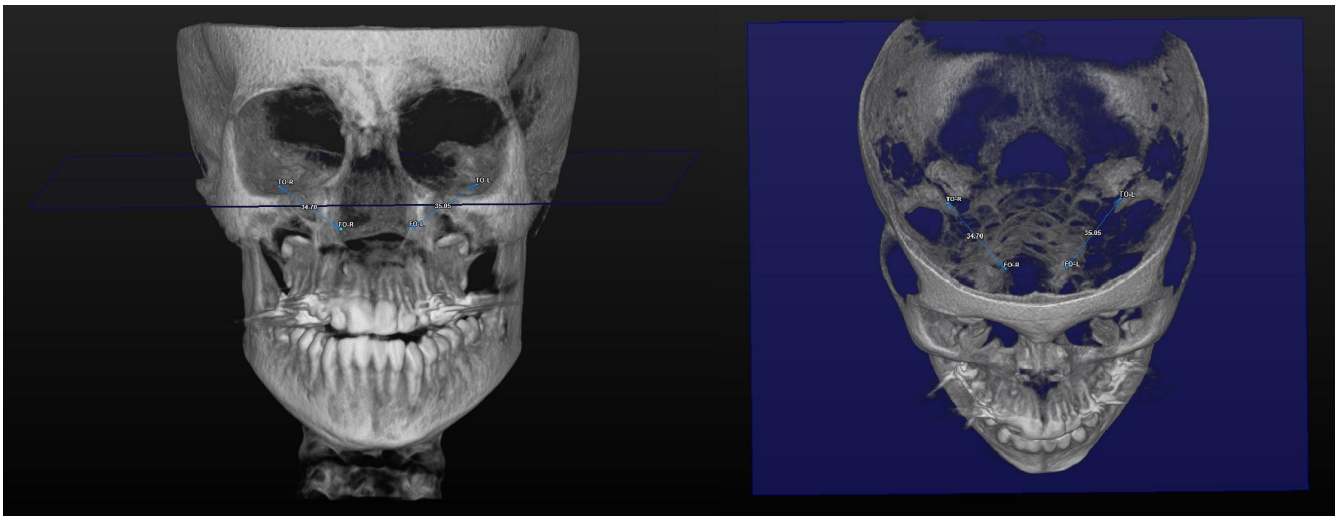

Fig. 2. CBCT measurement of the Eustachian tube (ET) length in three-dimensional (3D) space from different views

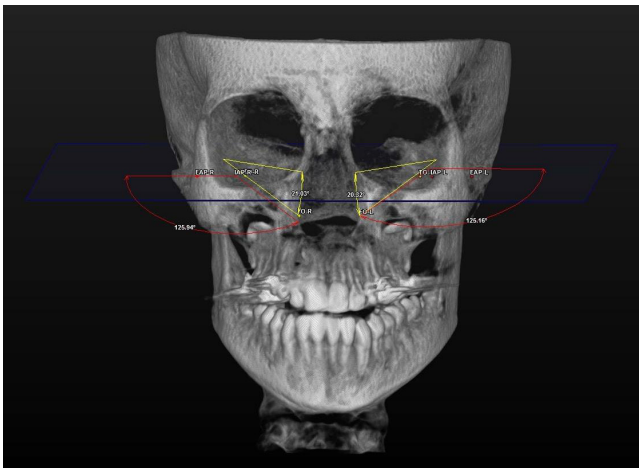

Fig. 3. CBCT measurement of the ET angle and the auditory tube angle in 3D space

Statistical analysis

The descriptive statistics were compared using Microsoft Excel (Microsoft Corp., Redmond, USA) (Table 2). The statistical analyses were conducted using MedCalc software for Windows, v. 17.5 (MedCalc Software Ltd, Ostend, Belgium). The Kolmogorov–Smirnov test indicated that the data was non-normally distributed. The paired samples *t*-test was used to evaluate the statistical significance of the observed differences between the T0 and T1 measurements. The significance level was set at $p < 0.05$.

In order to ensure the reliability of the methodologies, each measurement was collected twice, separated by a designated time period. The intraclass correlation coefficient (ICC) was used to determine the accuracy of the radiographic measurements in terms of intraobserver reliability. The mean ICC was 0.91, indicating that the radiographic measurements were accurate ($p > 0.05$).

Table 2. Demographic characteristics of the study subjects

Subjects, *n*	Age [years] *M* ±*SD*	Sex (male/female)
97	13.27 ±2.10	38/59

M – mean; *SD* – standard deviation.

Results

The patients included in the study were referred to the orthodontic clinic for treatment of transverse maxillary constriction. The subjects included individuals with unilateral or bilateral crossbites, maxillary transversal deficiencies, and deep palatal vaults. These patients received orthopedic therapy using a hybrid RME appliance.

The comparison of the 3D linear and angular data is presented in Table 3. The mean right ET lengths were 36.26 ±3.10 mm at T0 and 36.13 ±3.08 mm at T1. Before and after RME, the mean left ET lengths were 36.82 ±2.98 mm and 36.82 ±3.12 mm, respectively. The right and left ET lengths did not differ significantly between T0 and T1 ($p = 0.513$ and $p = 0.978$, respectively). The mean right ET angle was 22.23 ±3.72° before RME and 22.82 ±4.06° after RME. At T0, the mean left ET angle was 22.33 ±4.22°. At T1, the mean left ET angle was 22.74 ±3.84°, indicating a non-significant difference ($p = 0.077$ and $p = 0.184$, respectively). The right auditory tube angle at T0 exhibited a significant increase compared to that at T1 ($p = 0.000$). The mean left auditory tube angles were 127.87 ±3.28° at T0 and 127.53 ±3.45° at T1 ($p = 0.197$).

Table 3. Three-dimensional (3D) morphological linear and angular data before and after maxillary expansion

Variable	T0 *M* ±*SD*	T1 *M* ±*SD*	*p*-value
Right ET length [mm]	36.26 ±3.10	36.13 ±3.08	0.513
Left ET length [mm]	36.82 ±2.98	36.82 ±3.12	0.978
Right ET angle [°]	22.23 ±3.72	22.82 ±4.06	0.077
Left ET angle [°]	22.33 ±4.22	22.74 ±3.84	0.184
Right auditory tube angle [°]	128.82 ±3.42	128.01 ±3.31	0.000*
Left auditory tube angle [°]	127.87 ±3.28	127.53 ±3.45	0.197

* statistically significant ($p < 0.05$, paired samples *t*-test); T0 – before expansion; T1 – after expansion.

Discussion

In the present study, no statistically significant difference was identified between T0 and T1 in left or right angular or linear ET parameters and the left auditory tube angle. The T0 right auditory tube angle was significantly greater than the T1 angle. Rapid maxillary expansion did not modify the 3D ET parameters; only the right auditory tube angulation improved after expansion. The dentoskeletal indications for RME include a bilateral or unilateral crossbite, a cleft palate, and a maxillary deficiency. Maxillary expansion has been demonstrated to enhance respiration and the use of the nasal passage. Nasal structure enlargement reduces dehydration of the pharyngeal surface, infections of the upper respiratory region, and otitis media.[6] A meta-analysis revealed that an increase of 1,218.3 mm^3 in the volumetric upper airway is associated with RME.[17] Cheung et al. evaluated the effects of upper airway volume produced by Hyrax, Hybrid-Hyrax and Keles keyless expanders.[18] Maxillary

expansion caused enhancement in total airway volume in the Hybrid-Hyrax group (+5,902.1 mm^3) and a smaller rise in the Hyrax group (+2,537.9 mm^3) or the Keles group (+3,001.4 mm^3). Small increases in total upper airway volume were observed after RME.[18] Echarri-Nicolás et al. compared upper airway modifications in cases undergoing treatment with different methods of microimplant-assisted expansion.[19] A significant increase in total area and minimal section at the level of nasopharynx and oropharynx was observed in cases receiving bone-anchored maxillary expansion. An umbrella review has presented important increases in the nasal and oropharyngeal space volumes of growing children and adolescents after RME at 3-, 6- and 12-month follow-ups.[20] Shetty et al. reported notable differences in the soft tissue parameter after mini-implant assisted rapid palatal expansion.[21] Aljawad et al. emphasized that RME causes an increase in upper airway volume.[22]

A study by Braun demonstrated that a narrow maxilla induced oral breathing and impaired nasal respiration, thus affecting the ET and middle ear.[23] Several studies have revealed that maxillary expansion can modify the anatomical structures of the pharynx and palate, thereby allowing the ostium tuba auditiva to function more efficiently by equalizing the pressures on both regions of the tympanic membrane.[4] The tensor veli palatini muscle extends from the entrance of the ET to the soft palate. After RME, the muscle may enlarge to expand the ET orifice.[24] To the best of our knowledge, this is the first study to use ultra-low-dose CBCT to examine the effects of RME on the detailed angular and linear parameters of the right and left ETs.

Dinç et al. compared the ET angle and length in healthy subjects and those with otitis media. The ET angles in healthy individuals were 23.0° in females and 24.3° in males.[25] The Frankfort horizontal plane was used when measuring ET angles. The angles in our study were slightly lower. In other studies, the mean ET angles with the horizontal palatal plane averaged 34–36° in adults.[26–28] In adolescents, the ET angle with the Frankfort horizontal plane was approx. 22°. The difference may reflect age-related changes and the use of reference planes. The ET angle remained constant after RME on both sides. No prior study has measured the effect of RME on 3D ET angles. Therefore, there was no basis for comparison. We expected that any changes would be similar; symmetrical RME was applied to the mid-palatal suture. No differences in right and left ET length were observed between T0 and T1 (right: 36.26 ±3.10 mm at T0 and 36.13 ±3.08 mm at T1, left: 36.82 ±2.98 mm at T0 and 36.82 ±3.12 mm at T1; p = 0.513 and p = 0.978, respectively). Using two-dimensional images, Bluestone et al. found that the ET length increased as children grew, attaining a final size of 31–38 mm at approx. 7 years of age.[12] Takasaki et al. used conventional CT to compare the ET length between children and adults; the ET length in children was 38 mm and 43 mm in adults.[28] The CBCT data obtained in the current study was slightly different, presumably due to the imaging modalities. Ha et al. noted that the ET lengths revealed by conventional CT and CBCT differed due to subjects' positioning, whether in a supine or seated posture.[29] Ultra-low-dose CBCT has been demonstrated to optimally detect morphological structures and is associated with reduced radiation, sharper imaging and briefer viewing than CT. Many studies have shown that RME consistently reduces conductive hearing loss and improves ET function in adolescents with maxillary transversal constriction.[30] However, the present study found no significant effect of maxillary expansion on the ET length. There are 2 possible explanations for this state of affairs. Other studies have collected audiometric or tympanometric data; however, these studies did not include 3D CBCT linear and angular measurements. Secondly, RME inhibits the infection of the nasal and pharyngeal airways, which may contribute to a reduction in the incidence of otitis. Maxillary expansion improves conductive hearing loss and reduces the incidence of otitis media.

Duman et al. used an identical CBCT protocol to determine the auditory angle, which was significantly larger in patients with cleft lip and palate than in normal individuals; such patients often exhibit a severe maxillary transversal deficiency.[31] We also found that the right auditory tube angle was larger before RME in patients with maxillary constrictions. The effects of RME on Eustachian and auditory CBCT data require further research. Longitudinal studies would be instrumental in defining the effect of RME in patients without adenoidal or other nasopharyngeal disorders. The retrospective nature of the study constitutes a limitation, as it relied on a limited set of patient data. Additionally, the absence of auditory tests comparing measurements to patient data further restricts the study's conclusions.

Conclusions

Cone-beam computed tomography can yield morphological, angular and linear data on the ET and auditory structures. No significant differences were observed in linear or angular ET parameters based on maxillary expansion treatment status. The right auditory tube angle differed significantly between T0 and T1. Future studies should be conducted on larger populations and designed to compare measurements.

Ethics approval and consent to participate

The clinical research protocol was reviewed and approved by the Clinical Research Ethics Board of Eskisehir Osmangazi University, Turkey (approval No. 2018-58). Informed consent was obtained from all subjects and/or their legal guardian(s).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Leptin, fibronectin, MMP-1, and MMP-2 concentration in saliva during orthodontic treatment

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Abstract

Background. The composition of saliva serves as an indicator of the state of the oral cavity.

Objectives. The aim of the study was to examine the salivary levels of leptin, fibronectin (FN), matrix metalloproteinase (MMP)-1, and MMP-2 during orthodontic treatment.

Material and methods. A total of 50 patients scheduled for orthodontic treatment and 50 healthy volunteers were included in the study. The orthodontic patients underwent the placement of standard brackets (MINI MASTER® fixed braces; American Orthodontics) and self-ligating brackets (H4, 0.022″ × 0.028″; Orthoclassic). Non-stimulated saliva samples were collected before orthodontic treatment, 24 min after the procedure, and 1 month after the procedure. The levels of MMP-1, MMP-2, FN, and leptin in the saliva of patients were measured before and after orthodontic treatment.

Results. The levels of salivary leptin were significantly elevated before orthodontic treatment ($p < 0.001$), and a subsequent rise was also observed during orthodontic tooth movement. Salivary FN levels were significantly increased before orthodontic treatment ($p < 0.001$), followed by a decrease during orthodontic tooth movement, remaining outside the normal range. The concentration of MMP-1 in saliva was significantly higher before orthodontic treatment ($p < 0.001$) and decreased during orthodontic tooth movement, though this change was not statistically significant. Similarly, the concentration of MMP-2 in saliva was significantly higher before orthodontic treatment, but its levels were even higher during orthodontic tooth movement. However, this increase was not statistically significant.

Conclusions. The levels of leptin in saliva were elevated during orthodontic treatment. Leptin stimulates wound healing and angiogenesis in the oral cavity. In addition, it serves as a mediator in the orthodontic movement of teeth.

Keywords: leptin, orthodontic treatment, MMP-1, fibronectin, MMP-2

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Highlights

- Salivary leptin levels increased during orthodontic treatment.
- Leptin promotes wound healing and angiogenesis in the oral cavity.
- Leptin serves as a mediator in orthodontic tooth movement.

Introduction

The oral cavity is a site of continuous reactions between food, gland secretions and bacteria. Saliva is the most important fluid in this environment. Its main constituents are water (99.5%), as well as organic (0.3%) and non-organic factors (0.2%).[1] The composition of saliva reflects the state of the oral cavity and the entire organism.[2–6] Orthodontic treatment alters the oral environment, stimulates the flow rate of saliva, its buffer capacity, and pH level.

During orthodontic treatment, continuous pressure is applied on the tooth, leading to alveolar bone remodeling. The tooth undergoes movement within the interior of the bone, thereby causing the attached periodontal system and alveolar processes to be dragged along. The most widely accepted theory for orthodontic tooth movement is the pressure-tension theory. It is characterized by blood flow changes caused by constant pressure, which in turn serve as indicators of tooth movement. Firstly, it creates periodontal ligament (PDL) pressure, and secondly, it induces PDL extension. On the pressure side, the blood flow ceases and it is possible to notice chemical changes in gingiva liquid. Regarding the tension aspect, an increase in blood flow and oxygen level is observed. The process of bone remodeling is initiated by chemical changes that prompt the release and differentiation of specific bone remodeling cells. The application of force initiates the previously described mechanisms within few minutes.

Tooth movements can be categorized into 3 phases, as follows: alterations in blood flow and PDL tension; generation or release of chemical mediators; and cell activation, which is responsible for bone remodeling and is a result of tooth movement. The movement of teeth triggers an acute inflammatory reaction in periodontal tissues and stimulates cells of PDL to produce enzymes and cytokines necessary in the remodeling of connective tissue.[1] The remodeling of connective tissue is achieved by a complex process, in which matrix metalloproteinases (MMPs) play a major role.

Matrix metalloproteinases are enzymes that degrade the extracellular matrix (ECM) and components of the basement membrane. They are synthetized by connective tissue cells, including osteoblasts, odontoblasts and fibroblasts.[7–12] Matrix metalloproteinases circulate in gingival crevicular fluid (GCF) and saliva, and they can be found in the dentin of carious teeth and plaque.[9–12]

Several types of MMPs were identified in saliva. The levels of MMP-1 and MMP-2 were measured. Matrix metalloproteinase-1 is responsible for the breakdown of the ECM, including collagen types I, II and III, while MMP-2 is a collagenase that destroys collagen type IV and regulates inflammatory and vascular processes.[9–14]

In vivo, mechanical forces trigger the production of MMP-1 in the cells of the periodontal ligament.[11] Matrix metalloproteinase-1 is able to break the molecules of interstitial collagen, initiating cell remodeling.[11] Moreover, animal studies have demonstrated that the application of inhibitors of MMPs can delay the orthodontic tooth movement.[15]

The activity of MMPs is balanced by the catalysis of their proenzymes and the production of tissue inhibitors of metalloproteinases (TIMPs).[13–15] These inhibitors are produced by B-cells and are found in the composition of GCF.[13–15] The α2-macroglobulin, a non-specific inhibitor of MMP, is also found in GCF.[9–13] The balance between MMPs and TIMPs determines the extent of tissue destruction.[13–15]

Matrix metalloproteinases influence the resolution of acute inflammation. In the absence of MMPs, a progression to chronic inflammation occurs, resulting in tissue damage. One of the roles of MMP-2 in inflammatory processes is massive leukocyte infiltration and heightened levels of pro-inflammatory cytokines observed in MMP-2-devoid animals.[7–10]

Additionally, polypeptides, such as leptin and fibronectin (FN), take part in the remodeling of connective tissue that occurs in response to tooth movement.

Leptin is a polipeptide produced by adipocytes; however, it is also present in osteoblasts and muscles, the stomach, the brain, the salivary glands, and the placenta.[16–20] The polypeptide regulates energetic balance through its influence on the hypothalamus. When the leptin level is low, there is an increase in glucocorticoid production, which in turn stimulates agouti-related protein (AgRP) neurons and boosts appetite. Leptin has an influence on metabolism and weight, thermogenesis, hematopoiesis and angiogenesis, mineralization of bones, and wound healing.[16] Leptin was identified in both GCF and tooth pulp.[21–23] Furthermore, an interrelation was observed

between plasma or salivary leptin concentration and body mass index (BMI).[16] Obese individuals exhibit an elevated concentration of leptin in the serum, which has been demonstrated to influence bone remodeling.[24,25] According to the latest surveys, leptin concentration in GCF decreases during orthodontic tooth movement.[26,27]

Fibronectin is a glycoprotein that exists in 2 forms, a soluble form found in body fluids and an insoluble form found in cells. In cells, FN moderates cell–matrix attachments and facilitates cell migration and the differentiation of organs. Fibronectin is produced by osteoblasts and odontoblasts. It is involved in the interaction between the implant and the bio-matrix.[28–30]

The aim of the present study was to examine the levels of MMP-1, MMP-2, FN, and leptin in the saliva of patients before and after orthodontic treatment.

Material and methods

Patients

A total of 50 patients scheduled for orthodontic treatment to improve their dentofacial appearance and 50 healthy volunteers were included in the study. The orthodontic patients underwent the placement of standard brackets (MINI MASTER® fixed braces; American Orthodontics, Sheboygan, USA) and self-ligating brackets (H4, 0.022" × 0.028"; Orthoclassic, McMinnville, USA). The nickel titanium wires were used. There were no statistical discrepancies regarding the crowding levels, which were classified as mild to moderate. No extractions were scheduled before orthodontic treatment. The estimated duration of treatment was 2.5–3 years. More precisely, approx. 20 visits were planned for standard brackets, while 30 visits were planned for self-ligating brackets. The inclusion criteria were good oral hygiene and good health. Subjects taking long-term medications and smokers were excluded from the study. The eligibility criteria were assessed during interviews with the subjects. Written informed consent was obtained from all participants. The study received the approval of the Ethics Committee of Medical University of Bialystok, Poland (approval No. R-I-002/41/2019).

The study group (31 females and 19 males) had a mean age of 31.4 ±1.9 years, while the control group (36 females and 14 males) had a mean age of 21.3 ±1.8 years. In the latter, dental examinations were performed before the collection of saliva.

Methods

Patients referred for orthodontic treatment were scheduled for scaling and sandblasting of teeth (removal of plaque and tartar above and below the gumline) 1 day before treatment. The subjects were instructed to abstain from eating and were allowed only to drink water for 2 h before the extraction of saliva. Patients were seated and whole saliva samples were collected in plastic tubes by the passive spitting method in order to obtain approx. 1–2 mL of sample. Non-stimulated saliva samples were collected prior to orthodontic treatment, 24 min after the procedure, and 1 month after the procedure. The collection periods were selected on the premise that periodontium metabolism and factor secretion occur immediately after orthodontic force application.

The samples were centrifuged at 10,000 g for 5 min, and the supernatants were frozen and stored at –80°C. One researcher performed interviews, examinations of the oral cavity and saliva collection. All methods were performed in accordance with the relevant guidelines and regulations.

For the measurement of MMP-1, MMP-2 and FN levels, a highly selective surface plasmon resonance imaging (SPRI) biosensor was used, as previously described in other studies.[7,8,31] The SPRI biosensor measures the changes of the refractive index caused by molecules bound to the metal surface. Surface plasmon resonance (SPR) is generated at a thin metal surface when an analyte binds the ligand on the metal film, thereby causing a change in the interfacial architecture. The SPRI signal is dependent on the change in the wavelength and the angle of light polarization.

The main component of the biosensor for MMP-1 detection is an immobilized rabbit anti-human MMP-1 antibody, which binds the enzyme present in the sample. The analytical response signal of the biosensor is in the range of 0.05–20.00 ng/mL^{-1}. The detection limit is 9 ng/mL^{-1}, and the limit of quantification is 18 ng/mL^{-1}.[7,8] For MMP-2 measurements, MMP-2-specific inhibitor ARP 101 was used as the receptor to bind the enzyme from the sample.[31] The biosensor for FN used the specific reaction of rabbit anti-FN antibody. To evaluate the SPRI method, the levels of MMP-1, MMP-2 and FN were determined in the biological samples using enzyme-linked immunosorbent assay (ELISA). There was a strong correlation between the 2 methods. For example, for MMP-2, the correlation coefficient for healthy donors was 0.996, and for patients, it was 0.984.[7,8,31]

The quantitative measurement of leptin in saliva was performed using a leptin enzyme immunoassay or an ELISA kit.[2]

Statistical analysis

The normality of distribution was tested using the Shapiro–Wilk test. Non-Gaussian data was presented as a median (minimum–maximum) and analyzed using the non-parametric Kruskal–Wallis test. The differences were deemed statistically significant when $p < 0.05$. The calculations were performed using the GraphPad 7.04 Prism software (GraphPad Software, Boston, USA).

Results

Leptin

In the study group, salivary leptin levels were significantly higher before orthodontic treatment in comparison to controls ($p < 0.001$). Additionally, a notable increase in salivary leptin levels was noted during orthodontic tooth movement (Table 1, Fig. 1). There was no correlation between salivary leptin levels and BMI between the study group and the control group.

Fibronectin

Salivary FN levels were significantly elevated in the study group before orthodontic treatment when compared to the control group ($p < 0.001$). They decreased during orthodontic tooth movement, without reaching the normal range (Table 2, Fig. 2).

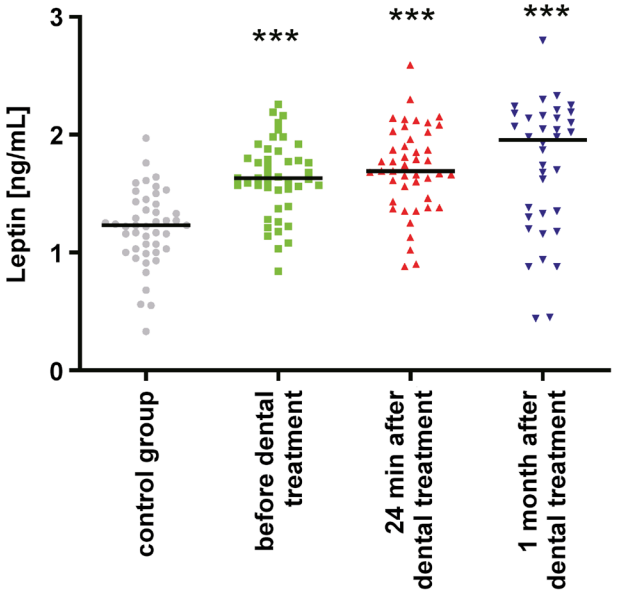

Fig. 1. Statistical parameters of leptin concentration in saliva before and after orthodontic treatment

*** statistically significant in comparison to controls ($p < 0.001$). Horizontal lines on the graph represent the mean values.

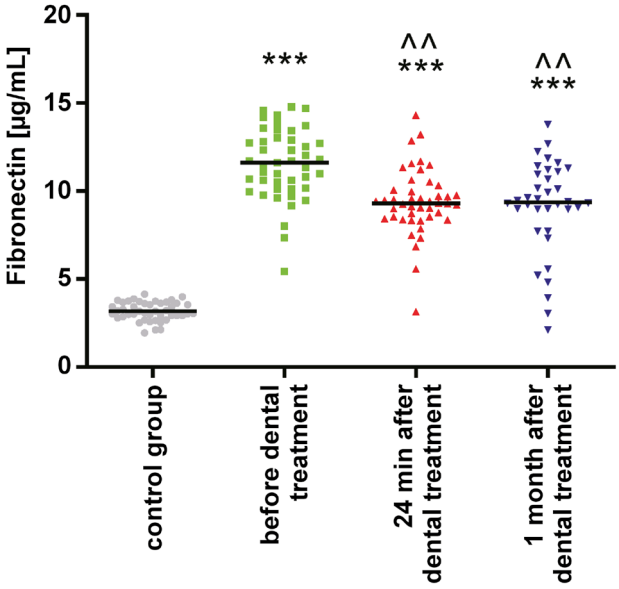

Fig. 2. Statistical parameters of fibronectin (FN) concentration in saliva before and after orthodontic treatment

*** statistically significant in comparison to controls ($p < 0.001$); ^^ statistically significant compared to the value before dental treatment ($p < 0.01$). Horizontal lines on the graph represent the mean values.

Table 1. Statistical parameters of leptin concentration in saliva before and after orthodontic treatment

Variable	Leptin [ng/mL]			
	control group	before dental treatment	24 min after dental treatment	1 month after dental treatment
Me	1.23	1.63*	1.69*	1.96*
Minimum	0.33	0.84	0.88	0.44
Maximum	1.97	2.26	2.59	2.80
Percentiles (25–75%)	1.00–31.40%	1.46–1.80%	1.44–1.99%	1.30–2.15%

* statistically significant in comparison to controls ($p < 0.001$, Kruskal–Wallis test); *Me* – median.

Table 2. Statistical parameters of fibronectin (FN) concentration in saliva before and after orthodontic treatment

Variable	Fibronectin [ng/mL]			
	control group	before dental treatment	24 min after dental treatment	1 month after dental treatment
Me	3.17	11.60*	9.30*#	9.35*#
Minimum	1.94	5.42	3.14	2.11
Maximum	4.14	14.77	14.31	13.78
Percentiles (25–75%)	2.89–3.62%	10.15–12.96%	8.49–10.18%	8.04–11.07%

* statistically significant in comparison to controls ($p < 0.001$, Kruskal–Wallis test); # statistically significant compared to the value before dental treatment ($p < 0.01$, Kruskal–Wallis test).

MMP-1

The concentration of MMP-1 in saliva was significantly higher in the study group before orthodontic treatment when compared to controls ($p < 0.001$). A decrease in the concentration was observed during orthodontic tooth movement; however, it was not statistically significant (Table 3, Fig. 3).

MMP-2

In the study group, the concentration of MMP-2 in saliva was significantly higher before orthodontic treatment as compared to the control group ($p < 0.001$). Its levels further increased during orthodontic tooth movement, but the rise was not statistically significant (Table 3, Fig. 3).

Discussion

The periodontal ligament is a membrane-like connective tissue that surrounds the root of a tooth. It is located between the hard tissues of the alveolar bone and the cementum of teeth, thereby anchoring the tooth to the alveolus.[9–12] During orthodontic treatment, forces compress the PDL fibres, leading to a reduction in the PDL space, which triggers local damage, repair and inflammation processes involving vascular activity, and the involvement of leukocytes and macrophages.[9–12]

In the present study, a significant rise in the levels of leptin, FN, MMP-1, and MMP-2 was observed during orthodontic tooth movement. Cantarella et al. observed that the forces exerted during orthodontic treatment stimulate the production of MMP-1 and MMP-2.[13] During the first hour of treatment, the levels of MMP-1 rise,

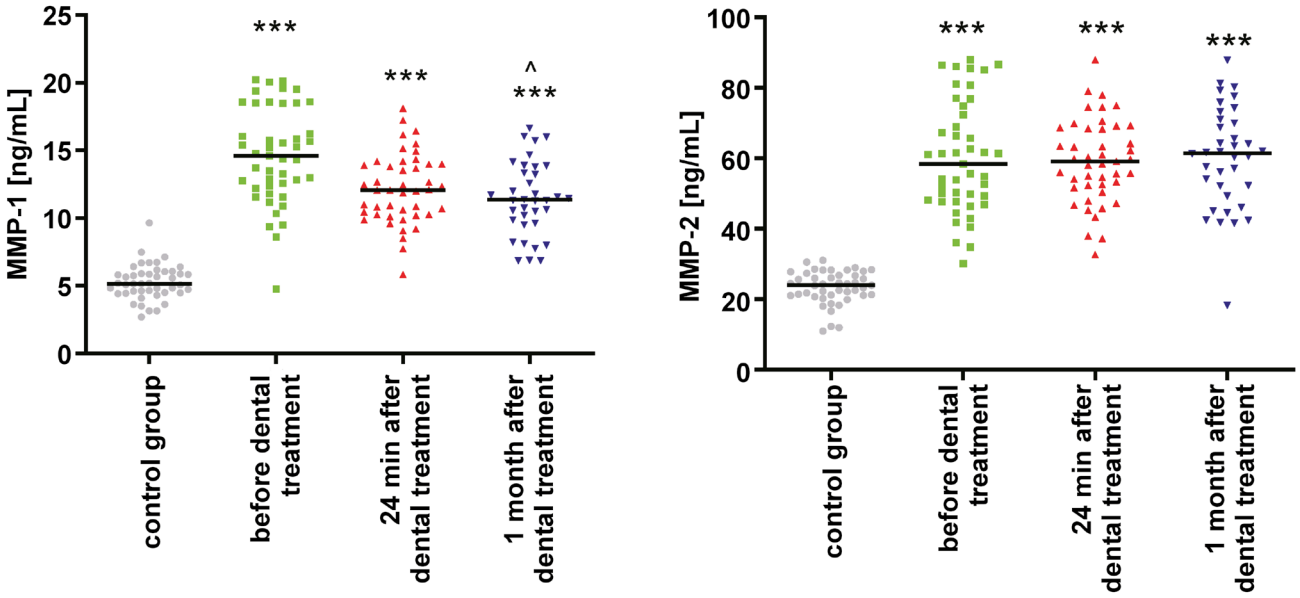

Fig. 3. Statistical parameters of matrix metalloproteinase (MMP)-1 and MMP-2 concentrations in saliva before and after orthodontic treatment

*** statistically significant in comparison to controls ($p < 0.001$); ^ statistically significant compared to the value before dental treatment ($p < 0.05$). Horizontal lines on the graph represent the mean values.

Table 3. Statistical parameters of matrix metalloproteinase (MMP)-1 and MMP-2 concentrations in saliva before and after orthodontic treatment

Variable		Control group	Before dental treatment	24 min after dental treatment	1 month after dental treatment
MMP-1 [ng/mL]	*Me*	5.16	14.62*	12.07*	11.36*#
	minimum	2.69	4.77	5.85	6.86
	maximum	9.66	20.24	18.11	16.65
	percentiles (25–75%)	4.49–5.90%	12.29–18.50%	10.26–13.00%	9.69–13.60%
MMP-2 [ng/mL]	*Me*	23.93	58.41*	59.09*	61.50*
	minimum	10.94	30.12	32.64	18.32
	maximum	31.06	87.94	87.92	87.87
	percentiles (25–75%)	21.09–27.10%	47.92–73.60%	51.97–68.50%	50.03–70.70%

* statistically significant in comparison to controls ($p < 0.001$, Kruskal–Wallis test); # statistically significant compared to the value before dental treatment ($p < 0.05$, Kruskal–Wallis test).

returning to its normal range within the next 3 h. With regard to MMP-2, its concentration remains higher for 8 h in the compression areas; however, in the tension area during the same period, it returns to the normal range.[13] Redlich et al. demonstrated higher MMP-1 activity in the compression side in the animal model.[14]

After the application of orthodontic forces, an increase in the concentration of pro-inflammatory cytokines is observed within the first 24 h.[1] Subsequently, a new biological equilibrium is established.[1,9,10] Similar observations were made in the current study. A paucity of human studies has focused on MMPs during orthodontic stress.[9–13] Garlet et al. found higher MMP-1 mRNA at the resorption and apposition sides compared with the controls.[32] This corresponds with the findings of the present study. As Takahashi et al. have stated: „At the resorption side, bone resorption takes place and extensive re-modelling of the PDL. At the apposition side, only bone apposition takes place and more limited PDL re-modelling".[33] In animal models, the MMP inhibitors delay orthodontic tooth movement.[15] Most probably, MMP-2 controls inflammatory signaling, influencing the resolution of acute inflammation. In the absence of MMPs, a progression to chronic inflammation occurs, resulting in tissue damage.

In the context of periodontal health, elevated levels of leptin have been observed in GCF. This observation suggests a potential role for leptin as a protective factor, potentially stimulating the immune system, impacting the osteoblasts and enhancing bone formation.[17–19] Although other researchers have noted a decrease in leptin concentration after orthodontic force application on teeth, our observations indicate an increase in salivary leptin levels along with tooth movement. Dilsiz et al., who evaluated the levels of leptin in GCF before and after the application of orthodontic force, also noted a decrease in GCF leptin levels, which contradicts the results of this study.[27]

In overweight and obese subjects, an elevated leptin concentration has been observed in the serum, which exerts an influence on bone metabolism.[34] Jayachandran et al. observed that the duration of orthodontic treatment was greater in obese individuals.[16] These subjects exhibited higher leptin levels in the serum and high mineral density of bones.[16] In the present study, no statistically significant correlation was identified between BMI and leptin levels in the control group and in the study group before orthodontic intervention.

Another pattern of leptin levels was observed by Srinivasan et al.[35] The researchers identified a biphasic change in leptin concentrations, with an apex 24 h after the application of orthodontic force, and the lowest level after 1 week.[35]

Most probably, leptin modulates processes of inflammation.[36–40] Bozkurt et al. observed lower leptin levels in GCF in smokers.[41] They showed that smoking damages the mechanism regulating leptin levels.[41]

Collagen and FN play a crucial role in tissue remodeling after orthodontic tooth movement.[28–30] Fibronectin is involved in the migration and attachment of the junctional epithelium and wound healing. It also promotes the growth of periodontal ligament cells and protects gingival tissues.[28–30] According to Huynh et al., "particles of FN found in GCF are the result of cleavage of FN by proteases such as MMPs during inflammation, wound healing and infections", so fragments of FN "may be used as an indicator of oral cavity status".[42] Kapila et al. proved that "FN and specific FN fragments induce the expression of proteinases in periodontal ligament cells, causing tissue degradation during periodontal disease".[43] Higher levels of FN were detected during acute infections and chronic diseases, e.g., liver cirrhosis or hepatic carcinoma.[28–30] Fibronectin, a non-specific salivary defense factor, has been shown to bind to bacteria and play a role in the formation of plaque.[28–30] Salivary levels of FN in subjects with oral lichen planus are lower than in the general population.[44] In the present study, we observed significantly higher salivary levels of FN before orthodontic treatment, likely attributable to an additional intervention performed 1 day earlier (scaling and sandblasting of teeth).[7,8] Levels of FN decreased during orthodontic tooth movement, both 24 min and 1 month after the application of braces.

The oral mucosa is particularly susceptible to mechanical injuries, e.g, during chewing and biting. In certain cases, ulceration may occur. Umeki et al. studied the role of leptin in the healing of the oral mucosa.[45] The healing of the ulcer was expedited in the group that received leptin in comparison to the control group.[45] Leptin stimulates angiogenesis and epithelial cells.[46–49] We speculate that the elevated salivary levels of leptin, FN, MMP-1, and MMP-2 in our patients before orthodontic treatment were caused by an additional intervention performed 1 day before, which involved scaling and sandblasting of teeth.

A notable limitation of this study is the fact that the force levels released by nickel titanium wires vary from subject to subject, even if the wires are from the same manufacturer and have equal diameters. Furthermore, the levels of orthodontic forces exerted by nickel titanium wires do not remain stable in the oral environment.[50] However, we believe that these factors do not diminish the value of our study.

Conclusions

Our study revealed an increase in the levels of leptin in saliva during orthodontic treatment. Leptin stimulates wound healing and angiogenesis in the oral cavity. It also serves as a mediator of orthodontic movement of teeth. Additionally, we observed significantly higher salivary levels of leptin, FN, MMP-1, and MMP-2 before orthodontic treatment, likely attributable to scaling and sandblasting of teeth performed before orthodontic treatment.

The concentration of FN and MMP-1 decreased during orthodontic tooth movement, 24 min and 1 month after the application of braces. In contrast, MMP-2 levels increased during orthodontic tooth movement; however, these changes were not statistically significant.

Ethics approval and consent to participate

Written informed consent was obtained from all participants. The study received the approval of the Ethics Committee of Medical University of Bialystok, Poland (approval No. R-I-002/41/2019).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Comparative evaluation of immediate implant placement and provisionalization (IIPP) with and without a concentrated growth factor-enriched bone graft: A randomized controlled trial

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Abstract

Background. The immediate placement of the implant into a fresh extraction socket site with immediate provisionalisation is considered to be a predictable and acceptable procedure. However, there are mixed results regarding the advantages of the immediate provisionalization of dental implants, using a biomaterial in the jump space (JS).

Objectives. This randomized controlled trial (RCT) aimed to evaluate the use of a concentrated growth factor (CGF)-enriched bone graft in the JS of immediate implant placement with provisionalization (IIPP) in the maxillary esthetic zone.

Material and methods. Forty immediate dental implants were placed with and without a CGF-enriched bone graft in the test and control groups, respectively, along with provisional restorations. The clinical evaluation of the modified plaque index (mPI), the modified sulcular bleeding index (mSBI), the probing depth (PD), the gingival thickness (GT), and the Testori implant esthetic score (TS) was done at baseline, and at 6 and 12 months postoperatively. The assessment of the crestal bone height (CBH), the buccal bone thickness (BBT), the ridge width (RW), the vertical distance (VD), JS, and the radiolucent area (RA) was carried out using cone-beam computed tomography (CBCT) at baseline and 12 months postoperatively. The visual analog scale (VAS) was used to assess pain and patient satisfaction.

Results. Highly significant differences were observed with regard to the change in RW at 4 mm from the crest (9.80 ±0.89 mm), VD-distal (1.35 ±0.43 mm), JS-mesial (0.38 ±0.34 mm), JS-distal (0.25 ±0.34 mm), JS-buccal (0.42 ±0.39 mm), RA-mesial (0.63 ±0.48 mm^2), and RA-buccal (0.19 ±0.47 mm^2) in the test group as compared to the control group at 12 months. The intergroup comparison for TS showed a statistically significant difference ($p < 0.05$).

Conclusions. It is recommended to use CGF-enriched bone grafting in JS, along with provisionalization, in the anterior esthetic zone.

Keywords: dental implants, allografts, cone-beam computed tomography, randomized controlled trial

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Highlights

- The study focused on immediate implant placement in the maxillary premolar-to-premolar region, combined with same-day provisionalization.
- In the test group, a concentrated growth factor (CGF)-enriched bone graft was used to fill the jump space.
- The gingival thickness slightly decreased over a 12-month period in both the test and control groups.
- The Testori score, an esthetic parameter, showed significant improvement in the test group.
- The results suggest that the use of CGF may lead to better soft tissue outcomes.

Introduction

One of the most effective treatment options for a failing tooth is its replacement with an implant. The long-term goal has changed from implant survival to better maintenance of both the soft and hard tissues. The practice of placing implants immediately after extraction in conjunction with prompt provisionalization is increasingly widespread.[1] The timing of implant insertion and provisionalization may have an impact on the peri-implant soft and hard tissues, which can affect the esthetic and patient-centered outcome. Wöhrle's immediate implant placement with provisionalization (IIPP) contributes eminently to maximizing the esthetic success by retaining the osseous and gingival structure, which is necessary for providing a temporary restoration. When a treatment strategy of flapless extraction and implant placement was combined with bone grafting, connective tissue grafting and the attachment of an immediate provisional crown, the least amount of variation in results was observed.[2]

Bone morphogenetic proteins (BMPs) are known to have osteoinductive properties, and the demineralized freeze-dried bone allograft (DFDBA) contains BMPs 2, 4 and 7. The DFDBA typically breaks down quickly, allowing the formation of new bone. The biological cascade that the BMPs are a part of involves chemotaxis, matrix attachment, cell proliferation, and cell differentiation into cartilage, bone, and marrow.[3] The main benefit of allografts is that, despite lacking vital cells, they may have mechanical properties similar to those of autogenous bone, and may contain the collagenous matrix and proteins seen in natural bone.

The process of osseointegration has been enhanced by a number of methods, including changing the implant's topography, surface morphology, roughness and energy, strain hardening, and chemical composition, as well as the presence of impurities, the thickness of the titanium oxide layer, and the presence of non-metal and metal composites.[4] The regulation of healing following implant placement is another strategy for hastening osseointegration. Herein lies the role of bioactive molecules known as growth factors (GFs). A natural source of GFs – platelets – contain insulin-like GF (IGF), platelet-derived GF (PDGF), transforming GF (TGF) -1 and -2, fibroblast GF (FGF), vascular endothelial GF (VEGF), and other GFs that promote angiogenesis, matrix remodeling and cell proliferation. Concentrated growth factor (CGF), introduced by Sacco in the year 2006, is obtained by centrifuging venous blood, which concentrates the platelets in a gel layer made of a fibrin matrix that is rich in GFs and leukocytes at alternating revolutions per minute, and yields a richer and denser fibrin matrix as compared to other autologous platelet concentrates. Concentrated growth factor releases GFs for at least 13 days and has demonstrated the stimulation of bone repair around implants in vitro.[5] A recent study by Guarnieri et al. compared the expression of pro-inflammatory cytokines in peri-implant crevicular fluid at two-piece/bone level vs. one-piece/tissue level single implants after at least 5 years of loading.[6] They reported that the two-piece implants presented a more profound pro-inflammatory condition, with higher levels of interleukins and higher crestal bone loss as compared to the one-piece implants.[6] A similar long-term implant function study found that the amount of early marginal bone remodeling could not be considered as an indicator of the subsequent onset of periimplantitis, whereas high levels of active matrix metalloproteinase-8 (aMMP-8) 6 months after loading could have a distinct ability to predict the same.[7] A recent study has demonstrated the improvement of the quality of life (QoL) of a Parkinson's patient after implant insertion, with an acceptable 12-month implant survival rate.[8]

There is a dearth of literature and in vivo research examining alterations in the soft and hard tissues when employing a CGF-enriched bone graft in comparison with the spontaneous healing of the jump space (JS), despite the fact that numerous studies have supported the usage of diverse graft materials in IIPP. Hence, this randomized controlled clinical trial prospectively evaluated the clinical and radiographic effects of IIPP with and without a CGF-enriched bone graft on the soft and hard tissues, using cone beam computed tomography (CBCT).

Material and methods

This comparative study was conducted as a double-blind randomized controlled clinical trial in accordance

with the World Medical Association (WMA) Declaration of Helsinki and the CONSORT (Consolidated Standards of Reporting Trials) guidelines, after obtaining written informed consent from the participants. The study flowchart is depicted in Fig 1. After receiving approval from the institutional Research Ethics Committee at VSPM Dental College and Research Centre, Nagpur, India (No. of approval: IEC/VSPMDCRC/02/2020), this trial was registered with the Clinical Trial Registry – India under the number CTRI/2021/01/030848.

The study population comprised patients requiring immediate implant placement in the anterior esthetic area and meeting the inclusion criteria: systemically healthy patients; an unrestorable tooth; the presence of the adjacent teeth and the opposing natural tooth; patients with a healthy and stable soft-tissue architecture of the site receiving intervention; intact alveolar bone extraction socket walls; the presence of bone apical to the root apex and palatal to the socket; the sites at which torque ≥35 N·cm was obtained at the time of implant insertion; and JS-buccal of more than 1.5 mm. The reasons for the exclusion of patients from the study were as follows: general contraindications to implant surgery; patients with a history of irradiation in the head and neck area within the last 6 months; patients treated or under treatment with intravenous amino-bisphosphonates; pregnant or lactating women; smokers or patients with poor oral hygiene; parafunctional habits and severe maxillo-mandibular discrepancies; and an active pathology of the adjacent teeth.

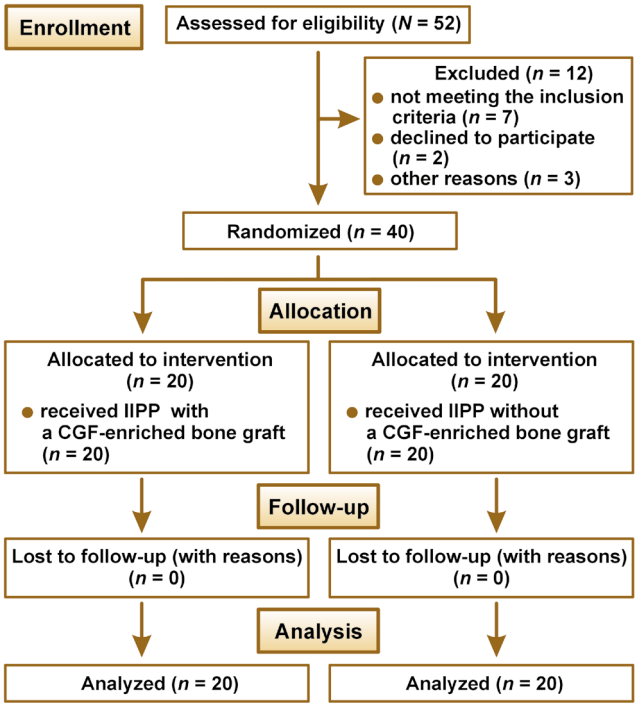

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart of the study

IIPP – immediate implant placement and provisionalization; CGF – concentrated growth factor.

The bone graft used was particulate DFDBA (500–1,040 μm) that was procured from the Tissue Bank of Tata Memorial Hospital and Research Centre, Mumbai, India. The protocol applied for the preparation of CGF[9] consisted in using 10 mL of intravenous blood, which was collected in 2 glass-coated plastic tubes with no anticoagulant addition and subjected to centrifugation (R-4C; Remi Lab World, Mumbai, India); it yielded 4 layers from bottom to top: the red blood cell (RBC) layer; the GF and stem cell layer (CGF); the buffy coat layer; and the serum layer. The jump space in the control group was left for spontaneous healing without any grafting material (Fig. 2). The CGF layer was separated using sterile surgical scissors, and then mixed with the bone graft material before placement over the target site in the test group (Fig. 3).

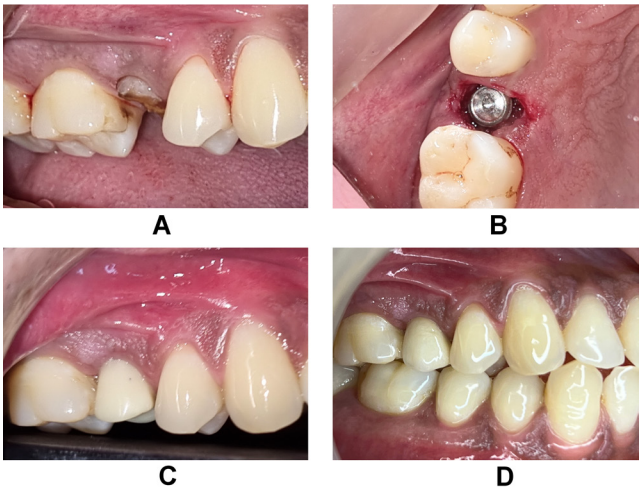

Fig. 2. Surgical protocol for the control group

A – preoperative baseline: maxillary right second premolar with a poor prognosis; B – immediate implant placement; C – provisional restoration at 4 months; D – buccal view of the final prosthesis in occlusion 12 months postoperatively.

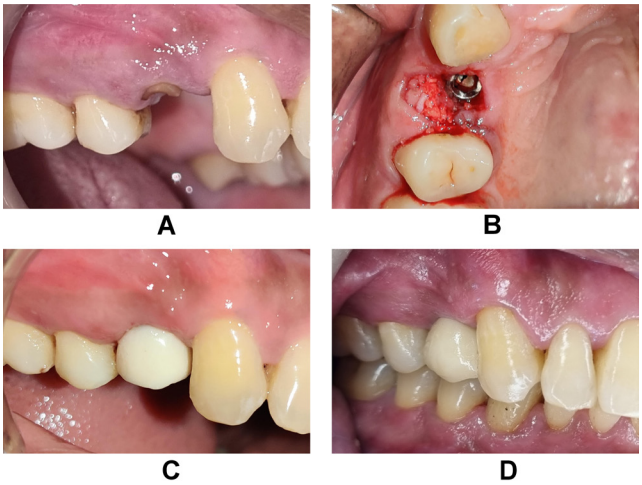

Fig. 3. Surgical protocol for the test group

A – preoperative baseline: maxillary right first premolar with a poor prognosis; B – immediate implant placement with the CGF-enriched bone graft placed in the jump space (JS); C – provisional restoration at 4 months; D – buccal view of the final prosthesis in occlusion 12 months postoperatively.

Referring to the study by Kabi et al.,[10] and considering an effect size of 1.0, a sample of 17 sites per group was established to obtain the desired effect with the 95% confidence and 80% power of the test. Further, considering a 15% loss to follow-up, the effective sample size was determined as 40 sites. All sites were randomly allocated with an allocation ratio 1:1 to one of the 2 groups by means of computer-generated random numbers at the time of surgery, using the 'blockrand' library from the R programming tool (https://www.r-project.org). The patient and the primary outcome assessor were blinded, as the CBCT images were just given codes, with no reference to patients or groups.

The clinical parameters assessed at baseline, and at 6 and 12 months were the modified plaque index (mPI),[11] the modified sulcular bleeding index (mSBI),[12] the probing

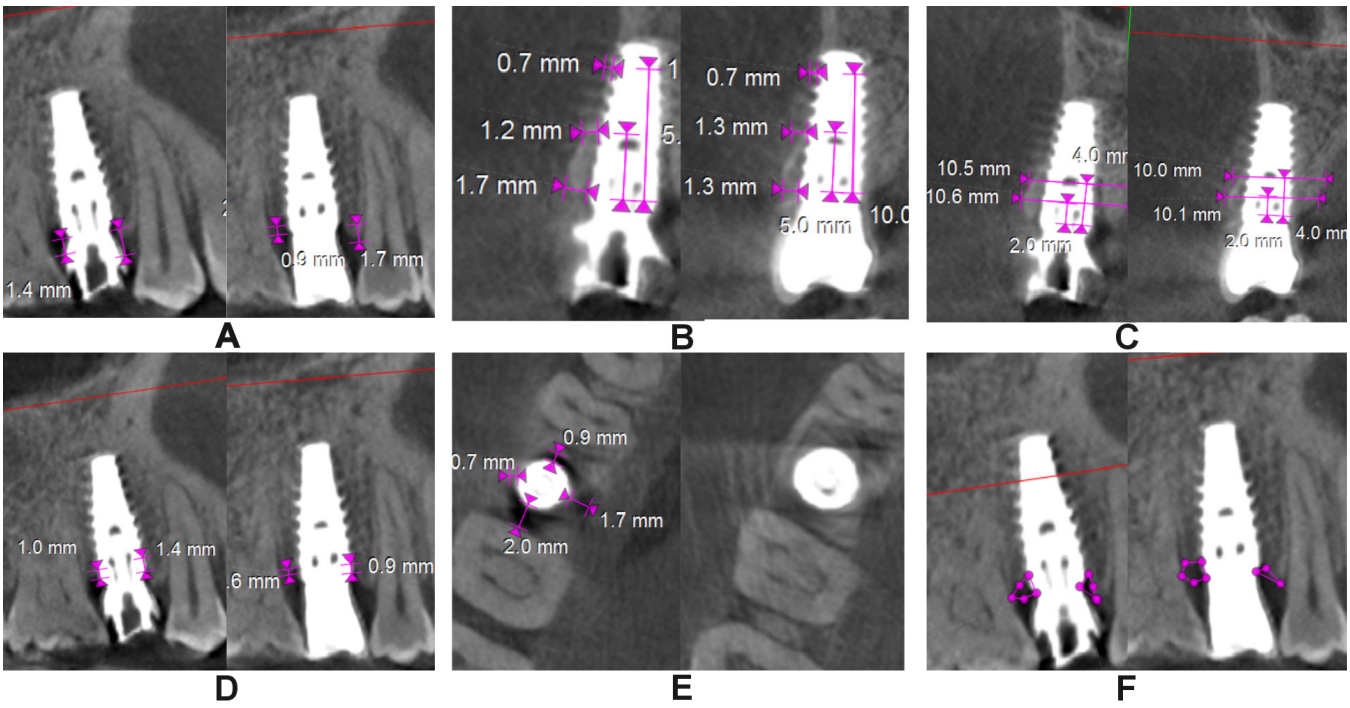

Fig. 4. Radiographic parameters at baseline and at 12 months postoperatively in the control group

A – crestal bone height (CBH); B – buccal bone thickness (BBT); C – ridge width (RW); D – vertical distance (VD); E – jump space (JS); F – radiolucent area (RA).

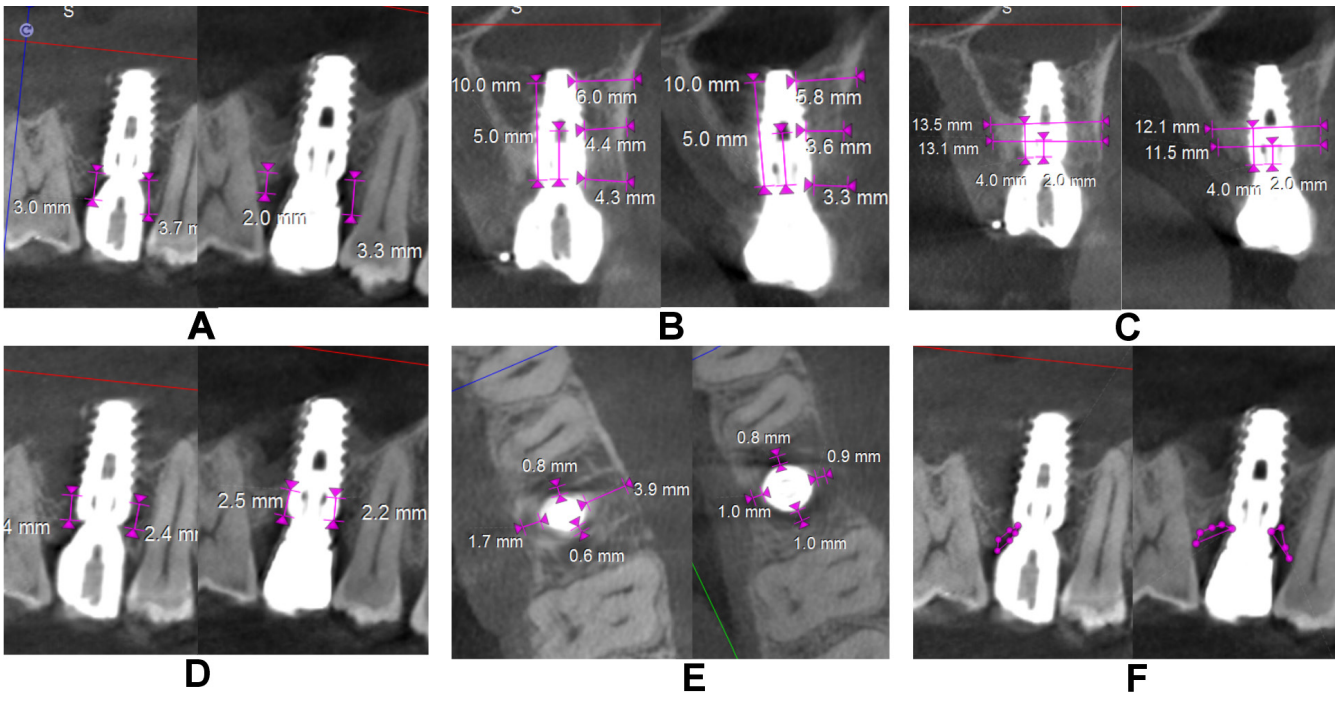

Fig. 5. Radiographic parameters at baseline and at 12 months postoperatively in the test group

A – CBH; B – BBT; C – RW; D – VD; E – JS; F – RA.

depth/implant sulcus depth (PD),[13] the gingival thickness (GT), and the Testori implant esthetic score (TS).[14] Using CBCT, the following radiographic parameters were assessed on the day of surgery after implant placement and at 12 months (Fig. 4 and 5): the crestal bone height (CBH) – the distance between the implant shoulder to the most coronal point of the interproximal crestal bone height in the sagittal view[15]; the buccal bone thickness (BBT), measured at the crest, 5 mm from the crest and 10 mm from the crest in the coronal view[16]; the ridge width (RW) – the buccolingual dimension of the osseous ridge in the coronal view; the vertical distance (VD) – the distance between the first radiographic bone implant contact and the first implant thread on the mesial and distal sides, and the amount of bone loss on the mesial and distal sides in the sagittal view[17]; JS – the perpendicular distance from the most coronal point of the mesial, distal, buccal, and palatal bone crest to the implant platform in the axial view; and the radiolucent area (RA) – the area between the implant shoulder and the bone crest in the sagittal and coronal views.[18] Discomfort/pain was assessed on the day of implant surgery, and patient satisfaction at 12 months postoperatively – both using the visual analog scale (VAS) (Fig. 6).

Surgical protocol

After 1 week of phase I therapy, atraumatic extraction was done without the elevation of the mucoperiosteal flap followed by a thorough degranulation of any soft tissue remnants, ensuring the integrity of the buccal bone plate. A presurgical radiograph performed with the use of radiovisiography and a clinical assessment of the intended implant site were used to estimate the necessary implant sizes. Following the manufacturer's instructions, the implant surgical drills from the Adin implant kit (Adin Dental Implant Systems Ltd., Afula, Israel) were used to drill 2–3 mm apical to the extraction socket. The Adin Touareg™ S implants were placed in. A calibrated-torque hand ratchet was used to secure the implant in place, at least 3 mm apical to the gingival margin and at the level of the alveolar crest. According to randomization, in the control group, IIPP without any biomaterial was conducted, while in the test group, JS was filled with a bone graft material enriched with CGF. For the test group, DFDBA and CGF were mixed together extraorally and the mix was allowed to settle for 2–3 min, which made the graft material slightly adherent to CGF, and then this CGF-enriched bone graft was placed into the space between the implant and the bone socket. The screw-retained provisional crowns were kept out of occlusal and eccentric contacts.

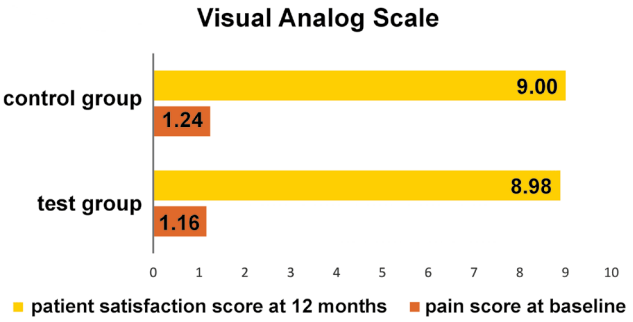

Fig. 6. Pain and patient satisfaction scores

All patients were given appropriate oral hygiene and post-surgery instructions. The participants were administered a capsule of amoxicillin trihydrate (500 mg) 3 times a day for 5 days. A tablet of aceclofenac (100 mg) and a tablet of paracetamol (325 mg) were prescribed to control postsurgical discomfort if needed. The patients were advised to use a chlorhexidine mouthwash (10 mL twice daily) for 15 days, and to abstain from chewing tough or sticky food stuff. The patients were advised to undergo CBCT (Orthophos SL 3D, FOV (field of view): 5×5 cm (85 Kv,7 mA); Dentsply Sirona, Charlotte, USA) within 24 h of implant placement. Such a FOV enables the clinician to obtain scans with high definition and finer details of the structures for precise evaluation. The scan image analysis was performed with the imaging software 3 DIEMME, v. 4.2 (Bioimaging Technologies, Figino Serenza, Italy). After 4–5 months, the interim crowns were replaced with the final prostheses.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 26.0 (IBM Corp., Armonk, USA), and the statistical significance level was set at 5%. The clinical and radiographic parameters at the end of study, i.e., after 12 months, were compared between the groups after adjusting to the baseline values, using the one-way analysis of covariance (ANCOVA). The within group comparisons of these parameters between the baseline and 12 months were performed using the paired *t* test. The change in the parameters from baseline to 6 months and 12 months, as well as from 6 months to 12 months, was evaluated; the mean change was compared between the groups with the use of the *t* test for independent samples.

Results

No implants were lost during the course of the study. During the follow-up period, neither the implants nor the bone graft material experienced any biological or mechanical issues. The demographic and implant characteristics of the 2 groups were comparable (Table 1).

The mPI values exhibited a substantial decline, i.e., 1.53 ±0.51, 0.63 ±0.50 and 0.37 ±0.50 in the test group, and 1.38 ±0.50, 0.67 ±0.58 and 0.33 ±0.48 in the control group

at baseline, and at 6 and 12 months, respectively. The average PD values were 2.26 ±0.49 mm, 2.63 ±0.37 mm and 2.72 ±0.42 mm in the test group, and 2.26 ±0.50 mm, 2.57 ±0.36 mm and 2.77 ±0.29 mm in the control group at baseline, and at 6 and 12 months, respectively. The GT parameter showed a decrease in the mean values from baseline to 6 months and 12 months, i.e., from 1.94 ±0.24 mm to 1.68 ±0.21 mm and 1.62 ±0.23 mm, and from 1.93 ±0.11 to 1.74 ±0.15 and 1.66 ±0.18 mm in both the test and control groups, respectively. The Testori score showed a statistically significant increase in the test group over time, with a mean score of 7.81 ±0.68 at baseline, and 8.44 ±1.15 at 6 and 12 months (Table 2). The intergroup comparison of the mean values of the clinical parameters at 12 months after adjusting to the respective baseline values did not show any significant differences except for TS, with 8.49 ±0.82 in the test group and 7.96 ±0.76 in the control group ($p < 0.05$) (Table 3).

Table 1. Demographic and implant characteristics of the patients in the 2 study groups

Characteristics		Test group ($n = 20$)	Control group ($n = 20$)	p-value
Age [years] *M ±SD*		36.20 ±13.01	38.31 ±12.69	0.650[1]
Gender *n* (%)	M	9 (60.0)	10 (62.5)	0.990[2]
	F	6 (40.0)	6 (37.5)	
Implant size *n* (%)	4.2 mm × 11.5 mm	0 (0.0)	2 (10.0)	0.670[2]
	4.2 mm × 13.0 mm	10 (50.0)	12 (60.0)	
	4.0 mm × 16.0 mm	1 (5.0)	0 (0.0)	
	4.2 mm × 16.0 mm	1 (5.0)	1 (5.0)	
	5.0 mm × 11.5 mm	3 (15.0)	2 (10.0)	
	5.0 mm × 13.0 mm	4 (20.0)	2 (10.0)	
	5.0 mm × 16.0 mm	1 (5.0)	1 (5.0)	
Implant sites *n* (%)	11	3 (15.0)	1 (5.0)	0.620[2]
	12	2 (10.0)	2 (10.0)	
	13	1 (5.0)	1 (5.0)	
	14	3 (15.0)	0 (0.0)	
	15	2 (10.0)	5 (25.0)	
	21	3 (15.0)	4 (20.0)	
	22	1 (5.0)	2 (10.0)	
	23	0 (0.0)	0 (0.0)	
	24	2 (10.0)	3 (15.0)	
	25	3 (15.0)	2 (10.0)	

M – mean; *SD* – standard deviation; M – male; F – female; [1] *t* test for independent samples; [2] Pearson's χ^2 test.

Table 3. Comparison of the clinical parameters at 12 months (the end of the study) between the 2 groups after adjusting to the respective baseline values (ANCOVA)

Parameter	Group	Adjusted values at 12 months	p-value
mPI	test	0.34 ±0.46 (0.13–0.55)	0.890
	control	0.36 ±0.44 (0.16–0.56)	
mSBI	test	0.69 ±0.48 (0.46–0.91)	0.840
	control	0.71 ±0.45 (0.50–0.92)	
PD [mm]	test	2.72 ±0.22 (2.62–2.83)	0.460
	control	2.77 ±0.21 (2.68–2.87)	
GT [mm]	test	1.62 ±0.17 (1.54–1.70)	0.380
	control	1.66 ±0.16 (1.59–1.74)	
TS	test	8.49 ±0.82 (8.11–8.87)	0.048*
	control	7.96 ±0.76 (7.61–8.32)	

Data presented as *M ±SD* (95% *CI*).
* statistically significant.

Table 2. Comparison of the clinical parameters within and between the groups

Parameter	Group	Baseline	6 months	12 months	p-value[1]
mPI	test	1.53 ±0.51 (1.28–1.77)	0.63 ±0.50 (0.39–0.87)	0.37 ±0.50 (0.13–0.61)	<0.001*
	control	1.38 ±0.50 (1.15–1.61)	0.67 ±0.58 (0.40–0.93)	0.33 ±0.48 (0.11–0.55)	<0.001*
	p-value[2]	0.360	0.830	0.820	–
mSBI	test	1.00 ±0.82 (0.61–1.39)	0.84 ±0.60 (0.55–1.13)	0.68 ±0.48 (0.45–0.91)	0.330
	control	1.05 ±0.67 (0.74–1.35)	0.95 ±0.59 (0.68–1.22)	0.71 ±0.46 (0.50–0.92)	0.075
	p-value[2]	0.840	0.560	0.840	–
PD [mm]	test	2.26 ±0.49 (2.03–2.50)	2.63 ±0.37 (2.45–2.81)	2.72 ±0.42 (2.52–2.93)	<0.001*
	control	2.26 ±0.50 (2.04–2.49)	2.57 ±0.36 (2.41–2.74)	2.77 ±0.29 (2.64–2.91)	<0.001*
	p-value[2]	0.990	0.600	0.660	–
GT [mm]	test	1.94 ±0.24 (1.82–2.05)	1.68 ±0.21 (1.58–1.79)	1.62 ±0.23 (1.51–1.73)	<0.001*
	control	1.93 ±0.11 (1.88–1.98)	1.74 ±0.15 (1.67–1.81)	1.66 ±0.18 (1.58–1.74)	<0.001*
	p-value[2]	0.880	0.350	0.530	–
TS	test	7.72 ±0.67 (7.39–8.05)	8.44 ±1.15 (7.87–9.02)	8.44 ±1.15 (7.87–9.02)	<0.001*
	control	7.81 ±0.68 (7.50–8.12)	8.00 ±0.84 (7.62–8.38)	8.00 ±0.84 (7.62–8.38)	0.200
	p-value[3]	0.560	0.250	0.170	–

Data presented as mean ± standard deviation (95% confidence interval) (*M ±SD* (*CI*)).
mPI – modified plaque index; mSBI – modified sulcular bleeding index; PD – probing depth; GT – gingival thickness; TS – Testori implant esthetic score; * statistically significant; [1] repeated measures ANOVA; [2] *t* test for independent samples; [3] Friedman's ANOVA.

Table 4. Comparison of the radiological parameters within and between the groups

Parameter	Group	Baseline	12 months	*p*-value[1]
CBH-mesial [mm]	test	2.60 ±1.19 (2.03–3.17)	2.67 ±1.29 (2.04–3.29)	0.820
	control	2.33 ±0.62 (2.04–2.61)	1.95 ±0.41 (1.76–2.14)	<0.001*
	p-value[2]	0.364	0.020*	–
CBH-midfacial [mm]	test	3.07 ±1.12 (2.53–3.61)	2.54 ±0.88 (2.12–2.96)	0.091
	control	2.49 ±0.73 (2.16–2.82)	1.88 ±0.49 (1.65–2.10)	<0.001*
	p-value[2]	0.054	0.006*	–
CBH-distal [mm]	test	2.30 ±0.81 (1.91–2.69)	1.91 ±0.92 (1.47–2.35)	0.128
	control	2.32 ±0.68 (2.01–2.63)	1.87 ±0.46 (1.66–2.08)	<0.001*
	p-value[2]	0.930	0.860	–
BBT at the crest [mm]	test	2.28 ±0.81 (1.89–2.68)	2.05 ±0.78 (1.68–2.43)	0.240
	control	1.46 ±0.53 (1.22–1.70)	1.18 ±0.41 (0.99–1.37)	<0.001*
	p-value[2]	<0.001*	<0.001*	–
BBT at 5 mm from the crest [mm]	test	2.18 ±0.86 (1.77–2.60)	2.06 ±0.76 (1.70–2.43)	0.540
	control	1.33 ±0.41 (1.14–1.51)	1.33 ±0.40 (1.15–1.51)	0.960
	p-value[2]	<0.001*	<0.001*	–
BBT at 10 mm from the crest [mm]	test	1.84 ±0.92 (1.39–2.28)	1.78 ±0.62 (1.48–2.08)	0.750
	control	1.85 ±0.64 (1.56–2.14)	1.82 ±0.39 (1.64–2.00)	0.820
	p-value[2]	0.960	0.830	–
RW at 2 mm from the crest [mm]	test	9.48 ±1.32 (8.84–10.12)	9.25 ±0.92 (8.81–9.69)	0.480
	control	8.19 ±1.07 (7.70–8.68)	7.89 ±0.78 (7.53–8.25)	0.010*
	p-value[2]	0.002*	<0.001*	–
RW at 4 mm from the crest [mm]	test	9.97 ±1.63 (9.19–10.76)	9.93 ±0.89 (9.50–10.36)	0.925
	control	8.57 ±1.01 (8.11–9.03)	8.10 ±0.82 (7.73–8.47)	<0.001*
	p-value[2]	0.002*	<0.001*	–
VD-mesial [mm]	test	2.14 ±0.56 (1.87–2.41)	1.91 ±0.76 (1.54–2.28)	0.301
	control	0.77 ±0.58 (0.51–1.04)	1.62 ±0.46 (1.42–1.83)	<0.001*
	p-value[2]	<0.001*	0.150	–
VD-distal [mm]	test	1.56 ±0.73 (1.21–1.92)	1.39 ±0.36 (1.22–1.57)	0.260
	control	1.43 ±0.91 (1.02–1.85)	2.15 ±0.87 (1.75–2.54)	<0.001*
	p-value[2]	0.620	0.001*	–
JS-mesial [mm]	test	1.23 ±0.44 (1.02–1.44)	0.36 ±0.32 (0.20–0.51)	<0.001*
	control	1.37 ±0.35 (1.21–1.53)	0.91 ±0.39 (0.73–1.09)	<0.000*
	p-value[2]	0.250	<0.001*	–
JS-distal [mm]	test	1.45 ±0.55 (1.19–1.72)	0.29 ±0.41 (0.09–0.49)	<0.001*
	control	1.06 ±0.47 (0.85–1.27)	0.94 ±0.25 (0.83–1.06)	0.250
	p-value[2]	0.020*	<0.001*	–
JS-buccal [mm]	test	2.34 ±0.58 (2.06–2.62)	0.43 ±0.40 (0.24–0.62)	<0.001*
	control	2.20 ±0.30 (2.06–2.33)	1.45 ±0.38 (1.28–1.62)	<0.001*
	p-value[2]	0.310	<0.001*	–
JS-palatal [mm]	test	1.11 ±0.48 (0.88–1.34)	0.42 ±0.34 (0.26–0.58)	<0.001*
	control	1.20 ±0.63 (0.91–1.49)	0.82 ±0.42 (0.63–1.01)	<0.001*
	p-value[2]	0.610	0.002*	–
RA-mesial [mm^2]	test	0.97 ±0.59 (0.69–1.26)	0.64 ±0.40 (0.45–0.83)	0.062
	control	0.90 ±0.68 (0.59–1.20)	1.20 ±0.58 (0.94–1.47)	0.023*
	p-value[2]	0.690	0.001*	–
RA-distal [mm^2]	test	0.81 ±0.65 (0.49–1.12)	0.85 ±0.49 (0.61–1.08)	0.780
	control	0.81 ±0.50 (0.58–1.04)	1.12 ±0.71 (0.80–1.45)	0.014*
	p-value[2]	0.980	0.160	–
RA-buccal [mm^2]	test	0.55 ±0.52 (0.30–0.80)	0.19 ±0.38 (0.00–0.37)	0.026*
	control	0.45 ±0.45 (0.24–0.65)	0.98 ±0.52 (0.74–1.21)	0.002*
	p-value[2]	0.500	<0.001*	–
RA-palatal [mm^2]	test	0.47 ±0.79 (0.10–0.85)	0.43 ±0.55 (0.16–0.69)	0.849
	control	0.55 ±0.53 (0.31–0.79)	1.23 ±0.82 (0.86–1.60)	0.006*
	p-value[2]	0.720	0.001*	–

Data presented as *M* ±*SD* (95% *CI*).
CBH – crestal bone height; BBT – buccal bone thickness; RW – ridge width; VD – vertical distance; JS – jump space; RA – radiolucent area;
* statistically significant; [1] paired *t* test; [2] *t* test for independent samples.

In the control group, CBH-mesial (2.33 ±0.62 mm, 1.95 ±0.41 mm), CBH-midfacial (2.49 ±0.73 mm, 1.88 ±0.49 mm), CBH-distal (2.32 ±0.68 mm, 1.87 ±0.46 mm), BBT at the crest (1.46 ±0.53 mm, 1.18 ±0.41 mm), and RW at 4 mm from the crest (8.57 ±1.01 mm, 8.10 ±0.82 mm) showed a highly significant decrease from baseline to 12 months in contrast to the test group. Similarly, VD-mesial and VD-distal showed an increase in the mean value in the control group ($p < 0.001$). A highly significant decrease from baseline to 12 months in JS-mesial (1.23 ±0.44 mm to 0.36 ±0.32 mm, and 1.37 ±0.35 mm to 0.91 ±0.39 mm), JS-distal (1.45 ±0.55 mm to 0.29 ±0.41 mm, and 1.06 ±0.47 mm to 0.94 ±0.25 mm), JS-buccal (2.34 ±0.58 mm to 0.43 ±0.40 mm, and 2.20 ±0.30 mm to 1.45 ±0.38 mm), and JS-palatal (1.11 ±0.48 mm to 0.42 ±0.34 mm, and 1.20 ±0.63 to 0.82 ±0.42 mm) was noted in both the test and control groups, respectively, except for JS-distal in the control group. A significant increase ($p < 0.05$) in RA on all sides was observed in the control group and on the buccal side for the test group (Table 4).

The intergroup comparison for the mean change in the radiographic parameters after adjusting to the respective baseline values showed statistically significant differences in all radiographic parameters except for CBH-distal, BBT at 5 mm and 10 mm from the crest, VD-mesial, and RA-distal. Highly significant differences were observed with regard to the change in RW at 4 mm from the crest (9.80 ±0.89 mm), VD-distal (1.35 ±0.43 mm), JS-mesial (0.38 ±0.34 mm), JS-distal (0.25 ±0.34 mm), JS-buccal (0.42 ±0.39 mm), RA-mesial (0.63 ±0.48 mm^2), and RA-buccal (0.19 ±0.47 mm^2) in the test group as compared to the control group at 12 months (Table 5).

Table 5. Comparison of the radiological parameters at 12 months (the end of the study) between the 2 groups after adjusting to the respective baseline values (ANCOVA)

Parameter	Group	Adjusted values at 12 months	*p*-value
CBH-mesial [mm]	test	2.59 ±0.19 (1.64–2.38)	0.033*
	control	2.01 ±0.18 (2.21–2.98)	
CBH-midfacial [mm]	test	2.45 ±0.16 (2.14–2.77)	0.028*
	control	1.95 ±0.15 (1.65–2.25)	
CBH-distal [mm]	test	1.91 ±0.15 (1.60–2.21)	0.810
	control	1.87 ±0.14 (1.58–2.14)	
BBT at the crest [mm]	test	1.84 ±0.57 (1.57–2.11)	0.022*
	control	1.37 ±0.54 (1.12–1.62)	
BBT at 5 mm from the crest [mm]	test	1.88 ±0.06 (1.60–2.16)	0.069
	control	1.50 ±0.56 (1.24–1.76)	
BBT at 10 mm from the crest [mm]	test	1.79 ±0.43 (1.59–1.99)	0.820
	control	1.82 ±0.41 (1.63–2.01)	
RW at 2 mm from the crest [mm]	test	8.99 ±0.77 (8.63–9.34)	0.002*
	control	8.13 ±0.73 (7.79–8.47)	
RW at 4 mm from the crest [mm]	test	9.80 ±0.89 (9.38–10.21)	<0.001*
	control	8.22 ±0.84 (7.83–8.61)	
VD-mesial [mm]	test	1.81 ±0.83 (1.42–2.20)	0.760
	control	1.72 ±0.77 (1.36–2.07)	
VD-distal [mm]	test	1.35 ±0.43 (1.15–1.55)	<0.001*
	control	2.19 ±0.41 (2.00–2.38)	
JS-mesial [mm]	test	0.38 ±0.34 (0.22–0.54)	<0.001*
	control	0.89 ±0.33 (0.74–1.04)	
JS-distal [mm]	test	0.25 ±0.34 (0.09–0.40)	<0.001*
	control	0.98 ±0.32 (0.83–1.13)	
JS-buccal [mm]	test	0.42 ±0.39 (0.24–0.61)	<0.001*
	control	1.46 ±0.37 (1.29–1.63)	
JS-palatal [mm]	test	0.44 ±0.34 (0.28–0.59)	0.002*
	control	0.81 ±0.32 (0.65–0.95)	
RA-mesial [mm^2]	test	0.63 ±0.48 (0.41–0.85)	<0.001*
	control	1.22 ±0.45 (1.01–1.43)	
RA-distal [mm^2]	test	0.85 ±0.54 (0.60–1.10)	0.115
	control	1.12 ±0.51 (0.89–1.36)	
RA-buccal [mm^2]	test	0.19 ±0.47 (–0.03–0.41)	<0.001*
	control	0.98 ±0.44 (0.77–1.18)	
RA–palatal [mm^2]	test	0.42 ±0.70 (0.09–0.75)	0.001*
	control	1.24 ±0.67 (0.92–1.55)	

Data presented as *M* ±*SD* (95% *CI*).
* statistically significant.

Discussion

The vascularity generated from the periodontal ligament (PDL) is disrupted during flapless tooth extraction, leaving 2 sources of blood supply behind. On the other hand, elevating a flap during surgical extraction damages the periosteum, another vascular source. Consequently, until angiogenesis takes place and the periosteal blood supply is restored, there is only one source of the blood flow left to the buccal bone (endosteal marrow). It is typical for the cortical and cancellous bone to make up the buccal bone for dental implants. Due to a reduced vascular supply, a thin facial bone, which has a higher proportion of the cortical bone than the cancellous bone, may be more prone to resorption. In contrast, a dense facial bone has a superior blood supply and the implant site is less likely to experience bone loss.[19] Hence, flapless immediate implant placement is advocated over raising a flap in such cases.

Several studies have shown that the one-stage technique has some clinical advantages when compared to the two-stage method,[17] comprising the following: the avoidance of a second surgical procedure; the lack of a micro-gap at the bone crest level, resulting in less crestal bone resorption; the prosthetic procedure is simplified and less chair time per patient is required; and the non-loaded, immediate-loading or delayed-loading protocol can be implemented.

A meta-analysis by Pitman et al. showed 0.87 mm less midfacial apical migration of the midfacial soft tissue when IIPP was done when compared to immediate implant placement alone, with the mean follow-up ranging

from 12 to 60 months.[20] Recession was not recorded in any of the cases in either the test or control groups, as all the treated sites in both groups received provisional restorations. A horizontal gap distance, i.e., JS of more than 1.5–2.0 mm, most likely requires the placement of a particulate bone substitute material, including an allograft covered with a membrane, for soft tissue in-growth, thus encouraging the osteogenic cells to engage in bone regeneration.[3] Hence, the same was tested against the no bone graft group in IIPP in the present study.

In ideal clinical circumstances, such as a fully intact facial bone wall with a thick wall phenotype (>1 mm) and a thick gingival biotype, Buser et al. advises using immediate implant insertion and states that the anterior maxilla hardly ever exhibits a thick wall phenotype.[21] The flattening of the orofacial soft-tissue profile and the recession of the facial mucosa are potential hazards. These recommendations are not supported by the findings of the current investigation, as the patients were not chosen for having thick facial bone walls, and thin gingival biotypes were also present. Despite the presence of these risk factors, the mid-term follow-up results were favorable.

The present study showed a statistically significant increase in the intragroup comparisons of PD around the immediate implants over time, from baseline to 12 months, for both groups, which is in accordance with the findings of Buser et al., where a slight increase in PD was seen, from 2.81 mm to 3.14 mm,[22] as PD can be influenced by variations in the gingival anatomy, and the distance between the implant margin and the mucosal margin (DIM). The evaluation of TS by Bhutani et al. yielded a score of 7.37 for the test group, which was more than in the control group (6.77), but the difference between the groups was statistically insignificant.[14] It is in contrast to the TS obtained in the current study, which may be due to the difference in follow-up timing. In the abovementioned study, the follow-ups were established only with the provisional restoration, at 3 and 6 months,[14] whereas the end outcome in the present study is with the final prosthesis, with a longer follow-up. Also, in all cases in the current investigation, screw-retained provisional restorations were strictly used, while in a study by Bhutani et al., the provisional restorations were either screw- or cement-retained[14]; it could alter the results as well.

Clinical studies have also shown that there is a significant amount of spontaneous filling of JS at the immediate implant site. Despite these findings, a recent recommendation is that marginal gaps should be filled with a bone replacement graft in order to get superior esthetic results. The clinical impact of such grafting, however, is a matter of debate, and few studies have been conducted to evaluate the spontaneous filling of JS as compared to the use of bone grafting. The present study is the first to use a CGF-enriched bone graft and evaluate it against a spontaneous gap fill in immediately placed implants in the anterior maxilla with provisionalization.

Kabi et al. reported that the mean alveolar bone loss was significantly greater in the sites left unfilled when compared to the sites filled with an autogenous graft at 6 and 9 months post implant placement,[10] which supports the results obtained in our study, where CBH showed a significant decrease from baseline to 12 months in the control group and the intergroup comparison also showed a statistically significant difference. The buccal bone thickness affects the degree of buccal plate resorption after immediate implant placement. In this study, the value of BBT at the crest in the control group showed a mean decrease from 1.46 ±0.53 mm to 1.18 ±0.41 mm, which is similar to the findings of Seyssens et al. – 0.59 mm (95% *CI*: 0.41–0.78; $p < 0.001$) or 54% less horizontal buccal bone resorption following immediate implant placement with socket grafting (IIP + SG) as compared to immediate implant placement alone.[23] In the same study, a trend toward less distal papillary recession was found (*MD* (mean difference) = 0.60 mm; 95% *CI*: –0.08–1.28; $p = 0.080$) when SG was performed, while mesial papillae appeared not significantly affected by SG.[23] This could be relevant with regard to the current study, where the change in the mean CBH-midfacial and CBH-distal was smaller in the test group than the control group, with a statistically significant difference between the groups; however, the change in the mean CBH-mesial over time remains statistically insignificant between the groups.

Patient-reported outcome measures are generally reliable, yet there are limited studies on that matter. The VAS was used to report patient discomfort/pain, as well as satisfaction, showing comparable scores for both groups. The emergence profile of the provisional restoration mechanically supports the soft tissue, preventing its collapse after tooth extraction. Clinical and histological studies that support the outcomes of our research show that an esthetic contour can be maintained both vertically and horizontally when the implant–socket gap is filled with a bone grafting material.[24–26]

In the present study, immediate provisionalisation was done in both groups. It provides benefits such as short treatment time, the elimination of a second surgery, which is required in the delayed-loading protocol, the protection of the gingival papillae, an immediate esthetic effect, and high patient satisfaction.[27]

A similar study was performed by Amam et al.[28] The authors compared radiologically the amount of bone gain and bone reduction by using tricalcium phosphate and calcium sulfate grafts mixed with advanced platelet-rich fibrin (A-PRF) in 18 maxillary sinus augmentation cases. They found no statistically significant differences between the 2 groups at a 6-month follow-up. However, a sufficient amount of bone was obtained when A-PRF was added to the 2 different bone grafts.[28]

It has been hypothesized that placing an implant right away after extraction and adding a graft material offers a scaffold on which blood clots can organize, aiding in maintaining the tissue volume. After a tooth is extracted, the soft tissue is mechanically supported by a temporary

restoration and its emergence profile, which prevents collapsing. The evaluation period of the current study is, however, brief, and long-term follow-up results are required. The findings indicate a limited presence of intact facial bone walls around the premolars in the anterior maxilla. Volumetric tissue changes that occur during the healing of the treated site could be measured on study casts for more accurate values, and parameters like the bone density and the implant stability quotient (ISQ) should also be taken into account in future research.

Conclusions

The effects of providing a provisional restoration with or without bone grafting were the primary focus of this research. It is evident that this clinical approach is essential to reduce the degree of facial contour alterations that may result from immediate implant placement. Additionally, it plays a crucial role in shaping both clinicians' and patients' perception of esthetic outcomes.

The results of the present clinical trial indicate that crestal bone resorption occurred in both groups. However, a significantly reduced resorption was observed in the test group, which is attributed to the augmentation of JS. This was reflected and confirmed by a marked reduction in JS and RA in the test group, along with a significantly lesser reduction in BBT, RW and VD as compared to the control group.

Immediate implant placement with provisionalization in the maxillary esthetic zone – both with and without the CGF-enriched DFDBA – demonstrated high patient satisfaction, particularly concerning the level of pain experienced during the surgical procedure.

It is recommended to graft JS after IIPP in the anterior esthetic zone with a CGF-enriched bone graft. However, an extended evaluation period is needed to determine whether such an approach offers long-term benefits.

Trial registration

The trial was registered with the Clinical Trial Registry – India under the number CTRI/2021/01/030848.

Ethics approval and consent to participate

The research was approved by the institutional Research Ethics Committee at VSPM Dental College and Research Centre, Nagpur, India (No. of approval: IEC/VSPMDCRC/02/2020). Written informed consent was obtained from all study participants.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Comparative evaluation of the effect of non-surgical periodontal therapy on serum levels of mannose-binding lectin, SIRT-1 and C-reactive protein in non-smokers and smokers with stage III periodontitis: A case–control study

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Abstract

Background. Host response to periodontal pathogens present in microbial plaque is characterized by the expression of various inflammatory and immune mediators known as biomarkers. There is a paucity of literature addressing the impact of non-surgical periodontal therapy (NSPT) on serum biomarkers, such as mannose-binding lectin (MBL), sirtuin 1 (SIRT-1) and C-reactive protein (CRP), in non-smokers and smokers with stage III periodontitis.

Objectives. The study aimed to evaluate and compare the effect of NSPT on serum levels of MBL, SIRT-1 and CRP in non-smokers and smokers with stage III periodontitis.

Material and methods. A total of 105 patients were equally divided into 3 groups, as follows: group I – periodontally healthy individuals; group II – non-smokers with stage III periodontitis; group III – smokers with stage III periodontitis. Probing pocket depth (PPD), clinical attachment level (CAL), plaque index (PI), gingival index (GI), and papillary bleeding index (PBI) were recorded, and serum MBL, SIRT-1 and CRP levels were analyzed using enzyme-linked immunosorbent assay (ELISA). The patients underwent NSPT, and all parameters were re-evaluated 6 weeks after the procedure.

Results. The mean change in MBL levels across the 3 groups from baseline to recall was significant. Conversely, SIRT-1 and CRP levels exhibited non-significant differences from baseline to recall, with p-values of 0.172 and 0.548, respectively. The mean differences in MBL and SIRT-1 levels between groups I and III at baseline ($p < 0.0001$ and $p = 0.041$ for MBL and SIRT-1, respectively) as well as in MBL between groups II and III at recall ($p < 0.0001$) were statistically significant.

Conclusions. A positive association of serum MBL levels and CRP levels as well as a negative association of SIRT-1 with the severity of periodontal disease may serve as a valuable, precise and feasible method of identifying individuals at risk of developing periodontal disease.

Keywords: smokers, periodontal diseases, non-surgical periodontal therapy

Highlights

- Serum sirtuin 1 (SIRT-1) levels were inversely associated with disease severity, suggesting their potential protective role despite non-significant changes after NSPT.
- C-reactive protein (CRP) levels correlated positively with disease severity, though 6 weeks of NSPT did not significantly reduce CRP levels.
- Smokers showed greater baseline biomarker imbalances, especially in MBL and SIRT-1 levels, highlighting the impact of smoking on periodontal inflammation and systemic biomarker expression.
- Mannose-binding lectin (MBL) may serve as a sensitive serum biomarker for periodontal disease monitoring, while CRP and SIRT-1 require further validation.

Introduction

Periodontal diseases are a group of microbial infections associated with a chronic inflammatory state. If left untreated, these infections cause extensive destruction of the adjacent tissues, ultimately resulting in tooth loss. Additionally, they are atypical in nature, with the associated inflammation being driven on by a complex biofilm of symbiotic and common pathogenic bacteria and their byproducts.[1] An increase in the periodontal pathogens disrupts the homeostasis, thereby affecting the susceptibility of the periodontal tissues.[2] The body's reaction to periodontal pathogens in microbial plaque is characterized by an expression of various inflammatory and immune mediators, known as biomarkers, which have been studied extensively over the recent past.[3,4] Thus, the monitoring of biomarkers in various biological samples, such as gingival crevicular fluid (GCF), saliva and/or serum in patients with periodontal disease can provide important conclusions into the pathogenesis of the disease. Similarly, the observation of the precocity of the peri-implant environment can facilitate the detection of an early peri-implant condition.[5,6]

Systemic alterations that ensue following an inflammatory response are referred to as the acute phase response,[7] despite the fact that they accompany both acute and chronic inflammation. Mannose-binding lectin (MBL), C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), complement proteins C3, C4 and C9, and fibrinogen are few examples of acute phase proteins that have both pro-inflammatory and anti-inflammatory properties. Acute phase reactants constitute an early, non-specific response to bacterial, viral or parasitic infection, mechanical or thermal trauma, ischemic necrosis, or malignant growth. The plasma concentration of these proteins undergoes a 25% increase or decrease in response to inflammation.[8] Mannose-binding lectin, a weak acute phase protein, is an important constituent of the innate immune system and one of the proteins of the complement system. Mannose-binding lectin functions as an opsonin and stimulates the traditional complement route.[7] The classical and lectin pathways are activated by the binding of complement-associated pattern recognition molecules to immune complexes (classical pathway) or carbohydrate subunits exposed on microbial or damaged host cells.[9] Mannose-binding lectin primarily recognizes and binds to specific polysaccharide groups that are present on the surface of microorganisms.[10] Interestingly, many of the periodontal pathogens also have mannan-containing polysaccharides on the cell surface, which can be a potential target for MBL binding. Following its binding to microorganisms, MBL activates the lectin pathway of the complement system.[11] Since inflammation is a central pathology in periodontal diseases, the estimation of MBL levels could be of importance for correlating these values pre- and post-non-surgical periodontal therapy (NSPT) and enable a better understanding of this biomarker.

Dental stem cell proliferation and osteoblast differentiation are key cellular processes involved in periodontal diseases. Preliminary studies have shown that sirtuin 1 (SIRT-1) regulates cellular differentiation and controls metabolic pathways in a wide variety of tissues.[12] Furthermore, it has been demonstrated that the levels of SIRT-1 influence the osteoblastic differentiation of human periodontal ligament stem cells (PDLSCs),[13] as well as the management of inflammation and oxidative stress. However, the role of SIRT-1 in periodontal disease activity and the impact of NSPT on SIRT-1 levels remains unclear.

Increased levels of CRP, an acute-phase inflammatory protein, have been observed in both acute and chronic conditions. C-reactive protein can activate the classical complement system as well as phagocytic cells via Fc receptors, facilitating the clearance of cellular debris, injured or dead cells, and foreign pathogens. The concentration of CRP closely reflects the progression of the acute-phase response to inflammation or tissue necrosis, and hence could serve as a useful biomarker for many disease processes.[7]

Smoking is a substantial risk factor for the progression of periodontal disease, which greatly enhances the likelihood of developing severe and chronic diseases.[14]

Furthermore, it can negatively affect the regenerative healing response by suppressing vascular growth, inhibiting fibroblast proliferation and adhesion, and reducing collagen production.[15]

Non-surgical periodontal therapy is universally regarded as the gold standard for treating periodontal disease, despite its evolution over time. The goal of eliminating pathogenic microbes may be overly ambitious.[16] However, a reduction in periodontal inflammation, attributable to a decreased microbial load, results in favorable clinical outcomes. There is a paucity of literature regarding the impact of NSPT on serum biomarkers, such as MBL, SIRT-1 and CRP. The present study was designed to evaluate and compare the effect of NSPT on serum levels of MBL, SIRT-1 and CRP in non-smokers and smokers with stage III periodontitis.

Material and methods

This case–control study was conducted from July 2021 to February 2023 in the Department of Periodontology at Ranjeet Deshmukh Dental College and Research Centre (Nagpur, India), following the approval of the Institutional Ethics Committee of Ranjeet Deshmukh Dental College and Research Centre, and in accordance with the Helsinki Declaration of 1975, as revised in 2013. This clinical study was registered with the Clinical Trial Registry-India (CTRI) (registration No. CTRI/2021/01/030833), the main registry of the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP). Informed consent was obtained from patients who were willing to participate in the trial after the objectives and procedures were outlined to them.

Sample size estimation

Caribé et al. observed the influence of non-surgical treatment of periodontal disease on serum concentration of SIRT-1 and MBL before and after intervention in the periodontitis group and the healthy group.[17] The effect size for these 2 parameters ranged between 0.10 and 0.55. The same 2 parameters, along with CRP, were measured before and after the intervention in these groups. According to the effect size of 0.53, the estimated sample size per group that can detect the effect with 95% confidence and 80% power is 28. However, considering 20% loss to follow-up, a sample of 105 (35 patients per group) was found appropriate.

Inclusion and exclusion criteria

The present study included 105 patients, comprising both males and females, divided equally into 3 groups and exhibiting good general health, generalized stage III periodontitis, and the presence of at least 15 natural teeth excluding third molars. Stage III periodontitis was diagnosed based on the current classification proposed by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) in 2018.[18] The study population was divided into 3 groups, each with specific inclusion criteria, as follows:

– group I: periodontally healthy patients with no history of smoking;
– group II: patients diagnosed with stage III periodontitis exhibiting probing pocket depth (PPD) ≥6 mm, interdental clinical attachment level (CAL) ≥5 mm and radiographic bone destruction extending almost up to the middle third of the root; no history of smoking;
– group III: patients diagnosed with stage III periodontitis exhibiting PPD ≥ 6 mm, interdental CAL ≥ 5 mm and radiographic bone destruction extending to or beyond the middle third of the root; and current smokers with a history of smoking more than 10 cigarettes per day.

Individuals who had undergone periodontal treatment within the past 6 months, those with any systemic disease, patients who had or were on immunosuppressive therapy, those with a history of antibiotic intake within the past 6 months or anti-inflammatory drug intake within the last 3 months, as well as pregnant and lactating women, were excluded from the study.

Clinical examination

Prior to the commencement of the clinical trial, the examiners were calibrated to ensure precise measurement recordings. A single examiner (RK) was responsible for taking a comprehensive case history and performing an intraoral examination using a manual periodontal probe (PCPUNC 15; Hu-Friedy, Chicago, USA). The intraoral examination consisted of clinical and radiological evaluations. Verbal interrogation was used to determine smoking status of the individuals. Periodontal parameters were charted for each patient. These included the plaque index (PI),[19] the gingival index (GI),[20] the papillary bleeding index (PBI),[21] PPD, and CAL. All participants underwent NSPT, which included scaling and root planing performed by a single operator (AP) along with oral hygiene instructions. The parameters were recorded at the conclusion of the observation period. For each periodontal parameter, the intraclass correlation coefficient (ICC) was calculated as a measure of reliability of observations of different parameters using a two-way mixed effects model. The ICC in the groups ranged from 0.92 to 0.99 ($p < 0.0001$), demonstrating high intraobserver reliability.

Assessment of biochemical parameters

Fresh blood was drawn from the antecubital fossa via venipuncture using a 20-gauge needle. A total of 10 mL of venous blood was collected from each subject and transferred to sterile test tubes. The samples were then

permitted to clot at room temperature for 20 min. The clot was removed by centrifuging at 2,000–3,000 rpm for 20 min. The collected serum was transferred to Eppendorf tubes (airtight plastic vials) using a clean pipette and stored at –20°C in a deep freezer until the final assay. The samples were analyzed for MBL, CRP and SIRT-1 levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Krishgen Biosystems, Mumbai, India) according to the manufacturer's instructions. Briefly, serum samples were diluted with the provided dilution buffer, and the levels of MBL, CRP and SIRT-1 were determined using a sandwich ELISA technique. Blood samples were collected at both time points, that is, at baseline and at the 6th week following the intervention.

Statistical analysis

Continuous demographic, clinical and biochemical parameters were expressed as mean and standard deviation (*M* ±*SD*). The distribution of subjects according to their sex was presented in terms of frequency and percentage (*n* (%)). The statistical analysis of continuous parameters across groups was conducted using one-way analysis of variance (ANOVA). The parameters exhibiting significant differences in means across the 3 groups were subjected to further comparison through the implementation of the Tukey's post hoc test. The distribution of subjects by sex was analyzed using Pearson's χ^2 test. The within-group comparison of clinical and biochemical parameters from baseline to recall was performed using the paired *t*-test. The statistical significance in clinical and biochemical parameters between groups II and III was determined by employing a *t*-test for independent samples. Furthermore, a one-way ANOVA was employed to compare the parameters at the study's conclusion (recall) across the 3 groups. The post-hoc analysis was carried out using Tukey's post-hoc test. All analyses were performed with the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). The significance level was set at 5%.

Results

A total of 105 patients attending the outpatient Department of Periodontology were included in the study. The demographic, clinical and biochemical parameters are depicted in Table 1. The mean age for groups II and III was found to differ insignificantly, whereas the mean age for group I was significantly lower compared to the other groups. The mean age of subjects in group I was 24.60 ±3.56 years, and it was 47.85 ±9.33 years in group II, and 45.08 ±7.89 years in group III. The distribution of sexes across groups also differed significantly ($p < 0.0001$). The mean biochemical parametric values of MBL, SIRT-1 and CRP were 809.22 ±109.41 ng/mL, 0.99 ±0.56 ng/mL and 2.61 ±0.76 mg/L, respectively, for group I. For groups II and III, the values for MBL were 978.75 ±275.39 ng/mL and 1,206.92 ±458.40 ng/mL, respectively. For SIRT-1, the values in groups II and III were 0.95 ±0.41 ng/mL and 0.71 ±0.46 ng/mL, respectively. Similarly, for CRP, the mean biochemical parametric values were determined to be 4.70 ±0.89 mg/L and 5.51 ±0.78 mg/L, respectively.

Table 1. Demographic, clinical and biochemical parameters of the study sample at baseline

Variable		Group I	Group II	Group III	*p*-value
Subjects, *n*		35	35	35	–
Age [years] *M* ±*SD*		24.60 ±3.56^a	47.85 ±9.33^b	45.08 ±7.89^b	<0.0001*
Sex, *n* (%)	male	24 (68.6)	11 (31.4)	31 (88.6)	–
	female	11 (31.4)	24 (68.6)	4 (11.4)	
Clinical parameter *M* ±*SD*	PPD [mm]	1.54 ±0.30^a	6.33 ±1.16^b	7.88 ±0.70^c	<0.0001*
	CAL [mm]	0.00 ±0.00	7.80 ±1.48	8.45 ±0.63	0.020‡
	PI [%]	0.71 ±0.29^a	2.08 ±0.54^b	2.63 ±0.49^c	<0.0001*
	GI [%]	0.44 ±0.35^a	2.23 ±0.48^b	1.70 ±0.51^c	<0.0001*
	PBI [%]	0.04 ±0.06^a	2.89 ±0.86^b	1.70 ±0.60^c	<0.0001*
Biochemical parameter *M* ±*SD*	MBL [ng/mL]	809.22 ±109.41^a	978.75 ±275.39^b	1,206.92 ±458.40^c	<0.0001*
	SIRT-1 [ng/mL]	0.99 ±0.56^a	0.95 ±0.41^a	0.71 ±0.46^b	0.034*
	CRP [mg/L]	2.61 ±0.76^a	4.70 ±0.89^b	5.51 ±0.78^c	<0.0001*

Different superscript letters indicate statistically significant differences between the groups. * one-way ANOVA; ‡ *t*-test for independent samples; *M* – mean; *SD* – standard deviation; PPD – probing pocket depth; CAL – clinical attachment level; PI – plaque index; GI – gingival index; PBI – papillary bleeding index; MBL – mannose-binding lectin; SIRT-1 – sirtuin 1; CRP – C-reactive protein.

The PI, PPD and CAL values were significantly higher, while the GI and PBI scores were lower in group III. The clinical attachment level in group II exhibited a mean of 7.80 ±1.48 mm at baseline, which was significantly lower than that in group III (8.45 ±0.63 mm; $p = 0.020$). A comparison of clinical parameters within groups II and III between 2 time points demonstrated statistically significant differences (Table 2).

At baseline, a statistically significant difference was observed in the mean PPD, CAL, PI, GI, and PBI values across the 3 groups ($p < 0.0001$). The mean PI for group I was the lowest (0.71 ±0.29), while for group III, it was the highest (2.63 ±0.49). Six weeks after NSPT, a significant improvement was noted in all the periodontal clinical parameters ($p < 0.0001$). There was a significant decrease in CAL values in group II and group III from baseline to recall (Table 2). Additionally, a reduction in PPD, PI, GI, and PBI levels was observed in smokers and non-smokers after NSPT. The mean change in MBL levels across

Table 2. Comparison of clinical and biochemical parameters within study groups

Parameter	Group II				Group III			
	baseline	recall	mean difference (95% *CI*)	*p*-value	baseline	recall	mean difference (95% *CI*)	*p*-value
PI [%]	2.08 ±0.54	1.20 ±0.50	0.88 (0.77, 1.00)	<0.0001*	2.63 ±0.49	1.57 ±0.42	1.06 (0.90, 1.22)	<0.0001*
GI [%]	2.23 ±0.48	1.03 ±0.44	1.20 (1.09, 1.31)	<0.0001*	1.70 ±0.51	0.78 ±0.38	0.92 (0.79, 1.05)	<0.0001*
PPD [mm]	6.33 ±1.16	5.24 ±1.25	1.09 (0.97, 1.21)	<0.0001*	7.88 ±0.70	6.24 ±0.54	1.65 (1.50, 1.79)	<0.0001*
CAL [mm]	7.80 ±1.48	6.64 ±1.58	1.16 (0.95, 1.38)	<0.0001*	8.45 ±0.63	6.90 ±0.51	1.55 (1.39, 1.71)	<0.0001*
PBI [%]	2.89 ±0.86	1.92 ±0.75	0.96 (0.80, 1.13)	<0.0001*	1.70 ±0.60	0.56 ±0.36	1.14 (0.97, 1.31)	<0.0001*
MBL [ng/mL]	978.75 ±275.39	721.71 ±118.32	257.04 (168.41, 345.67)	<0.0001*	1,206.92 ±458.4	844.91 ±111.49	362.01 (198.67, 525.35)	<0.0001*
SIRT-1 [ng/mL]	0.95 ±0.41	1.22 ±0.47	−0.27 (−0.47, −0.07)	0.01*	0.71 ±0.46	1.12 ±0.46	−0.41 (−0.55, −0.26)	<0.0001*
CRP [mg/L]	4.70 ±0.89	2.60 ±0.72	2.10 (1.72, 2.47)	<0.0001*	5.51 ±0.78	2.76 ±0.61	2.74 (2.45, 3.04)	<0.0001*

* statistically significant ($p < 0.05$, paired *t*-test); *CI* – confidence interval.

Table 3. Comparison of clinical and biochemical parameters 6 weeks after non-surgical periodontal therapy (NSPT) across the study groups

Parameter	Group I	Group II	Group III	*p*-value
PI [%]	0.71 ±0.29[a]	1.20 ±0.50[b]	1.57 ±0.42[c]	<0.0001*
GI [%]	0.44 ±0.35[a]	1.03 ±0.44[b]	0.78 ±0.38[c]	<0.0001*
PPD [mm]	1.54 ±0.30[a]	5.24 ±1.25[b]	6.24 ±0.54[c]	<0.0001*
CAL [mm]	0.00 ±0.00	6.63 ±1.58	6.90 ±0.51	0.348
PBI [%]	0.04 ±0.06[a]	1.92 ±0.75[b]	0.56 ±0.36[c]	<0.0001*
MBL [ng/mL]	809.22 ±109.41[a]	721.71 ±118.32[b]	844.91 ±111.49[c]	<0.0001*
SIRT-1 [ng/mL]	0.99 ±0.56	1.22 ±0.47	1.12 ±0.46	0.172
CRP [mg/L]	2.61 ±0.76	2.60 ±0.72	2.76 ±0.61	0.548

* statistically significant ($p < 0.05$, one-way ANOVA). Different superscript letters indicate statistical differences between the groups.

the 3 groups from baseline to recall was significant. Conversely, SIRT-1 and CRP levels did not demonstrate a statistically significant difference from baseline to recall, with *p*-values of 0.172 and 0.548, respectively. A comparison of the CAL values between groups II and III revealed no statistically significant differences. (Table 3).

Table 4 presents the pairwise comparison of biochemical parameters between the 3 groups at 2 time points. The mean difference in MBL between groups I and II at recall (87.51 ng/mL (95% confidence interval (*CI*): 23.19, 151.84)) was statistically significant ($p = 0.005$). Furthermore, the difference between groups II and III was significant (−123.21 ng/mL (95% *CI*: −187.53, −58.88)), with a *p*-value <0.0001. The results of the pairwise analysis for SIRT-1 indicated that the mean difference at baseline between groups I and III was 0.28 ng/mL (95% *CI*: 0.01, 0.56), which was significant ($p = 0.041$). For CRP, significant differences were observed at baseline among all groups, yet no significant differences were noted at recall.

Table 4. Pairwise comparison of biochemical parameters between the study groups at baseline and 6 weeks after non-surgical periodontal therapy (NSPT)

Biochemical parameter	Group		Baseline		Recall	
			mean difference (95% *CI*)	*p*-value	mean difference (95% *CI*)	*p*-value
MBL [ng/mL]	group I	group II	−169.53 (−348.72, 9.64)	0.068	87.51 (23.19, 151.84)	0.005*
		group III	−397.71 (−576.88, −218.53)	<0.0001*	−35.73 (−100.02, 28.63)	0.387
	group II	group III	−228.17 (−407.35, −49.01)	0.009*	−123.21 (−187.53, −58.88)	<0.0001*
SIRT-1 [ng/mL]	group I	group II	0.04 (−0.23, 0.32)	0.922	−0.23 (−0.51, 0.06)	0.146
		group III	0.28 (0.01, 0.56)	0.041*	−0.12 (−0.41, 0.16)	0.558
	group II	group III	0.24 (−0.03, 0.51)	0.100	0.11 (−0.18, 0.39)	0.667
CRP [mg/L]	group I	group II	−2.08 (−2.55, −1.62)	<0.0001*	0.01 (−0.39, 0.41)	0.998
		group III	−2.91 (−3.36, −2.43)	<0.0001*	−0.15 (−0.55, 0.24)	0.630
	group II	group III	−0.81 (−1.27, −0.35)	<0.0001*	−0.16 (−0.56, 0.23)	0.589

* statistically significant ($p < 0.05$, Tukey's post hoc test).

Discussion

Periodontitis is defined as the dynamic interplay among pathogenic microbes and the host inflammatory response, which promotes the destruction of connective tissue and alveolar bone.[22] Clinical parameters and radiographs that measure alveolar bone levels only provide information about past periodontal tissue destruction and do not elucidate current nor predict future disease activity due to their low sensitivity and positive predictive value. Recently, molecular determinants including enzymes, cytokines, receptors, and other proteins, have been used as potential biomarkers to establish a more biologically-based diagnostic approach and explain associations between periodontitis and systemic diseases.[23]

Groups II and III were characterized by a higher average age and a comparatively poor level of oral hygiene. Group III exhibited a preponderance of male participants, attributable to social and religious inhibitions concerning smoking among female subjects. The group demonstrated a higher degree of tissue destruction, as evidenced by the values of PPD and CAL, which are indicative of an increased severity of the disease. The patients in group III also exhibited lower GI and PBI and greater PI values, which is consistent with the findings of the previous studies.[14,24,25] Nicotine has been shown to stimulate the sympathetic ganglia, leading to the production of neurotransmitters such as catecholamines. These neurotransmitters activate alpha receptors on blood vessels, resulting in vasoconstriction and further enhancing periodontal tissue breakdown. Smokers exhibit a reduced manifestation of gingivitis and diminished gingival blood flow when compared to non-smokers.[26] Thus, smoking reduces the clinical signs of inflammation, including bleeding on probing, while concealing gingival inflammation.

In the current study, a significant improvement in the clinical parameters (PPD, CAL, PBI, PI, and GI) was observed 6 weeks after NSPT, which is consistent with the findings reported in previous literature.[27] Clinical studies have demonstrated that NSPT reduces the overall number of gingival sites that exhibit bleeding on probing, allowing the oral microbiota to shift from gram-negative to gram-positive bacteria.[27]

The present study is one of the few trials evaluating periodontal parameters and correlating them with the serum levels of MBL, SIRT-1 and CRP in smokers and non-smokers with stage III periodontitis, as well as determining the influence of NSPT on these biomarkers. It highlights the role of MBL, SIRT-1 and CRP in regulating inflammation and oxidative stress among smokers and non-smokers with severe periodontitis.

The enhanced severity of the periodontal disease, as manifested through various clinical parameters, is consistent with the results of the current study. The study indicates that systemic MBL levels were significantly higher in groups III and II than in group I. This rise can be attributed to an increased inflammatory burden. The findings corroborate the results of previous studies.[28] However, Maffei et al. did not demonstrate elevated serum MBL levels in periodontitis when compared with healthy controls.[29] Instead, they identified higher plasma MBL levels in smokers,[29] which is consistent with the results of the present study. In this study, the mean MBL levels were higher in group III (844.91 ±111.49 ng/mL) than in group II (721.71 ±118.32 ng/mL). Similarly, Louropoulou et al. reported an increase in serum MBL levels in patients with periodontitis, including those with deficient MBL production.[30] The possible explanations for these discrepancies may be found in the inclusion of smokers in the control group in the aforementioned study. Additionally, some authors have identified MBL deficiency inadvertently with serum MBL levels. Six weeks after the intervention, the results demonstrated a significant decrease in MBL concentrations compared to the baseline levels, as well as a significant mean difference across the 3 groups. This finding points to anti-inflammatory benefits of NSPT on serum MBL levels.

Sirtuin 1 is the most studied member of the sirtuin family. Recent investigations have identified considerable shifts in the levels of several sirtuin family proteins in periodontitis.[31] In the present study, the levels of SIRT-1 were found to be the highest in group I, and further decreased in groups II and III. It has been previously demonstrated that mitochondrial oxidative stress was caused by a decrease in SIRT-1 expression, which can be linked to the oxidative stress seen in smokers.[32] Thus, smoking might have decreased SIRT-1 levels to activate lung fibroblasts by promoting mitochondrial oxidative stress, which dysregulated lipid metabolism due to impaired autophagy flux. Additionally, an increase in serum levels of SIRT-1 after NSPT was evident in groups II and III. This phenomenon can be attributed to a reduction in the severity of periodontitis due to decreased oxidative stress. This finding aligns with the studies suggesting that NSPT leads to elevated serum concentrations of SIRT-1 in individuals with periodontitis, irrespective of their smoking status.

The concentration of CRP is the greatest in serum, with certain infectious diseases raising its levels up to 1,000-fold. When the stimuli are removed, CRP levels drop exponentially over 18–20 h, a timeframe that closely aligns with the CRP half-life.[33] Thus, this study analyzed serum CRP levels to ensure the attainment of more accurate results. C-reactive protein, an acute phase marker of inflammation, was significantly increased in group II and III participants with stage III periodontitis, as compared to group I. At baseline, greater CRP levels were observed in group III when compared to group II. The mean difference between the 2 groups was –0.81 mg/L ($p < 0.0001$). The results of the present study indicate an improvement in CRP levels after NSPT, which could be attributed to the beneficial effects of NSPT that ultimately reduce the inflammatory burden within the periodontium. The available literature on the

above aspects also supports this understanding. Mechanical periodontal treatment has been shown to reduce serum CRP and markers of systemic inflammation.[34] In contrast to the present study, Ide et al. conducted a study to determine whether treatment of periodontal disease would result in a decrease in circulating acute phase proteins.[35] However, the authors did not observe a reduction in circulating CRP following NSPT.[35] A potential rationale for the persistent elevation of CRP, even subsequent to scaling and root planning, is that this procedure may prove inadequate in fully controlling the progression of periodontal disease in subjects with periodontitis. The complete elimination of microorganisms and deposits from deep inaccessible pockets may require surgical intervention and/or the use of antimicrobial agents.

Results of the present study indicate a direct correlation between MBL and CRP levels, as well as an inverse relationship between MBL and CRP values with SIRT-1.

Limitations

The limitations associated with this case–control study include a relatively small sample size with an unequal age and sex distribution. Due to the limited follow-up, timely changes in serum biomarker levels cannot be identified. Longitudinal investigations are required to examine potential clinical benefits and to identify the phased alterations that will enable us to draw definitive and consistent conclusions. The sample was representative of a severe periodontitis population, exhibiting high levels of clinical disease, bleeding and dental plaque. Consequently, the findings of this study may only be applicable to patients with milder forms of periodontitis.

Conclusions

Non-surgical periodontal therapy is an effective modality for the reduction in clinical parameters of PPD and PBI, along with a gain in CAL. It also leads to a decrease in serum concentrations of MBL and CRP and an increase in SIRT-1 levels in smokers and non-smokers diagnosed with stage III periodontitis. A positive association of serum MBL levels, CRP levels, and a negative association of SIRT-1 with the severity of periodontal disease may be a valuable, precise and feasible method for identifying individuals at risk of developing periodontal disease. In addition, these markers could facilitate the confirmation of a diagnosis and the assessment of periodontitis severity. Additionally, they may aid in determining the prognosis of the smoking-associated inflammatory burden on the periodontium. These can be used as predictive biomarkers for disease development and as diagnostic markers in the evaluation of inflammation within periodontal tissues. Further clinical trials assessing the periodontal status in patients with periodontitis are needed to establish the links between MBL, SIRT-1 and CRP and the severity of periodontal disease.

Trial registration

This clinical study was registered using the Clinical Trial Registry-India (CTRI), the main registry of the WHO International Clinical Trials Registry Platform (ICTRP) (registration No. CTRI/2021/01/030833).

Ethics approval and consent to participate

This clinical study received the approval of the Institutional Ethics Committee of Ranjeet Deshmukh Dental College and Research Centre, and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from patients who were willing to participate in the trial after the objectives and procedures were outlined to them.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Evaluation of the periodontal status and systemic diseases in dental patients in Turkey

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Abstract

Background. Periodontal diseases are chronic inflammatory diseases related to many hereditary, environmental, physical, and psychological factors.

Objectives. The aim of the present study was to determine the demographic data, periodontal status and systemic conditions of patients who reported to the Department of Oral and Maxillofacial Radiology, and to evaluate the relationship between the periodontal status and the associated risk factors.

Material and methods. The study included a total of 2,000 patients (974 males and 1,026 females). The anamnesis data on the patients' systemic diseases/conditions and periodontal diagnoses were analyzed statistically.

Results. Upon evaluating the clinical and radiological examination data, the findings were as follows: 9.20% of patients had clinically healthy gingiva; 15.20% had gingivitis on an intact periodontium; 22.75% had gingivitis on a reduced periodontium (in total, 37.95% had gingivitis); 47.20% had mild to moderate periodontitis (stages I and II, grades A and B); and 3.45% had severe periodontitis (stages III and IV, grades B and C) (in total, 50.65% had periodontitis). Additionally, 2.20% of patients were diagnosed with necrotizing periodontal diseases (NPD). Gingival health was significantly poorer in patients with hypertension, diabetes, gastrointestinal system diseases (GID), cardiovascular system diseases (CVD), gynecological disorders, psychiatric disorders, hypothyroidism, rheumatological diseases, osteoporosis, chronic respiratory diseases/asthma, anemia, a history of cancer, and dermatological problems ($p < 0.001$).

Conclusions. Periodontitis is a significant public health concern among the Turkish population, with high incidence rates of mild to moderate periodontitis attributed to risk factors such as age, smoking and various systemic diseases.

Keywords: epidemiology, diabetes, periodontal disease, risk factors, systemic disease

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Highlights

- Periodontitis is highly prevalent in the Turkish population, with over 50% of patients affected, mostly in mild to moderate stages.
- Systemic diseases, such as hypertension, diabetes and hypothyroidism, are significantly associated with poorer gingival health ($p < 0.001$).
- These findings highlight the importance of a multidisciplinary approach in periodontal care, especially for patients with systemic health conditions.

Introduction

Periodontal diseases are chronic inflammatory diseases primarily caused by microbial dental plaque; they are related to various hereditary, environmental, physical, and psychological factors.[1] Host-related factors, such as age, gender, systemic diseases, smoking, and the psychological status, can have a significantly impact on disease progression. Therefore, obtaining a detailed patient history is essential for establishing a treatment plan for a periodontal disease, and identifying modifiable and non-modifiable risk factors.[1–6]

Numerous studies have shown that smoking, a modifiable risk factor, increases the development and progression of periodontal diseases.[7–10] In addition, research indicates improvement in the prognosis of periodontal diseases among patients who quitted smoking.[10,11]

Age and gender are non-modifiable risk factors associated with periodontal diseases. Studies show an increase in the prevalence and severity of periodontal diseases with advancing age.[12,13] Furthermore, periodontal diseases are more prevalent in males than females, which is attributed partly to genetic predisposition and differences in oral hygiene practices among male individuals.[6,14] Educating patients about smoking cessation and oral hygiene is important for improving the prognosis of periodontal diseases.[2,5,7,8]

Recent research confirms that periodontal diseases affect systemic health beyond oral cavity involvement. Studies have shown a link between periodontitis and preterm birth, low birth weight and systemic diseases, such as cardiovascular system diseases (CVD), diabetes, inflammatory joint disorders, and chronic kidney disease.[1,15–19] Moreover, deficiencies in essential vitamins and imbalance in the antioxidant–oxidant level, including conditions such as hyperhomocysteinemia, influence the development and prognosis of periodontitis.[20]

In 2017, the American Academy of Periodontology and the European Federation of Periodontology (AAP-EFP) introduced a new classification. In comparison with the AAP's 1999 classification, this updated version is more comprehensive and includes more detailed clinical diagnostic information. The AAP 1999 classification categorized patients based on the clinical pocket measurements (the level of periodontal attachment loss), distinguishing between localized or generalized periodontitis according to disease involvement, and between aggressive or chronic periodontitis according to disease severity.[21] In contrast, the AAP-EFP 2017 classification categorizes patients by staging (stages I–IV) and grading (grades A–C) according to the clinical attachment level (CAL) or interdental bone loss (IBL) and the number of lost teeth.[22] With the use of the new classification, the current periodontal disease can be addressed in various aspects.[22–25]

There are very few studies analyzing both the systemic and periodontal status, using the new AAP-EFP 2017 classification. Epidemiological studies need to be updated periodically to incorporate new information, including emerging epidemics, advances in medicine and technology, and the use of natural products in traditional medicine and their effects.[26–28] The null hypothesis was that there would be no difference in the distribution of diagnoses made by means of the AAP-EFP 2017 periodontal disease classification in the presence of systemic health or systemic diseases among the Turkish population in 2017–2022.

This retrospective study aimed to assess the periodontal disease and health status, systemic diseases/conditions, and the relationship between the periodontal status and patient risk factors among individuals visiting the Department of Oral and Maxillofacial Radiology at Antalya Bilim University, Turkey, in 2017–2022.

Material and methods

Our study adhered to the principles outlined in the Declaration of Helsinki adopted in 1975 (revised in 2013). The study protocol (No. 2022/08) was approved by the Clinical Research Ethics Committee at Niğde Ömer Halisdemir University, Turkey. This retrospective study analyzed the records of individuals who underwent their

initial dental examination at the Department of Oral and Maxillofacial Radiology at Antalya Bilim University between January 2017 and January 2022. Following their initial assessment, patients requiring periodontal treatment were referred to the periodontology clinic. The researchers retrieved data from the archives of the Faculty of Dentistry, encompassing the records of 3,000 patients, of whom 2,000 met the inclusion criteria.

The inclusion criteria specified patients who visited the clinic between January 2017 and January 2022, with complete demographic data, information about smoking habits, a medical history, and comprehensive clinical periodontal examination data, including the whole periodontal pocket depth (PPD), the gingival index (GI),[29] the plaque index (PI),[29] the number of missing teeth, and tooth mobility. In addition, complete radiological examination data on interdental alveolar bone loss was required. Patients diagnosed under the new periodontal disease classification were eligible for inclusion. The exclusion criteria encompassed individuals with missing data, those unwilling to participate in the study, patients diagnosed under the 1999 periodontal disease classification, and pregnant or lactating women.

Demographic and medical status data

Patient data was extracted from standard patient files, and included demographic details (age, gender), smoking habits, and self-reported systemic diseases or their absence.

Periodontal evaluation

Periodontal disease diagnoses were made by expert periodontologists at the Faculty of Dentistry of Antalya Bilim University in accordance with the following and were recorded on the patient forms. These patient forms were the primary data source for our retrospective study. The diagnoses were categorized according to the AAP-EFP 2017 classification, using clinical and radiological examination data, and the patients were divided into specific diagnostic groups.

Patients with no bleeding on probing (BoP) or BoP ≤ 10%, no signs of inflammation (edema, hyperemia), and no clinical or radiological evidence of CAL or IBL were included in the "gingival health on an intact periodontium" (GH+iP) group. Patients without gingival inflammation, with PPD ≤ 3 mm, but with decreased periodontium due to a previous periodontal disease were included in the "gingival health on a reduced periodontium" (GH+rP) group. These 2 subgroups constituted the "gingival health" group. Patients with gingival inflammation and PPD ≤ 3 mm were included in the "gingivitis on an intact periodontium" (G+iP) group if there was no bone loss and no decrease in the periodontium; if there was a decrease in the periodontium, they were included in the "gingivitis on a reduced periodontium" (G+rP) group. These 2 subgroups together constituted the "gingivitis" group.

Periodontitis was categorized into stages (I–IV) and grades (A–C) based on CAL or IBL and the number of lost teeth. Stages were determined by the severity and complexity of periodontitis as follows:

- stage I: Onset of periodontitis (0–15% IBL);
- stage II: Moderate periodontitis (15–33% IBL);
- stage III: Severe periodontitis (>33% IBL) with possible tooth loss; and
- stage IV: Severe periodontitis (>33% IBL) with potential for a complete loss of dentition.

The mild to moderate periodontitis (MP; chronic periodontitis according to the AAP's 1999 classification) group included patients classified as stages I and II, and grades A and B (IBL: 0–33% and %IBL/age: 0–1).

The severe periodontitis (SP; aggressive periodontitis according to the AAP's 1999 classification) group included patients classified as stages III and IV, and grades B and C (IBL > 33% and %IBL/age > 0.25).[22]

In addition, the "necrotizing periodontal diseases" (NPD) group comprised "necrotizing gingivitis" (NG) and "necrotizing periodontitis" (NP) patients,[30] as per the classification system.

Sample size estimation

Sample size estimation and power analysis were performed using the G*power software, v. 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), and the one-way analysis of variance (ANOVA) test. The calculations indicated a requirement of at least 228 patients for the 4 main diagnostic groups (gingival health, gingivitis, periodontitis, and NPD) and a minimum of 296 patients for the 8 detailed subgroups (GH+iP, GH+rP, G+iP, G+rP, MP, SP, NG, NP), with 90% power and a significance level (α) of 0.05.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA). Descriptive statistics for quantitative variables are presented as median (*Me*) (min–max), since the data was not normally distributed. In addition, the mean and standard deviation ($M \pm SD$) values are provided as well. Qualitative data is reported as frequency and percentage (n (%)). Normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Group comparisons were done using the non-parametric Kruskal–Wallis test, followed by post hoc pairwise comparisons to identify significant differences. The χ^2 test was used to analyze relationships and ratios with regard to qualitative data. Statistical significance was determined at $p < 0.05$.

Results

In our study, data from 2,000 patients was analyzed. The sample consisted of 974 (48.70%) males and 1,026 (51.30%) females, with a mean age of 42.33 ±14.11 years (40.15 ±11.66 years for males and 38.22 ±10.11 years for females). The mean age across different periodontal health status groups were as follows: 25.93 ±11.33 years for the periodontally healthy individuals; 25.33 ±7.66 years for the gingivitis patients; 46.54 ±13.33 years for the periodontitis patients; and 33.47 ±10.33 years for the patients with NPD. The periodontitis patients were on average significantly older as compared to other groups ($p < 0.001$). In addition, a significant difference in terms of age was found between the healthy and periodontitis groups (post hoc) ($p < 0.05$).

The periodontal status according to gender is shown in Table 1. Among male patients, 72 were periodontally healthy (12 GH+iP, 60 GH+rP), 338 had gingivitis

Table 1. Periodontal status according to gender

Gender	Periodontal status													*p*-value
	gingival health			gingivitis			periodontitis			NPD			total	
	GH+iP	GH+rP	total	G+iP	G+rP	total	MP	SP	total	NG	NP	total		
Male	12 (0.60)[a]	60 (3.00)[a]	72 (3.60)[a]	121 (6.05)	217 (10.85)[a]	338 (16.90)	516 (25.80)	27 (1.35)[a]	543 (27.15)	12 (0.60)[a]	9 (0.45)[a]	21 (1.05)[a]	974 (48.70)	<0.001*
Female	30 (1.50)[a]	82 (4.10)[a]	112 (5.60)[a]	183 (9.15)	238 (11.90)[a]	421 (21.05)	428 (21.40)	42 (2.10)[a]	470 (23.50)	16 (0.80)[a]	7 (0.35)[a]	23 (1.15)[a]	1,026 (51.30)	<0.001*
Total	42 (2.10)	142 (7.10)	184 (9.20)	304 (15.20)	455 (22.75)	759 (37.95)	944 (47.20)	69 (3.45)	1,013 (50.65)	28 (1.40)	16 (0.80)	44 (2.20)	2,000 (100.00)	<0.001*

Data presented as number (percentage) (*n* (%)).
Groups: GH+iP – gingival health on an intact periodontium; GH+rP – gingival health on a reduced periodontium; G+iP – gingivitis on an intact periodontium; G+rP – gingivitis on a reduced periodontium; MP – mild to moderate periodontitis; SP – severe periodontitis; NG – necrotizing gingivitis; NP – necrotizing periodontitis. NPD – necrotizing periodontal diseases; * statistically significant; [a] no statistically significant difference between the groups.

Table 2. Distribution of periodontal diagnoses by patient groups (systemic diseases)

Systemic disease	Periodontal status													*p*-value
	gingival health*			gingivitis*			periodontitis*			NPD*			total	
	GH+iP*	GH+rP*	total	G+iP*	G+rP*	total	MP*	SP*	total	NG*	NP*	total		
None*	26 (1.30)	62 (3.10)	88 (4.40)	163 (8.15)	134 (6.70)	297 (14.85)	125 (6.25)	27 (1.35)	152 (7.60)	8 (0.40)[a]	0 (0.00)[a]	8 (0.40)[a]	545 (27.25)	<0.001*
Hypertension	3 (0.15)[a]	13 (0.65)[a]	16 (0.80)[a,b]	8 (0.40)	47 (2.35)	55 (2.75)[b]	296 (14.80)[c]	5 (0.25)	301 (15.05)[c]	0 (0.00)	0 (0.00)[a]	0 (0.00)	372 (18.60)	<0.001*
Diabetes	5 (0.25)[a]	14 (0.70)[a]	19 (0.95)[a,b]	21 (1.05)	33 (1.65)	54 (2.70)[b]	107 (5.35)[c]	13 (0.65)	120 (6.00)[c]	5 (0.25)[a]	2 (0.10)	7 (0.35)[a]	200 (10.00)	<0.001*
GID	3 (0.15)[a]	10 (0.50)	13 (0.65)	16 (0.80)	56 (2.80)	72 (3.60)	34 (1.70)	3 (0.15)[a]	37 (1.85)	3 (0.15)[a]	2 (0.10)[a]	5 (0.25)	127 (6.35)	<0.001*
CVD	2 (0.10)[a]	3 (0.15)[a]	5 (0.25)	6 (0.30)[a]	34 (1.70)	40 (2.00)	76 (3.80)	4 (0.20)[a]	80 (4.00)	0 (0.00)[a]	0 (0.00)[a]	0 (0.00)	125 (6.25)	<0.001*
Gynecological disorders	0 (0.00)	8 (0.40)	8 (0.40)	19 (0.95)	34 (1.70)	53 (2.65)	30 (1.50)	1 (0.05)	31 (1.55)	2 (0.10)	0 (0.00)	2 (0.10)	94 (4.70)	<0.001*
Psychiatric disorders	2 (0.10)	3 (0.15)	5 (0.25)	20 (1.00)	16 (0.80)	36 (1.80)	28 (1.40)	4 (0.20)	32 (1.60)	7 (0.35)	1 (0.05)	8 (0.40)	81 (4.05)	<0.001*
Hypothyroidism	1 (0.05)	11 (0.55)	12 (0.60)[a]	22 (1.10)	28 (1.40)	50 (2.50)	13 (0.65)[a]	1 (0.05)	14 (0.70)	1 (0.05)	0 (0.00)	1 (0.05)	77 (3.85)	<0.001*
Rheumatological diseases	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.10)	8 (0.40)	10 (0.50)	61 (3.05)	4 (0.20)	65 (3.25)	1 (0.05)	0 (0.00)	1 (0.05)	76 (3.80)	<0.001*
Osteoporosis	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	5 (0.25)	5 (0.25)	50 (2.50)	4 (0.20)	54 (2.70)	0 (0.00)	0 (0.00)	0 (0.00)	59 (2.95)	<0.001*
Chronic respiratory diseases/asthma	0 (0.00)	1 (0.05)	1 (0.05)	6 (0.30)	12 (0.60)	18 (0.90)	26 (1.30)	0 (0.00)	26 (1.30)	1 (0.05)	0 (0.00)	1 (0.05)	46 (2.30)	<0.001*
Anemia	0 (0.00)	6 (0.30)	6 (0.30)	3 (0.15)	15 (0.75)	18 (0.90)	20 (1.00)	0 (0.00)	20 (1.00)	0 (0.00)	0 (0.00)	0 (0.00)	44 (2.20)	<0.001*
History of cancer	0 (0.00)	3 (0.15)	3 (0.15)	4 (0.20)	5 (0.25)	9 (0.45)	10 (0.50)	1 (0.05)	11 (0.55)	0 (0.00)	4 (0.20)	4 (0.20)	27 (1.35)	<0.001*
Dermatological problems	0 (0.00)	3 (0.15)	3 (0.15)	0 (0.00)	5 (0.25)	5 (0.25)	16 (0.80)	0 (0.00)	16 (0.80)	0 (0.00)	0 (0.00)	0 (0.00)	24 (1.20)	<0.001*

Data presented as *n* (%).
GID – gastrointestinal system diseases; CVD – cardiovascular system diseases; * statistically significant difference; [a] no statistically significant differences among the periodontal status groups; [b] no statistically significant differences between the hypertension and diabetes subgroups; [c] statistically significant differences between the hypertension and diabetes subgroups.

(121 G+iP, 217 G+rP), 543 had periodontitis (516 MP, 27 SP), and 21 had NPD (12 NG, 9 NP). Among female patients, 112 were periodontally healthy (30 GH+iP, 82 GH+rP), 421 had gingivitis (183 G+iP, 238 G+rP), 470 had periodontitis (428 MP, 42 SP), and 23 had NPD (16 NG, 7 NP). Gender differences were significant in diagnosing gingivitis and periodontitis ($p < 0.001$), but no significant difference was found between the genders in diagnosing other diseases ($p > 0.05$).

The distribution of periodontal diagnoses by patient groups is shown in Tables 2 and 3. Systemic diseases in the tables are listed from the most to the least common. Of all patients included in the study, 72.75% had a systemic disease. The presence of a systemic disease significantly increased the likelihood of a periodontitis diagnosis ($p < 0.001$).

In the comparison of the hypertension and diabetes patients, a statistically higher percentage of patients with MP was found among the hypertensives than in the diabetes subgroup (14.80% vs. 5.35%, respectively). However, in contrast to the hypertensive patient subgroup, among the diabetes patients, some had NG (0.25%) or NP (0.10%). Except for the MP and NPD diagnoses, no significant differences between the 2 subgroups were found in the distribution of periodontal diagnoses. In the patients with gastrointestinal system diseases (GID), gingivitis prevalence was significantly higher as compared to other periodontal conditions ($p < 0.001$). Among the CVD patients, significant differences between periodontal conditions were found ($p < 0.001$); NPD were absent in the CVD subgroup. The patients with gynecological disorders showed significant differences in periodontal conditions ($p < 0.001$). The patients with psychiatric disorders, hypothyroidism and chronic respiratory diseases/asthma showed significant differences with regard to the gingival health, gingivitis, periodontitis, and NPD groups ($p < 0.001$). Periodontitis was notably higher than other conditions in the rheumatological disease and osteoporosis patient subgroups ($p < 0.001$). The patients with anemia also showed significant differences among the gingival health, gingivitis and periodontitis groups ($p < 0.001$).

In our study, gingival health was significantly poorer in patients with hypertension, diabetes, GID, CVD, gynecological disorders, psychiatric disorders, hypothyroidism, rheumatological diseases, osteoporosis, chronic respiratory diseases/asthma, anemia, a history of cancer, and dermatological disorders (Table 2).

The distribution and number of patients in the mental retardation and disability, kidney disease, hepatitis B, hypotension, human immunodeficiency virus (HIV)-positive, familial Mediterranean fever (FMF), hyperthyroidism, Parkinson's disease, Alzheimer's disease, and hepatitis C subgroups were not suitable for statistical analysis in our study (p-value unavailable) (Table 3).

The distribution of smoking according to diagnoses is shown in Table 4. Of all patients, 662 (33.10%) were smokers. All patients diagnosed with NPD were smokers. Statistically significant differences were found between the clinical gingival health, gingivitis, periodontitis, and NPD (both NG and NP) groups regarding the smoking status ($p < 0.001$). In the gingival health group there were more non-smokers than smokers ($p < 0.001$).

Table 3. Distribution of periodontal diagnoses by patient groups (other systemic diseases)

Systemic disease	Periodontal status												total	*p*-value
	gingival health			gingivitis			periodontitis			NPD				
	GH+iP	GH+rP	total	G+iP	G+rP	total	MP	SP	total	NG	NP	total		
Mental retardation and disability	0 (0.00)	0 (0.00)	0 (0.00)	7 (0.35)	2 (0.10)	9 (0.45)	10 (0.50)	1 (0.05)	11 (0.55)	0 (0.00)	0 (0.00)	0 (0.00)	20 (1.00)	unavailable
Kidney diseases	0 (0.00)	1 (0.05)	1 (0.05)	1 (0.05)	2 (0.10)	3 (0.15)	12 (0.60)	1 (0.05)	13 (0.65)	0 (0.00)	0 (0.00)	0 (0.00)	17 (0.85)	unavailable
Hepatitis B	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.15)	8 (0.40)	11 (0.55)	4 (0.20)	0 (0.00)	4 (0.20)	0 (0.00)	0 (0.00)	0 (0.00)	15 0.75)	unavailable
Hypotension	0 (0.00)	2 (0.10)	2 (0.10)	0 (0.00)	5 (0.25)	5 (0.25)	7 (0.35)	0 (0.00)	7 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	14 (0.70)	unavailable
HIV-positive	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.05)	1 (0.05)	2 (0.10)	0 (0.00)	2 (0.10)	0 (0.00)	7 (0.35)	7 (0.35)	10 (0.50)	unavailable
FMF	0 (0.00)	1 (0.05)	1 (0.05)	3 (0.15)	2 (0.10)	5 (0.25)	4 (0.20)	0 (0.00)	4 (0.20)	0 (0.00)	0 (0.00)	0 (0.00)	10 (0.50)	unavailable
Hyperthyroidism	0 (0.00)	1 (0.05)	1 (0.05)	0 (0.00)	1 (0.05)	1 (0.05)	5 (0.25)	0 (0.00)	5 (0.25)	0 (0.00)	0 (0.00)	0 (0.00)	7 (0.35)	unavailable
Parkinson's disease	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.20)	0 (0.00)	4 (0.20)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.20)	unavailable
Alzheimer's disease	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.05)	1 (0.05)	3 (0.15)	0 (0.00)	3 (0.15)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.20)	unavailable
Hepatitis C	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.05)	1 (0.05)	1 (0.05)	0 (0.00)	1 (0.05)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.10)	unavailable

Data presented as *n* (%).
HIV – human immunodeficiency virus; FMF – familial Mediterranean fever.

Table 4. Distribution of periodontal diagnoses according to the smoking status

Smoking status	Periodontal status													*p*-value
	gingival health*			gingivitis*			periodontitis*			NPD			total	
	GH+iP*	GH+rP*	total	G+iP*	G+rP*	total	MP*	SP*	total	NG[a]	NP[a]	total		
Non-smokers	36 (1.80)	95 (4.75)	131 (6.55)	268 (13.40)	360 (18.00)	628 (31.40)	437 (21.85)	60 (3.00)	497 (24.85)	0 (0.00)	0 (0.00)	0 (0.00)	1,256 (62.80)	<0.001*
Current smokers	4 (0.20)	16 (0.80)	20 (1.00)	28 (1.40)	75 (3.75)	103 (5.15)	486 (24.30)	9 (0.45)	495 (24.75)	28 (1.40)	16 (0.80)	44 (2.20)	662 (33.10)	<0.001*
Ex-smokers	2 (0.10)	31 (1.55)	33 (1.65)	8 (0.40)	20 (1.00)	28 (1.40)	21 (1.05)	0 (0.00)	21 (1.05)	0 (0.00)	0 (0.00)	0 (0.00)	82 (4.10)	<0.001*
Total	42 (2.10)	142 (7.10)	184 (9.20)	304 (15.20)	455 (22.75)	759 (37.95)	944 (47.20)	69 (3.45)	1,013 (50.65)	28 (1.40)	16 (0.80)	44 (2.20)	2,000 (100.00)	<0.001*

Data presented as *n* (%).
* statistically significant difference; [a] no statistically significant difference between the groups.

Discussion

Periodontal diseases are chronic infectious diseases caused by periodontopathogenic bacteria. They are prevalent in societies and influenced by various physical and environmental factors.[7,31] Systemic diseases, such as diabetes and CVD, are host-associated risk factors. The literature confirms that the systemic conditions of the host can impact the onset and prognosis of periodontal diseases.[1–4,7,15,16,23] The prevalence of systemic diseases in the population is relatively high, reported between 39.9% and 52.5%.[32] Advancement in technology and medicine have led to increased lifespans for patients with systemic diseases, consequently increasing the demand for periodontal treatment to improve their quality of life (QoL).[5,7,13]

Epidemiological studies should be periodically repeated to take into account the changing living conditions.[26,28,33,34] In 2017, the classification of periodontitis was revised by AAP-EFP. The staging of periodontitis provides information on disease severity, extent and distribution within the dentition, as well as the complexity of management. The grading system provides supplemental information on future disease risk and progression.[22,24,25] This new classification also refers to systemic diseases (particularly diabetes) and the smoking status.[22] Our study utilized this classification to diagnose and evaluate the systemic diseases, smoking habits and periodontal status of Turkish patients with regard to the period between 2017 and 2022. Our study contributes to epidemiological research in the Turkish population by aligning with the current literature and the updated disease classifications.

The percentage of IBL was used to determine the stages of periodontitis in the study patients. The ratio %IBL/age was used to indirectly assess periodontitis progression. The new classification also provides information about disease severity and progression rates in patients diagnosed with periodontitis, indicating whether treatment may require both non-surgical and surgical approaches.[22,24,25] Whereas debridement and subgingival curettage may be sufficient treatment for stages I and II, surgical periodontal treatment is additionally recommended for stages III and IV.[22,24,25] In our study, IBL exceeded 33% in 7% of the periodontitis patients. In 93% of cases, IBL ranged from 0% to 33% and the ratio %IBL/age was <1. This suggests that non-surgical periodontal treatment is generally sufficient for treating periodontitis in this population. Although MP was significantly higher in the patients with systemic diseases as compared to the healthy group, the presence of MP in the systemically healthy group reveals the multifactorial nature of periodontitis.

In order to include periodontally healthy individuals in our study, we scanned the records of patients visiting the Department of Oral and Maxillofacial Radiology, where the initial examinations were conducted. Most studies in the literature on the Turkish population did not include periodontally healthy groups.[28,33,34] The periodontally healthy individuals in our study were referred to the periodontology clinic due to gingival recession, esthetic smile design, gingival operations prior to fixed prostheses, and implant requirements. Patient forms with data from periodontal clinical evaluations were filled by expert periodontologists. In our study, 2.10% of patients were in the GH+iP group, 7.10% in the GH+rP group (totaling 9.20% with healthy gingiva), 15.20% had G+iP, 22.75% had G+rP (totaling 37.95% with gingivitis), 47.20% had MP, 3.45% had SP (totaling 50.65% with periodontitis), 1.40% had NG, and 0.80% had NP (totaling 2.20% with NPD). Similarly, Akpınar et al. found gingivitis in 51.9% of patients and periodontitis in 47.8%.[28] Sezgin et al. reported 50.0% gingivitis patients, 39.8% periodontitis patients and 9.9% G+rP patients in their study.[34] Another study conducted at Adıyaman University, Turkey, reported gingivitis in 19.6% of patients and periodontitis in 79.3%.[33] These proportional discrepancies may be due to differences in the mean patient age, environmental conditions, geographic factors, the years of epidemiological research, and the use of alternate diagnostic classifications.[12,33] Although our study gave similar results to those in previous studies, it provided more comprehensive insights into periodontal disease severity and complexity, using a new classification.

In our study, the mean age of the periodontally healthy individuals was 25.93 ±11.33 years, it was 25.33 ±7.66 years

for the gingivitis patients, 46.54 ±13.33 years for the periodontitis patients, and 33.47 ±10.33 years in patients with NPD. Similar to other studies in the literature, it was observed that the prevalence of periodontal diseases increases with age.[7,12,28,33,34] This is thought to be due to changes in the immune system with aging and the cumulative effects of periodontal disease agents.[7,28]

In our study, MP was observed mainly in male patients, whereas more G+iP cases were diagnosed among female patients, and this difference was statistically significant ($p < 0.001$). There were no significant gender differences in diagnosing other diseases. In the study of Çalışır and Talmaç, similar to ours, the rate of gingivitis was 21.7% in females and 17.5% in males, and the rate of periodontitis was 81.7% in males and 77.0% in females.[33] Although not fully elucidated in the literature, this difference is attributed to periodontitis being more prevalent in males, possibly due to poorer oral hygiene, higher smoking rates and genetic factors.[7,11,14,23,28]

It has been widely demonstrated in studies that smoking is the strongest modifiable risk factor for periodontal diseases after bacterial plaque, and its role in the pathogenesis of periodontal diseases has been investigated extensively. Many studies indicate a reduced treatment response among smokers. In addition, approx. 40% of periodontitis cases are believed to be associated with active smoking.[35] The increased risk of periodontitis in smokers is associated with the negative effects of smoking on the immune system.[7,9,11] In our study, 51.48% of patients diagnosed with MP were smokers, whereas Çalışır and Talmaç reported a rate of 61.2%.[33] In addition, all patients diagnosed with NPD were smokers.

The incidence of NG varies among populations, depending on living conditions, sociocultural factors, the socioeconomic status, and genetic factors.[36–38] Recent data shows that the prevalence of necrotizing ulcerative gingivitis varies widely, from 6.70% in Chilean students aged 12–21 years[38] to 0.11% in the British Armed Forces.[37] In our current study, the rate of NG in the Turkish population was 1.40%. Furthermore, 25.0% (7/28) of NG patients in our study suffered from psychiatric disorders, such as depression.

Although the prevalence of NP varies across populations, it has been reported to range from 0% to 11%.[39] Its incidence increases notably in patients with HIV, immunosuppression and malnutrition.[40] In our study, NP was detected in 16 patients (0.80%). Among these patients, 2 had diabetes, 2 had GID, 1 had a psychiatric disorder, 4 had a history of cancer, and 7 had HIV. The inclusion of a relatively large number of HIV-positive patients in our study may have contributed to the higher incidence of NP.

Studies have shown that periodontitis, a chronic infectious disease, is associated with many systemic diseases and conditions, such as diabetes, CVD, and preterm birth/low birth weight.[1,7,15,16,23,32] In our study, 72.75% of patients had at least one systemic disease, and clinical gingival health was significantly lower in the patients with hypertension, diabetes, GID, CVD, gynecological disorders, psychiatric disorders, hypothyroidism, rheumatological diseases, osteoporosis, chronic respiratory diseases/asthma, anemia, a history of cancer, and dermatological problems.

Respiratory diseases associated with periodontal diseases include chronic obstructive pulmonary disease (COPD), emphysema, pneumonia, bronchitis, and asthma.[41] It was previously believed that the aspiration of periodontopathogenic bacteria into the lungs, along with enzymes present in saliva, facilitated their adhesion and colonization, thereby altering mucosal surfaces.[42] In a study by Yang et al., the risk of pneumonia was lower in periodontally treated patients with MP as compared to the general population.[43] In our study, gingivitis and MP were higher in the patients with respiratory tract disorders as compared to those with clinically healthy gingiva. Our current findings support a potential relationship between respiratory tract diseases and periodontal diseases.

It has been reported that the periodontal status is associated with GID (e.g., gastroesophageal reflux, gastritis, ulcerative colitis). *Helicobacter pylori*, one of the most important bacteria causing GID, can be found in saliva, the dorsum of the tongue and dental plaque, potentially being a risk factor for chronic periodontitis.[44,45] In our study, G+rP and MP were significantly more prevalent in the patients with GID. Vitamin deficiencies, often due to malabsorption, may explain the higher incidence of bone loss observed in these patients as compared to healthy individuals.[20]

Hormonal changes during puberty, the menstrual cycle, pregnancy, or menopause can affect gingival epithelium, connective tissue, microvascular structures, bone mineral density, and inflammatory responses, potentially contributing to periodontal diseases and severe tissue destruction.[46,47] In our study, the gynecology patient group included individuals receiving hormone supplements, such as progesterone and estrogen, for treatment. In these patients, the rates of gingivitis (53/94) and MP (30/94) were significantly higher than in those periodontally healthy (8/94).

Individuals with thyroid dysfunction are reported to be more susceptible to dental caries and periodontal diseases.[48] Although the exact reason for the increased incidence and severity of periodontal diseases in these patients is still unknown, it is hypothesized that decreased serum levels of thyroid hormones may contribute to increased bone loss due to periodontitis by promoting the activity of resorbing cells.[49] In our study, significant differences in the periodontal status were observed in the patients with hypothyroidism.

A higher incidence of periodontitis was found in patients with hypertension and diabetes, which is consistent with the literature. Similarly, an increased prevalence of periodontal diseases is observed in the presence of any

systemic disease. Çalışır and Talmaç detected systemic diseases in 46.0% of patients with gingivitis and 63.0% of patients with periodontitis.[33] The association between hypertension, diabetes, CVD, smoking and periodontal diseases has been well documented for years.[15,50] Although systemic diseases were not considered in the diagnosis under the 1999 classification of periodontal diseases, they were included in the new classification as of 2017. This emphasizes the importance of taking into consideration systemic diseases in diagnosis and encourages physicians to conduct more comprehensive medical interviews. The relationship between systemic and periodontal diseases, and the distribution of periodontal diagnoses are shown in Table 2. The presented differences in disease distribution under the new classification warrant further investigation in future studies.

Limitations

Physicians learn about the presence of systemic diseases from the patient's medical history, which is based on the patient's statement. The presence of undiagnosed systemic diseases, the management of systemic diseases, the specific medications used, and the routes of their administration could all affect the results.

Therefore, the limitations of our study include self-reported medical data, the lack of patient medication records, and no evaluation of the relationship between socioeconomic factors and other confounding variables and periodontal diseases. More comprehensive studies should be carried out using a multidisciplinary approach, involving medical doctors, as well as the analysis of gingival crevicular fluid (GCF) and saliva, and blood tests.

Conclusions

Current patient-oriented epidemiological approaches are important to understanding the status, distribution, risk factors, and treatment of periodontal diseases in the population. In comparison with the 1999 classification, the 2017 classification of periodontal diseases offers more comprehensive information for the diagnosis of periodontal diseases and should be used in current epidemiology studies, as it also aids in determining the treatment modality. Periodontitis is a public health problem in the Turkish population, with high incidence rates of mild to moderate periodontitis due to risk factors such as age, smoking and various systemic diseases. Given these risk factors, a multidisciplinary treatment approach is important. In addition, physicians should be aware of various risk factors that may exacerbate periodontal diseases and, therefore, take a comprehensive medical history. Early diagnosis enables the stabilization of periodontitis with minimal alveolar bone loss and facilitates the management of systemic diseases.

Ethics approval and consent to participate

The study adhered to the principles outlined in the Declaration of Helsinki adopted in 1975 (revised in 2013). The study protocol (No. 2022/08) was approved by the Clinical Research Ethics Committee at Niğde Ömer Halisdemir University, Turkey. Written informed consent was obtained from the patients for the study.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Frequency of odontogenic lesions in patients with impacted maxillary canines and the association with impaction characteristics

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Abstract

Background. Since impacted canines are a frequent eruptive anomaly, it is imperative to study their etiological aspects. A possible cause of impaction is the presence of odontogenic lesions close to the impacted canine.

Objectives. The aim of this study was to evaluate the frequency of odontogenic lesions in patients with impacted canines and their association with the characteristics of impaction.

Material and methods. This cross-sectional study was carried out with the scans of 93 impacted maxillary canines obtained from cone-beam computed tomography (CBCT) studies performed in 3 radiological centers. The selection criteria regarded male and female patients older than 12 years, and were based on the CBCT scans of impacted canines, showing unilateral or bilateral maxillary impaction, with or without odontogenic lesions. The position of the impacted canines, the sector of impaction, the presence or absence of a dentigerous cyst, an odontoma or follicular enlargement, as well as the total diameter of the lesion, were evaluated. The χ^2 test and logistic regression analysis were performed, and the level of statistical significance was set at $p < 0.05$.

Results. The frequency of odontogenic lesions near the canine impaction area was generally low (7.5% for a dentigerous cyst, 6.5% for follicular enlargement and 3.2% for a mesiodens). However, there was a significant association between the presence of a dentigerous cyst and buccal or mid-alveolar impacted canines ($p = 0.032$). The alpha and beta angles influenced the possibility of the occurrence of dentigerous cysts, with the alpha angle increasing the risk (B = 1.22; $p = 0.041$) and the beta angle decreasing the chance of developing a dentigerous cyst (18%) ($p = 0.024$).

Conclusions. The presence of odontogenic lesions in impacted maxillary canine cases is low, and involves mainly dentigerous cysts and follicular enlargement in buccal or mid-alveolar impacted canines. The alpha and beta angles may influence the development of dentigerous cysts.

Keywords: cone-beam computed tomography, impacted maxillary canines, odontogenic lesions

Highlights

- The occurrence of odontogenic lesions near the canine impaction area was generally low.
- Significant associations were found between dentigerous cysts and impacted canines in the buccal or mid-alveolar regions.
- The alpha angle influences the likelihood of developing dentigerous cysts.

Introduction

Eruptive processes can occasionally present anomalies due to obstruction in the trajectory of eruption. Dental impactions are primarily asymptomatic, and in the orthodontic diagnosis, they are initially identified by means of panoramic radiographs.[1–3] The maxillary canine is the 2nd most frequently impacted tooth (0.8–2.8%), and its position can divert into the palatal or buccal direction, inside or outside the arch, with the palatal position being the most prevalent with a ratio of 2:1, although some authors report a ratio of 3:1.[2–5] Even greater significant differences were reported with regard to canine eruption anomalies between age-matched individuals with and without Down syndrome.[6]

Impacted canines can be associated with odontogenic lesions that cause a mechanical obstacle preventing the normal eruption of the tooth; on the other hand, impaction damages the surrounding tissues and structures, and, in turn, lead to the occurrence of lesions.[3,4] These associations are determined using cone-beam computed tomography (CBCT), showing that the prevalence of canine impaction associated with some pathology ranges between 1.0% and 9.9% as compared to other teeth, and is surpassed only by third molars.[3–5] Likewise, previous studies assessed the thickness of the dental follicle of the impacted maxillary canine, and found that in 22% of cases, the dental follicle thickness exceeded 3 mm,[5] but no significant correlation was found between the dental follicle width and sex, the impaction side and localization.[7] Although panoramic and periapical radiographs are frequently used for the initial diagnosis, odontogenic tumors may require more detailed imaging observation. Therefore, greater visual capacity is required for this type of finding to avoid tissue overlapping and to perform a three-dimensional (3D) analysis.[8]

It is essential to know the prevalence of odontomas, supernumerary teeth, root dilacerations, lesions of traumatic origin, deciduous teeth without root resorption, congenital deformities, and cysts in association with impacted maxillary canines.[9–11] Three-dimensional images can help determine the anatomical variations presented by impacted teeth or the areas invaded by the above-mentioned lesions, and provide a more precise diagnosis.[12,13] It is worth mentioning that dentigerous cysts are the most frequent lesions, with a prevalence of 9.9%, and they affect the adjacent structures, including bones and roots.[14,15]

For the treatment planning of impacted maxillary canines, it is crucial to assess the presence of associated injuries and know the characteristics of the surrounding structures, as well as their condition before any procedure.[16,17] It also facilitates the determination of appropriate treatment alternatives for each case. Although some studies report data on the frequency of odontogenic lesions in patients with impacted maxillary canines, few carry out 3D evaluations or have an adequate sample size for extrapolation.[18–21] In this way, it would be possible to determine exactly what percentage of maxillary canine impactions could be the consequence of an odontogenic lesion and to know the real impact of lesions on the etiology of this condition. Thus, the aim of the present study was to evaluate the frequency of odontogenic lesions in patients with impacted maxillary canines, as well as the association between odontogenic lesions and impaction characteristics, using CBCT.

Materials and methods

This cross-sectional study was approved by the institutional Ethics and Research Committee at the School of Dentistry of the Scientific University of the South (Universidad Científica del Sur), Lima, Peru, with approval number 704-2021-POS70. The study was carried out in accordance with the Declaration of Helsinki.

The study sample included the CBCT scans of 93 impacted maxillary canines (30 male patients aged 16.92 ±4.24 years and 63 female patients aged 16.70 ±4.58 years), obtained from 3 radiological centers in 3 Latin American countries (Mexico, Colombia and Peru). The CBCT images were performed for reasons external to the present study. The inclusion criteria comprised CBCT scans of patients with impacted maxillary canines, patients over 12 years of age (at this age, the eruption of the maxillary canine must have occurred), of both sexes, with unilateral or bilateral maxillary impaction, in any sector of impaction according to the Ericson and Kurol classification,[22] with and without associated pathology, such as a compound odontoma, a dentigerous cyst or

a follicular cyst. The CBCT scans of patients with previous orthodontic treatment, syndromes or craniofacial anomalies, dental agenesis, ankylosis, or localized infection were excluded.

The sample size calculation was performed using a formula, with a confidence level of 95%, precision of 5%, and an estimated proportion of 6.5% for the frequency of odontogenic lesions associated with impacted maxillary canines (data obtained from a pilot study). Thus, a minimum of 93 impacted maxillary canines were necessary.

Collection of CBCT scans

Three different radiological centers located in Mexico, Colombia and Peru were requested for CBCT images with a field of view (FOV) of 8×8 cm and 10×10 cm. The images were analyzed using the CS 3D Imaging (Carestream Health, Inc., Rochester, USA), Blue Sky Plan (https://www.blueskyplan.com) and Xelis Dental-3DViewer (INFINITT Europe, Frankfurt am Main, Germany) software, chosen for their compatibility with the image output formats of the CBCT machines used (Vatech Co., Ltd., Yongin, South Korea, and Carestream Health, Inc.).

Training and calibration

A researcher was trained and calibrated by 3 orthodontists with more than 10 years of experience to carry out all the measurements of the qualitative variables. The weighted Cohen's kappa test was applied to determine the intra- and inter-evaluator calibration values, until values greater than 0.9 were achieved in all measurements.

Measurement of impacted maxillary canines

The position of the impacted maxillary canine (palatal, buccal or mid-alveolar), as well as the location of the impaction, whether unilateral or bilateral, were established. In addition, the sector of impaction was registered as follows according to the Erikson and Kurol classification:[22] sector 1 – between the mesial side of the first premolar to the distal side of the lateral incisor; sector 2 – between the distal side of the lateral incisor and its median axis; sector 3 – between the median axis of the lateral incisor and the distal side of the central incisor; sector 4 – between the distal of the central incisor and its median axis; and sector 5 – between the median axis of the central incisor and the dental midline (Fig. 1A).[22–25]

The height of impaction in millimeters in relation to the occlusal plane, as well as the angulation of the impacted maxillary canine in relation to the mid-sagittal plane (the alpha angle) and to the medium axis of the lateral incisor (the beta angle), were also measured (Fig. 1B).[22]

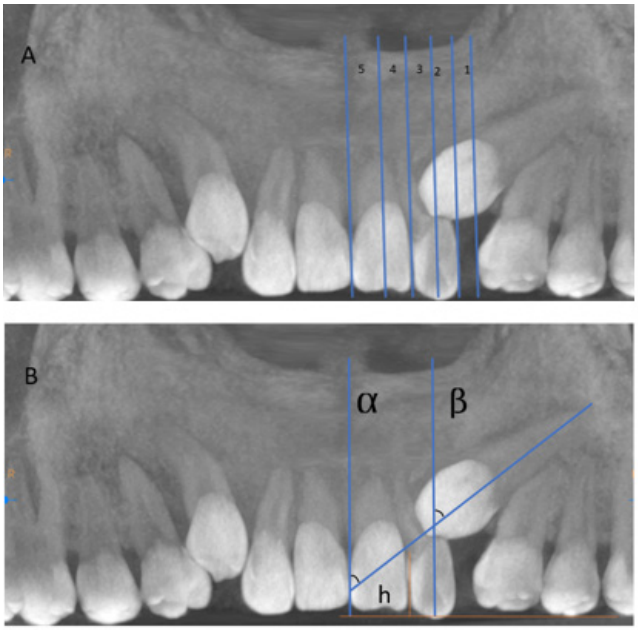

Fig. 1. Sector of impaction according to the Erikson and Kurol classification (A),[22] and the height of impaction [mm] in relation to the occlusal plane, as well as the angulation of the impacted maxillary canine in relation to the mid-sagittal plane (the alpha angle – α) and to the medium axis of the lateral incisor (the beta angle – β) (B)

Measurement of odontogenic lesions

The presence or absence of odontogenic lesions – dentigerous cysts, follicular cysts and compound odontomas – was evaluated. A dentigerous cyst was considered as the presence of the epithelium formed around the crown of the impacted tooth, showing a hypodense image with a well-defined sclerotic border around the crown of the tooth and a diameter larger than 5 mm. A follicular cyst was defined as the epithelial growth of the eruption membrane of the canine, not larger than 3 mm in diameter. Lastly, a compound odontoma was determined to be a set of miniature rudimentary dental structures in the epithelial tissue, obstructing or associated with the maxillary canine, with small, hyperdense masses similar to the teeth observed in CBCT. Sizes of 4 mm and 5 mm were not taken into account in order to ensure well-defined criteria for diagnosing a follicular cyst or follicular enlargement, and to avoid borderline cases.

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA) was employed for statistical analysis. The associations between the study variables was analyzed using the χ^2 test. Also, the multivariate analysis was performed with the logistic regression test ($p < 0.05$).

Table 1. Characteristics of maxillary canine impaction in the sample evaluated

Impaction type	Impaction sector					Total	*p*-value
	1	2	3	4	5		
Palatal	1 (2.3)	8 (18.2)	12 (27.3)	8 (18.2)	15 (34.1)	44 (100.0)	0.001*
Buccal	8 (34.8)	3 (13.0)	8 (34.8)	3 (13.0)	1 (4.3)	23 (100.0)	
Mid-alveolar	11 (42.3)	1 (3.8)	3 (11.5)	4 (15.4)	7 (26.9)	26 (100.0)	
Total	20 (21.5)	12 (12.9)	23 (24.7)	15 (16.1)	23 (24.7)	93 (100.0)	

Data presented as number (percentage) (*n* (%)).
* statistically significant (χ^2 test).

Table 2. Frequency of odontogenic lesions near the area of canine impaction in the sample evaluated

Odontogenic lesion	Condition	Percentage [%]
Dentigerous cyst	absent	92.5
	present	7.5
	total	100.0
Follicular enlargement	absent	93.5
	present	6.5
	total	100.0
Odontoma	absent	100.0
	present	0.0
	total	100.0
Other alterations	none	94.6
	mesiodens	3.2
	sinusitis	2.2
	total	100.0

Results

The characteristics of maxillary canine impaction showed that palatally impacted canines were mainly found in impaction sectors 3 and 5, while buccally impacted canines were mainly identified in sectors 1 and 3 ($p = 0.001$) (Table 1). The frequency of odontogenic lesions near the canine impaction area was generally low, showing the presence of a dentigerous cyst in 7.5%, follicular enlargement in 6.5% and a mesiodens in 3.2% of the sample (Table 2). There were no associations between the type of impaction (palatal, buccal or mid-alveolar) and follicular enlargement or other lesions ($p > 0.05$). However, there was a significant association between a dentigerous cyst and maxillary canine impaction, with this lesion found in approx. 15% of buccal and mid-alveolar cases ($p = 0.032$) (Table 3). When evaluating the influence of the predictor variables (sex, impaction type, impaction sector, alpha and beta angles, and impaction height) on the occurrence of odontogenic lesions, only the alpha and the beta angles had an impact with regard to dentigerous cysts, showing that for each degree of increase in the alpha angle, the risk of a dentigerous cyst increased 1.22 times ($p = 0.041$). On the contrary, for each degree of increase in the beta angle, the possibility of developing a dentigerous cyst decreased by 18% ($p = 0.024$) (Table 4).

Table 3. Association between the type of impaction and the presence of an odontogenic lesion

Odontogenic lesion	Condition	Impaction type				*p*-value
		palatal	buccal	mid-alveolar	total	
Dentigerous cyst	absent	44 (100.0)	20 (87.0)	22 (84.6)	86 (92.5)	0.032*
	present	0 (0.0)	3 (13.0)	4 (15.4)	7 (7.5)	
	total	44 (100.0)	23 (100.0)	26 (100.0)	93 (100.0)	
Follicular enlargement	absent	41 (93.2)	22 (95.7)	24 (92.3)	87 (93.5)	0.885
	present	3 (6.8)	1 (4.3)	2 (7.7)	6 (6.5)	
	total	44 (100.0)	23 (100.0)	26 (100.0)	93 (100.0)	
Other alterations	none	43 (97.7)	23 (100.0)	22 (84.6)	88 (94.6)	0.091
	mesiodens	1 (2.3)	0 (0.0)	2 (7.7)	3 (3.2)	
	sinusitis	0 (0.0)	0 (0.0)	2 (7.7)	2 (2.2)	
	total	44 (100.0)	23 (100.0)	26 (100.0)	93 (100.0)	

Data presented as *n* (%).
* statistically significant (χ^2 test).

Table 4. Binary logistic regression analysis to evaluate the occurrence of a dentigerous cyst according to the predictor variables

Predictor variable		*p*-value	Exp (B)	95% *CI* to Exp (B)	
				inferior	superior
Sex	F	–	–	–	–
	M	0.315	2.97	0.36	24.76
Impaction type		0.174	2.90	0.62	13.46
Impaction sector	1	0.865	–	–	–
	2	0.999	0.00	0.00	.
	3	0.957	1.07	0.08	13.80
	4	0.930	1.15	0.06	23.86
	5	0.334	0.18	0.01	5.87
Alpha angle		0.041*	1.22	1.01	1.48
Beta angle		0.024*	0.82	0.70	0.98
Impaction height		0.647	1.06	0.84	1.34

CI – confidence interval; F – female; M – male; * statistically significant.

Discussion

Taking into account the high prevalence of impacted maxillary canines observed during the initial radiographic diagnosis of patients over 12 years of age, we considered the determination of the occurrence of odontogenic lesions as one of the possible causes of maxillary canine impaction to be a priority, since the presence of odontogenic lesions can originate mechanical barriers to the normal eruption of the maxillary canine.[26–29] Dentigerous cysts, compound odontomas and the enlargement of the follicular sac were the lesions evaluated in our study, as they are most commonly associated with the occurrence of an impacted maxillary canine in the literature.[29,30] Additionally, in order to better understand and treat this challenging condition, the present study evaluated the severity of canine impaction, the alpha and beta angles, and the impaction sector as intervening variables.[31]

This study evaluated the CBCT scans of patients older than 12 years with impacted maxillary canines, given that the eruption of maxillary canines must have already occurred at this age. Likewise, the impaction could be in any sector according to the Ericson and Kurol classification,[22] which, although based on panoramic radiographs, has been applied in various studies with regard to panoramic images derived from tomography, reinforcing its reliability.[23–25] It is also important to highlight that the researchers were trained and calibrated to carry out all the measurements of the variables evaluated.

One of the consequences of untreated canine impaction can be the root resorption of the neighboring teeth, which may be severe. For this reason, it is essential to carry out a radiographic evaluation of the eruption of this tooth at the age of 9–11 years as a diagnostic test or for treatment planning if necessary. The results of this study show that the most frequent impaction sectors were 3 and 5 for palatally impacted canines, and sectors 1 and 3 for buccally impacted canines ($p = 0.001$). Orthodontists should take this into consideration, even though odontogenic lesions associated with canine impaction mainly involve buccally impacted maxillary canines. In addition, the frequency of the lesions related to canine impaction – 7.5% for a dentigerous cyst, 6.5% for follicular enlargement and 3.5% for a mesiodens – should be regarded by orthodontists as a possible etiological factor for this condition despite the low prevalence values.

The results of the present study are useful for clinical practice, since there is a lack of evidence in the scientific literature on the exact percentage of maxillary canine impaction due to the occurrence of cystic lesions. We observed that dentigerous cysts were present in approx. 8% of cases, and were exclusively found in patients with buccal or mid-alveolar canine impaction, demonstrating that the presence of these odontogenic lesions is scarce in the case of palatally impacted canines. Likewise, the alpha and beta angles were found to affect the appearance of an impacted canine. It was observed that for each degree of increase in the alpha angle, the risk of the presence of a dentigerous cyst increased 1.22 times ($p = 0.041$). In contrast, for each degree of increase in the beta angle, the likelihood of presenting a dentigerous cyst decreased by 18% ($p = 0.024$). Orthodontists should consider both angular results in the early planning of the treatment of such cases. In this regard, it is essential to note that the early diagnosis and treatment of maxillary canine impaction reduce treatment complexity and possible complications. Besides, the development of additional complications, such as the root resorption of the neighboring teeth in buccal or mid-alveolar impacted maxillary canines, must also be considered.[32–34]

The scientific literature provides information on the measurement methods, differential radiographic and tomographic diagnosis, the characteristics and classification of odontogenic lesions, diagnostic imaging interpretation, and even genetic information that can guide and enrich clinical practice.[35–38] In this regard, the results of the present study provide data that can help develop metric and positional analysis methods for volumetric images, and CBCT storage guidelines for imaging findings, among other aspects.

Finally, according to the present results, the probability of maxillary canine impaction being dependent on the presence of an odontogenic lesion is very low. Although these lesions may be circumstantial in cases of buccal and mid-alveolar impaction, there is no absolute association between these lesions and impaction, and more studies regarding this research line should be done in different racial groups.

Conclusions

The presence of odontogenic lesions as a possible factor for the appearance of impacted maxillary canines is low, and involves mainly dentigerous cysts and follicular enlargement, which are primarily observed in buccal or mid-alveolar impacted canines. In addition, the alpha and beta angles could influence the development of dentigerous cysts, with the alpha angle increasing and the beta angle decreasing the risk of the lesion.

Ethics approval and consent to participate

The present study was approved by the institutional Ethics and Research Committee at the School of Dentistry of the Scientific University of the South (Universidad Científica del Sur), Lima, Peru (No. of approval: 704-2021-POS70). The study was carried out in accordance with the Declaration of Helsinki.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Proprioceptive test for the temporomandibular joint: An intra- and inter-rater reliability study with healthy adults

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Abstract

Background. Temporomandibular disorders (TMDs) are challenging to manage due to their multifactorial nature. As with other joints, the function of temporomandibular joint (TMJ) is influenced by proprioception, making it an essential factor in TMD management. However, previous studies on the joint position error (JPE) in TMD patients lacked a proper diagnosis and reliability assessment.

Objectives. The aim of the present study was to develop a reliable JPE test in healthy adults as a foundation for future evaluation in TMD patients.

Material and methods. Two examiners conducted the JPE assessment, utilizing a methodology encompassing between-days intra-rater and within-day inter-rater reliability assessments. A total of 22 healthy participants (7 men and 15 women) with a mean unassisted mouth opening of 46 ±5 mm were recruited. The evaluation targeted specific distances (10 mm, 20 mm and 30 mm) within the participants' mouth opening range, with proprioceptive accuracy evaluated through the measurement of the absolute error (AE) and the constant error (CE).

Results. The CE was consistently lower than AE across all the targeted distances, although with higher standard deviations. Intra-rater reliability varied across the distances, with poor reliability observed for AE at 10 mm, moderate reliability for CE at 10 mm, good reliability for both AE and CE at 20 mm, and moderate reliability for both AE and CE at 30 mm. Inter-rater reliability was moderate at 10 mm and good at 20 mm for both AE and CE, with equally moderate reliability levels at 30 mm.

Conclusions. The 20 mm target distance demonstrated good intra- and inter-rater reliability, warranting its evaluation in patients with TMDs in the subsequent investigation phase.

Keywords: temporomandibular joint (TMJ), reliability, proprioception, temporomandibular disorders (TMDs), joint position error (JPE)

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Highlights

- A joint position error (JPE) test for the temporomandibular joint (TMJ) was developed and tested for reliability in healthy adults.
- The 20 mm mouth opening target showed the most consistent and reliable results, with good intra- and inter-rater reliability.
- The constant error (CE) was generally lower than the absolute error (AE), though it exhibited higher variability.
- The 10 mm and 30 mm targets showed limited reliability, suggesting they are less suitable for clinical use.
- The validated 20 mm JPE protocol provides a solid foundation for future testing in patients with temporomandibular disorders (TMDs).

Introduction

Temporomandibular disorders (TMDs) are a group of conditions that affect the temporomandibular joint (TMJ), masticatory muscles and other related structures.[1] These disorders can cause pain, and facial and jaw dysfunction.[2] Temporomandibular disorders are challenging to diagnose and manage due to their multi-etiological nature, including biomechanical, genetic, psychosocial, sleep, and neurobiological factors.[2–4] According to a large multisite prospective cohort study conducted in the USA, called the OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) study, it is estimated that each year, 4% of TMD-free adults aged 18–44 years develop a clinically confirmed first-onset painful TMD.[5] The annual incidence of TMDs increases with age, with 2.5% for adults aged 18–25 years, 3.7% for adults aged 25–34 years, and 4.5% for adults aged 35–44 years.[5] Despite recent advances in research and clinical management, TMDs still present a challenge, requiring ongoing investigation and collaboration among healthcare professionals to optimize and personalize patient care and outcomes.[6–8]

The sensorimotor system comprises 3 primary inputs for motor control – visual, vestibular and somatosensory.[9] Proprioception is a critical aspect of the somatosensory component, providing the ability to sense the body parts' position, movement and orientation in space.[10] In the context of joint function, proprioception is vital in providing feedback to the central nervous system (CNS), regarding the relative positions of the articulating surfaces, and the muscle length and tension.[10] This feedback helps perform precise movements and coordinate them.[10] The joint position error (JPE) measures how accurately an individual perceives and reproduces a specific joint position.[11] Several factors, such as aging, injury, hypermobility joint syndrome, and neurological conditions, can impact the proprioceptive function and contribute to alterations in JPE.[10,12] The JPE serves as a critical, valid and reliable outcome measure for several joints in the human body during the rehabilitation process.[13–15]

When we move our jaw around TMJ without the visual feedback or the vestibular input, we rely entirely on somatosensory information. This means that the proprioceptive component is crucial for the normal function of the masticatory system. Some studies suggest that patients with TMDs may have a deficit in proprioception.[16] Still, previous studies that evaluated JPE in TMD patients failed to subdivide patients properly and demonstrated a high risk of bias.[16] A recent study assessed JPE in a specific subgroup of TMD patients (those with intra-articular disorders),[17] and while it reported some clinically meaningful deficit in the TMD group, it did not provide any intra- or inter-rater reliability values, which weakens its external validity.

Therefore, the present study aimed to develop a specific and reliable test for JPE in healthy adults, which may be conducted on TMD patients in the future.

Methods

This observational reliability study involved a between-days (two-day gap) intra-rater and within-day inter-rater reliability design. The study followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) reporting standards, as well as the three-phase reliability protocol recommended by the International Academy of Manual/Musculoskeletal Medicine (IAMMM).[18] Data was collected from April to June 2023. The study was approved by the ethics committee at Recanati School for Community Health Professions of Ben Gurion University of the Negev, Be'er Sheva, Israel, and all the participants provided written informed consent.

Participants

All the participants were recruited from the student population at the Department of Physical Therapy of Ben-Gurion University of the Negev. To ensure eligibility for the study, volunteers were initially asked to fill out and email a health screening questionnaire.

The study included individuals aged 18–40 years, currently enrolled as physical therapy students at the university. They were generally healthy and could open their mouths without reporting pain (pain-free opening) at a minimum of 45 mm.

The exclusion criteria were as follows: individuals younger than 18 years old; those with a diagnosed systemic or neurological condition; individuals with a history of neck surgery or jaw surgery directly involving the jaw joint (e.g., arthroplasty); individuals with a history of cancer affecting the neck, jaw, face, or mouth; individuals who had experienced trauma affecting the neck or head (e.g., concussion) with ongoing neck or facial pain over the past 6 months; individuals with a history of neck or jaw fractures in the past 3 months; those with major dental procedures or orthodontic treatment in the past 4 weeks; and any individuals with missing or artificial molars, excluding wisdom teeth.

Examiners

Two qualified physical therapists (N.R. and D.B.) conducted the study's examinations. A senior physical therapist with 20 years of clinical experience in cervical-cranio-mandibular rehabilitation, holding DPT and Ph.D. in Physical Therapy degrees (T.G.), individually trained them for the examination protocol.

Testing procedure

The TMJ position sense was evaluated using the active assisted positioning–active replication method. The measurement was taken using a ruler, and regarded the distance between the top and bottom incisors,[19,20] as illustrated in Fig. 1. To determine the reliability rates, the joint position sense was tested at 3 target positions – 10 mm, 20 mm and 30 mm of mouth opening – representing the inner, middle and outer range of motion, respectively. The participant performed 3 repetitions at each target (10/20/30 mm), and the absolute error (AE) for the 3 trials at each target was the outcome measure (Fig. 1).

During the examination, the examiner directed the participant to sit on a chair with their back supported and both feet on the ground. The head was intentionally unsupported to stimulate a real functional scenario. Then, the examiner instructed the participant to open their mouth until told to stop. Once the participant reached a 10-millimeter distance according to a ruler, the examiner led them to hold the position for 3 s, and then close their mouth. Subsequently, the participant was asked to reopen their mouth to the same point as before, as accurately as possible, 3 times. The examiner measured the mouth opening each time with a ruler. The same procedure was repeated for mouth openings of 20 mm and 30 mm.

Fig. 1. Measurement of mouth opening up to the 10/20/30 mm targets

For intra-rater reliability, the same examiner repeated the test 2 days later. For inter-rater reliability, 2 examiners performed the test for each participant on each study day. Data was collected immediately from the participant's JPE tests, as described above.

Data analysis

The difference between the starting position (zero) and the point of return in the plane of movement was measured in millimeters. The average of the 3 trials was calculated and taken forward for data analysis. Both AE and the constant error (CE) were determined. The AE was defined as the mean of total deviation from the target, ignoring the positive and negative values.[21] The CE was defined as the mean of total deviation from the target, considering positive and negative values.[21]

Statistical analysis

To evaluate the reliability and validity of our study, we used G*Power, v. 3.1.9.4 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower) to determine the required sample size. The sample size for the reliability analysis was established based on a significance level of 0.05, a true reliability exceeding 0.7, and a power of 0.8. This resulted in a required minimum sample size of 19 participants.[22]

Data analysis was conducted using IBM SPSS Statistics for Windows, v. 27.0 (IBM Corp., Armonk, USA). The normality of data distribution was assessed with the Shapiro–Wilk test. The interclass correlation coefficient (ICC) and the standard measurement error (SEM) were also calculated. The two-way mixed model ICC (3,k) with absolute agreement and average measures was used for analysis. According to the interpretation criteria, an ICC score <0.50 indicates poor reliability, 0.50–0.74 indicates moderate reliability, 0.75–0.90 indicates good reliability, and >0.91 indicates excellent reliability.[23]

To evaluate intra-rater reliability, ICC between the 2 assessment days was calculated for each examiner. The total intra-rater ICC was computed as an average of the 2 examiners. To evaluate inter-rater reliability, ICC between the 2 examiners was calculated for each assessment day. The total inter-rater ICC was computed as an average of the 2 assessment days.

Results

Twenty-two participants (7 men and 15 women; a mean age of 26.2 ±1.5 years; a mean body mass index (BMI) of 23.4 ±2.5) with a mean unassisted mouth opening of 46 ±5mm were included (Table 1).

Table 1. Demographics of the study participants ($N = 22$)

Characteristics	Description/value
Gender	7 M/15 F
Age [years] *M* ±*SD*	26.2 ±1.5
Height [cm] *M* ±*SD*	169.0 ±9.0
Weight [kg] *M* ±*SD*	67.2 ±10.9
BMI [kg/m^2] *M* ±*SD*	23.4 ±2.5

M – mean; *SD* – standard deviation; M – male; F – female; BMI – body mass index.

Table 2 displays the average values of AE and CE for the 3 different target distances (10 mm, 20 mm and 30 mm). For all targets, the CE means were consistently lower than the AE means (1.7 vs. 3.1 for 10 mm; 1.5 vs. 3.2 for 20 mm; and 1.6 vs. 3.1 for 30 mm), whereas the CE standard deviations were consistently higher (3.6 vs. 2.6 for 10 mm; 3.7 vs. 2.5 for 20 mm; and 3.3 vs. 2.3 for 30 mm).

Table 3 reports the intra-rater reliability levels, as indicated by ICCs, based on the mean results of 2 examiners across 3 target distances (10 mm, 20 mm and 30 mm). The results demonstrate that at the 10 mm target distance, the reliability was poor for AE (ICC = 0.38) and moderate for CE (ICC = 0.72). At the 20 mm target distance, both AE and CE showed good reliability (ICC = 0.83). At the 30 mm target, the reliability was moderate for AE (ICC = 0.61) and CE (ICC = 0.74).

Table 4 presents the inter-rater reliability levels evaluated at 3 target distances (10 mm, 20 mm and 30 mm). The results indicate moderate reliability at the 10 mm target distance for AE (ICC = 0.72) and CE (ICC = 0.71). At the 20 mm target distance, the reliability levels were equally good for AE and CE (ICC = 0.77). Finally, at the 30 mm target distance, the reliability levels were equally moderate for both AE and CE (ICC = 0.70).

Table 3. Intraclass correlation coefficient (ICC) for the intra-rater reliability of each targeted mouth opening

Target distance	Parameter	Examiner 1	Examiner 2	Total intra-rater ICC (mean value)
10 mm	AE	0.38	0.39	0.38 (poor)
	CE	0.73	0.71	0.72 (moderate)
20 mm	AE	0.88	0.78	0.83 (good)
	CE	0.88	0.78	0.83 (good)
30 mm	AE	0.49	0.74	0.61 (moderate)
	CE	0.74	0.74	0.74 (moderate)

Table 4. Intraclass correlation coefficient (ICC) for the inter-rater reliability of each targeted mouth opening

Target distance	Parameter	Day 1	Day 2	Total inter-rater ICC (mean value)
10 mm	AE	0.68	0.76	0.72 (moderate)
	CE	0.55	0.87	0.71 (moderate)
20 mm	AE	0.72	0.83	0.77 (good)
	CE	0.72	0.83	0.77 (good)
30 mm	AE	0.63	0.78	0.70 (moderate)
	CE	0.63	0.78	0.70 (moderate)

Discussion

This study aimed to develop a proprioceptive test for TMJ. It was decided to check its reliability first on healthy individuals as a baseline for further studies on the TMD patient population. The results showed that CE was lower than AE for all target distances (10 mm, 20 mm and 30 mm), though with higher standard deviations. Intra-rater reliability varied across the distances, with poor reliability observed for AE at 10 mm, moderate reliability for CE at 10 mm, good reliability for both AE and CE at 20 mm, and moderate reliability for both AE and CE at 30 mm. Inter-rater reliability was moderate at 10 mm and good at 20 mm for both AE and CE, with equally moderate reliability levels at 30 mm.

Table 2. Absolute error (AE) and constant error (CE) values for the 3 different target distances [mm]

Parameters AE and CE for the target distances	Examiner 1 Day 1	Examiner 1 Day 2	Examiner 2 Day 1	Examiner 2 Day 2	Overall
AE for 10 mm	3.7 ±3.4	3.1 ±2.5	3.1 ±1.9	2.8 ±2.9	3.1 ±2.6
CE for 10 mm	2.6 ±3.9	1.5 ±3.6	1.4 ±3.2	1.4 ±3.7	1.7 ±3.6
AE for 20 mm	4.0 ±2.6	3.1 ±2.5	3.0 ±2.7	3.0 ±2.4	3.2 ±2.5
CE for 20 mm	2.6 ±3.9	0.8 ±3.9	2.0 ±3.5	0.8 ±3.7	1.5 ±3.7
AE for 30 mm	3.2 ±1.7	3.4 ±2.5	3.3 ±3.1	2.8 ±2.0	3.1 ±2.3
CE for 30 mm	2.2 ±2.8	1.6 ±3.8	2.0 ±3.5	0.7 ±3.3	1.6 ±3.3

Data presented as mean ± standard deviation (*M* ±*SD*).

In their recent study, Dinsdale et al. were the pioneers in assessing proprioceptive performance among patients with intra-articular TMDs in comparison with controls.[17] They utilized 50% of maximal mouth opening (MMO) as the targeted range of motion and did not gauge the reliability of their proposed novel JPE test.[17] In the present reliability study, the targeted range of motion was not linked to the percentage of MMO, but to 3 distinct distances (10 mm, 20 mm and 30 mm), representing a broader functional range of motion rather than a personalized one. Most essential human jaw functions, such as speech and mastication, operate within this range. Hence, proprioception evaluation is necessary with regard to those target distances. For instance, a patient with a MMO of 25 mm would perform the majority of their daily jaw activities within the 20 mm range (and not 12.5 mm, which is 50%), making it a more clinically meaningful target for assessment.

Interestingly, when analyzing the measurements, CE was consistently lower (more accurate measurement) than AE, while the standard deviations from the mean values were consistently higher. The probable explanation is that the participants consistently tended to make the same error while performing their tasks (crossing the targeted range of motion), but were relatively accurate regarding their overall mean.[24] However, the larger standard deviations for CE show that there was higher variability or inconsistency in the magnitude of the errors the participants made during the tasks. Regarding reliability, it may suggest less precise or stable measurements, as there was more significant fluctuation in the errors made by the participants.[24] After considering both factors (accuracy and standard deviations from means), CE and AE are equally valid parameters for evaluating JPE.

The only targeted range of motion for which good intra- and inter-reliability was found is 20 mm. In general, the inner range of a joint relies more on the somatosensory input from the muscle spindles (10 mm opening of TMJ), while the outer range on the somatosensory input from the joint mechanoreceptors (30 mm opening of TMJ).[25] That could explain why the ultimate somatosensory input is reached in the mid-range (20 mm mouth opening) with an ideal combination of muscles and the TMJ somatosensory input, which results in better proprioceptive performance (accurate and reproducible in terms of reliability). Clinically, most TMD patients are likely to have a MMO of at least 20 mm,[26] making this test feasible in a clinical setting.

Limitations

The reliability varied across different targets, requiring further investigation for a deeper understanding. Generalizability could be limited due to the relatively young and physically active participants. The lips of the participants could touch each other and provide an additional tactile input.

Conclusions

This study aimed to address the existing gap by developing a specific and reliable test for JPE in healthy adults, laying the groundwork for future investigations in TMD patients. The between-days intra-rater and within-day inter-rater reliability study protocol adhered to international standards, ensuring robust methodology. The results demonstrated variable reliability across different target distances, with the 20 mm target showing the most consistent and reliable outcomes.

Overall, this study advances our understanding of proprioception in TMJ and lays the groundwork for future research in TMD patients. By establishing a reliable assessment tool in healthy adults, this study paves the way for investigating proprioceptive deficits, and their impact on TMD pathophysiology and treatment outcomes. Future studies should include a larger and more diverse population with different TMDs.

Ethics approval and consent to participate

The study was approved by the ethics committee at Recanati School for Community Health Professions of Ben Gurion University of the Negev, Be'er Sheva, Israel, and all the participants provided written informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Relationship between periodontitis and cerebral atrophy: A cross-sectional study

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Abstract

Background. Periodontitis is a chronic inflammatory condition caused by the bacterial infection of the gums that leads to tissue destruction, bone loss and tooth loss. Various risk factors, including smoking, age, diabetes, and obesity, contribute to its development and progression. Recent studies have revealed systemic effects of periodontitis, linking it to diabetes, atherosclerosis, stroke, and dementia.

Objectives. The study aimed to assess the relationship between periodontitis and cerebral atrophy, an indirect marker of brain health.

Material and methods. A cross-sectional study was conducted to examine the association between cerebral atrophy and periodontitis. A total of 166 participants were included in the study. All individuals underwent computed tomography (CT) of the head and a full-mouth periodontal assessment to verify if they met the diagnostic criteria for periodontitis. They also underwent a complete neurological examination to rule out dementia.

Results. Sixty-four patients (38.6%) had periodontitis, 85 individuals (51.2%) had cerebral atrophy, and 43 patients presented with both conditions. The study sample included 89 females (53.6%), and the median age of the participants was 67 ±10 years. Patients diagnosed with periodontitis showed a higher grade of cerebral atrophy, as measured using the global cortical atrophy (GCA) scale. An independent association was identified between periodontitis and cerebral atrophy (odds ratio (*OR*): 2.56; 95% confidence interval (*CI*): 1.29–5.07).

Conclusions. Cerebral atrophy, even in the absence of cognitive decline, is significantly associated with periodontitis.

Keywords: dementia, periodontitis, cerebral atrophy

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Highlights

- Periodontitis was significantly associated with cerebral atrophy, even without evident cognitive decline.
- Chronic inflammation caused by periodontitis may contribute to brain tissue damage via systemic inflammation and microvascular injury.
- Although periodontitis has been linked to cardiovascular and neurological diseases, few studies have explored the direct relationship between periodontitis and cerebral atrophy, highlighting the need for longitudinal research.

Introduction

Periodontitis is a chronic inflammatory condition in which bacteria commonly found in the mouth infect the gums, causing inflammation, tissue destruction, bone loss, and ultimately, the loss of the affected tooth.[1] Several phases of periodontitis are defined, with the first being gingivitis, which is reversible and confined to the gums. While it was once accepted that bacteria were the sole factor in the development of this condition, in recent years, it has been shown that various personal[2] and genetic factors also play a role. In fact, the presence and distribution of bacteria do not always correlate with the onset and progression of periodontitis.

Periodontitis, one of the most common diseases in human population, has been increasingly associated with other conditions such as diabetes, atherosclerosis, heart attacks, strokes, and dementia.[3,4] Although numerous epidemiological studies have been conducted to determine the prevalence of periodontitis, the results vary greatly,[5,6] likely due to the cross-sectional nature of most of the studies. According to recent research, the prevalence of periodontitis ranges from 8% to 47% of the population; furthermore, up to 10% of the population may be affected by advanced periodontitis.[7] Periodontitis can become one of the top 5 most common diseases in some parts of the world, such as South America, with an incidence of up to 700 cases per person-year.[8]

Periodontitis is associated with numerous risk factors. Smoking has been linked to this condition, even when other confounding factors related to smoking are eliminated.[9] Moreover, smokers have significantly greater bone loss and tooth mobility.[9] On the other hand, factors such as age, diabetes and obesity have also been related to periodontitis.[10–12] Diabetes is associated with a worse prognosis once the disease is established.[13]

Although periodontitis was initially considered a localized infection, recent studies indicate that this condition may contribute to the systemic circulation of various pro-inflammatory proteins, leading to a state of mild but chronic systemic inflammation. This chronic inflammation could be responsible for the relationship between several systemic pathologies and periodontitis. It has been demonstrated that these pathogenic bacteria are capable of colonizing other areas of the body by entering the bloodstream. Additionally, it has been hypothesized that they may significantly contribute to plaque formation in arteries, with bacterial DNA from these microorganisms being identified in such plaques.[14] Various studies have shown that patients with periodontitis exhibit elevated levels of multiple blood markers of inflammation.[14,15]

Other studies have shown that patients suffering from periodontitis have the capacity to produce antibodies in response to periodontal infection. Some of these antibodies, in addition to fighting the infection within the oral cavity, have the potential to cross-react, increasing the pro-inflammatory state of the system or even blocking protective molecules that usually try to prevent the formation of cholesterol plaque in the arterial walls. Consequently, numerous studies in recent decades have identified periodontitis as a potential risk factor for ischemic stroke.[16,17] It has also been demonstrated that patients with severe periodontitis are at an increased risk of hemorrhagic transformation once an ischemic stroke is established.[18]

Finally, various studies have shown a relationship between periodontitis, cognitive decline and dementia.[19,20] Cerebral atrophy, or cortical atrophy, is characterized by the widening of sulci, narrowing of gyri, decreased grey matter thickness, diminished white matter volume, and/or enlargement of the cerebral ventricles and subarachnoid spaces. Normal brain aging exhibits some of the same changes, although age-related atrophy is typically less rapid and less severe than that observed in individuals with neurodegenerative diseases.[21] Importantly, cerebral atrophy has an impact on cognitive function, affecting memory, attention, language, and motor skills, even in the absence of dementia. Cerebral atrophy has been identified as an initial process of neurodegenerative diseases, such as Alzheimer's. It may also result from other condition, potentially leading to cognitive decline.

To the best of our knowledge, no studies have investigated the effect of periodontitis on cerebral atrophy in the absence of dementia or neurodegenerative disease. The hypothesis of this study was that patients diagnosed with periodontitis have a higher degree of atrophy than those diagnosed with age-related atrophy. The aim of the study was to investigate whether there is an association between cerebral atrophy and periodontitis in the absence of cognitive decline.

Material and methods

A cross-sectional study was carried out from July 2021 to October 2023 at the University Clinical Hospital of Santiago de Compostela (Santiago de Compostela, Spain) and the Hospital Universitario Lucus Augusti (Lugo, Spain). The study was conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[22] Patients aged ≥50 years who underwent computed tomography (CT) of the head as part of the care process were offered the opportunity to participate in this study. The exclusion criteria were: previous history of dementia, intracerebral tumor, malignancy, any other severe medical condition, or any other medical condition that could potentially be associated with cerebral atrophy, such as chronic renal failure; <10 teeth present, which would render the periodontal examination unreliable; those who, after anamnesis conducted by the principal investigator (neurologist), were deemed to have possible or probable dementia; and patients who did not consent to participate. The neurological criterion for possible or probable dementia was based on the latest international dementia diagnostic guidelines[23] and relied on medical history, as well as at least 1 additional scale commonly used for cognitive testing: Montreal Cognitive Assessment (MoCA); or Mini-Mental State Examination (MMSE). The present study was conducted in accordance with the World Medical Association (WMA) Declaration of Helsinki (2013) and approved by the Research Ethics Committee of Santiago-Lugo (protocol No. 2022/18). All participants provided written consent.

Demographic data such as sex and age was collected. The medical history of the subjects was thoroughly reviewed to ascertain the presence or absence of various risk factors, including hypertension, type 2 diabetes, smoking, and alcohol consumption. The variables such as smoking, alcohol consumption, hypertension, periodontitis, and diabetes were dichotomous and recorded as "yes" or "no".

Assessment of cerebral atrophy

The presence of cerebral atrophy was evaluated based on the head CT scans by a neurologist and a radiologist, who have experience measuring cerebral atrophy according to the global cortical atrophy (GCA) scale. A minimum of 13 brain regions were assessed separately in each hemisphere, with scores ranging from 0, indicating "normal volume", to 3, representing "knife blade" atrophy. The sum of all regions' scores constituted the final score.[24] The 13 brain regions evaluated were as follows: sulcal dilatation in the frontal, parieto-occipital and temporal regions; ventricular dilatation in the frontal, parieto-occipital, temporal, and third ventricle regions. The evaluation of all regions was conducted bilaterally, with the exception of the third ventricle. The sum of the individual region scores, with a range of 0–3 points for each region, resulted in a total score of 0–39. The values equal or greater than 1 in each region were considered cerebral atrophy. The initial measurement of these scores was conducted by the radiologist, who was blinded to the clinical data, followed by the evaluation of the neurologist.

Assessment of periodontal disease

The diagnosis of periodontitis was made by a periodontist. A full-mouth periodontal examination was conducted on all subjects. Probing pocket depth (PPD) and clinical attachment level (CAL) were measured in all teeth, with the exception of third molars. The measurements were recorded at 6 sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, distolingual, and midlingual). Slight periodontitis was defined as the presence of at least 2 interproximal sites with CAL ≥ 3 mm and at least 2 interproximal sites with PPD ≥ 4 mm (not on the same tooth) or 1 site with PPD ≥ 5 mm. Moderate periodontitis was defined as the presence of at least 2 interproximal sites with CAL ≥ 4 mm (not on the same tooth) or at least 2 interproximal sites with PPD ≥ 5 mm, also not on the same tooth. Severe periodontitis was defined as the presence of ≥2 interproximal sites with CAL ≥ 6 mm (not on the same tooth) and ≥1 interproximal site with PPD ≥ 5 mm.[25] Total periodontitis comprised slight, moderate and severe cases. Bleeding on probing (BOP) and the presence of dental furcations were not assessed.

Statistical analysis

Categorical data was reported as percentages and compared using the χ^2 test. The mean and standard deviation values ($M \pm SD$) were calculated for the continuous variables, and a comparison was made using an independent t-test after confirming normality through the Kolmogorov–Smirnov test. Finally, a multivariate analysis was performed using logistic regression to ascertain the relationship between the variables and cerebral atrophy.

Results

A total of 231 patients were initially selected to participate in the study. Of these, 29 were excluded on the basis of the eligibility criteria. Nine participants were not included after anamnesis, conducted by the principal investigator, as they were considered to show signs of dementia. Twenty-seven individuals declined to participate or did not consent to undergo periodontal examination. The study sample comprised 166 patients who underwent cranial CT as part of their standard care regimen. Of these, 64 subjects (38.6%) had been diagnosed with periodontitis, while 85 individuals (51.2%) had been diagnosed with cerebral atrophy. Forty-three patients presented with both conditions. The study sample included

89 females (53.6%), and the median age of the participants was 67 ±10 years. With the exception of 1 patient, all subjects were diagnosed with hypertension and prescribed either an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin II receptor blocker (ARB) at the time of recruitment.

No statistically significant differences in sociodemographic characteristics were observed between patients with periodontitis and those without the condition. The study also revealed no significant differences for the vascular risk factors between the periodontitis and no periodontitis groups, with the exception of smoking (34.4% vs. 19.6%; $p = 0.033$). This finding is consistent with the established correlation between tobacco use and periodontal disease. The noted difference in the prevalence of diabetes, hypertension and alcohol consumption between the 2 groups was not statistically significant. The characteristics of the study population are summarized in Table 1.

Table 1. Characteristics of the study sample

Variable	Group		*p*-value
	no periodontitis ($n = 102$)	periodontitis ($n = 64$)	
Age [years]	65.6 ±9.6	69.5 ±11.4	0.098
Females, *n* (%)	51 (50.0)	38 (59.4)	0.238
Hypertension, *n* (%)	40 (39.2)	33 (51.6)	0.119
Smoking habit, *n* (%)	20 (19.6)	22 (34.4)	0.033*
Diabetes, *n* (%)	40 (39.2)	34 (53.1)	0.079
Alcohol consumption, *n* (%)	39 (38.2)	32 (50.0)	0.136
Cerebral atrophy, *n* (%)	42 (41.2)	43 (67.2)	<0.01*

* statistically significant ($p < 0.05$, χ^2 test).

The direct analysis of variables using the χ^2 test demonstrated a relationship between cerebral atrophy and hypertension ($p = 0.017$) and periodontal disease ($p < 0.001$). The relationship between cerebral atrophy and type II diabetes was moderate but not significant ($p = 0.056$). The present study did not include any patients with type I diabetes.

The results of the multivariate logistic regression analysis are presented in Table 2. The logistic regression analysis demonstrated a statistically significant positive correlation between periodontitis and cerebral atrophy (odds ratio (*OR*): 2.56; 95% confidence interval (*CI*): 1.29–5.07). Hypertension and age were also related to cerebral atrophy, but to a lesser extent (*OR*: 1.94; 95% *CI*: 1.00–3.75 and *OR*: 1.04; 95% *CI*: 1.01–1.08, respectively).

To conclude, the data was examined using the Hosmer–Lemeshow test, yielding a *p*-value of 0.599. Additionally, the variability of the data was assessed by performing Nagelkerke's R^2, which resulted in an R^2 value of 0.143.

Table 2. Results of the multivariate logistic regression analysis

Variable	*OR*	95% *CI*	*p*-value
Age	1.04	1.01–1.08	0.022*
Sex	0.80	0.41–1.56	0.519
Hypertension	1.94	1.00–3.75	0.049*
Smoking habit	0.91	0.41–2.01	0.823
Diabetes	1.54	0.80–2.97	0.195
Alcohol consumption	1.55	0.79–3.04	0.202
Periodontitis	2.56	1.29–5.07	0.007*

OR – odds ratio; *CI* – confidence interval; * statistically significant ($p < 0.05$).

Discussion

The findings of the present study suggest that periodontitis may contribute to cerebral atrophy, even in the absence of cognitive decline. In models implemented with logistic regression, we observed that periodontitis is strongly correlated with cerebral atrophy, even when accounting for confounding factors.

The relationship between periodontitis and various pathologies has been extensively examined in the literature. Numerous studies, including meta-analyses of epidemiological studies, have indicated that individuals diagnosed with periodontitis have a higher risk of developing coronary heart disease, ischemic stroke, dementia, and peripheral arterial disease when compared to those without periodontitis.[17,26–29] In recent years, the elevation of periodontitis markers, such as the periodontal inflamed surface area (PISA), has also been linked to cardiac involvement in hypertensive patients.[30] Although the direct relationship between cardiovascular diseases and periodontitis is not known, efforts have been made to understand the underlying mechanisms. A number of studies have investigated biomarkers such as pentraxin-3, C-reactive protein or paraoxonase-1 in patients with periodontitis, with the objective of understanding the heightened risk of atherosclerosis observed in these individuals.[31,32]

Despite the paucity of research in this area, periodontal disease has also been linked to other neurological conditions such as multiple sclerosis and epilepsy. One article attempted to correlate the severity of seizures with oral hygiene practices. Additionally, a relationship between periodontitis and multiple sclerosis has been identified, which is consistent with the inflammatory nature of these diseases.[33,34]

Despite the clear relationship between cerebral atrophy and cognitive decline, and numerous articles linking periodontitis with Alzheimer's disease, only 1 paper was found relating periodontitis to cerebral atrophy. In the aforementioned study, 468 patients with periodontitis were analyzed, and the thickness and volume of the cerebral cortex were measured on magnetic resonance

imagining, although this was not the study's primary objective.[35] Similar to our study, the article suggested a direct relationship between cerebral volume loss and periodontitis.

Other studies have correlated tooth loss with cognitive decline and cerebral volume loss.[36,37] However, they employed a methodology that differed from the present study. Some of them focused on patients with genetic Alzheimer's disease in the prodromal phase, while others examined the correlation between tooth loss and cerebral atrophy, excluding the influence of periodontitis. Multiple studies have demonstrated a relationship between periodontitis and leukoaraiosis or small vessel brain disease. Additionally, other articles have linked periodontitis to ischemic stroke and other cardiovascular diseases.[38,39]

The relationship between cerebral atrophy and cognitive decline is well-established. Although it is not always directly related to the degree of cognitive decline, it is an interesting factor to consider in the prevention of the condition. In the present study, we found that periodontitis is directly and independently related to the degree of cerebral atrophy, even in the absence of cognitive decline.

While the precise underlying mechanism through which periodontitis leads to cerebral atrophy remains unclear, it is likely that the continuous inflammatory state caused by this condition plays a pivotal role. Numerous studies have shown that the effects of inflammation are not limited to the oral cavity but can affect the entire body. In fact, the destruction of periodontal epithelium by pathogens enables the entry of endotoxins and exotoxins into the bloodstream, thereby increasing systemic inflammatory response, which can result in brain tissue damage. Other studies have shown the presence of periodontal pathogens in various tissues and organs of the cardiovascular system, including heart valves.[40–42] One study sought the presence of various periodontitis-causing microorganisms in cerebrospinal fluid, but was unable to detect them.[43]

Limitations

Some limitations of the present study should be addressed. Firstly, the design of the study precluded the testing of causality regarding the association between periodontitis and cerebral atrophy. Secondly, the patients were recruited following a consultation with a neurologist. Patients who might have presented dementia or another condition directly affecting cerebral atrophy were excluded, which represents a selection bias that could influence the results. Thirdly, given the nature of the study, it was not possible to assess the severity of diabetes in each patient; it was simply considered a dichotomous variable. In future studies, it would be ideal to examine its influence using each patient's glycated hemoglobin levels, for example. Lastly, the diagnosis of periodontitis was made based solely on PPD and CAL measurements. However, other indicators, such as BOP or PISA, may be of more importance when discussing systemic inflammation and the relationship between cerebral atrophy and periodontitis.

Future studies, especially prospective studies on healthy subjects with comprehensive periodontal exploration, are needed to confirm our results. However, this study, along with the recent investigations conducted on the relationship between systemic disease and periodontitis, underscore the importance for ongoing research in this area, focusing on the prevention and treatment of this condition.

Conclusions

Cerebral atrophy, even in the absence of observable cognitive decline, demonstrates a significant association with periodontitis. To substantiate this finding, further research is warranted, preferably through prospective studies, to establish and clarify the nature of this relationship.

Ethics approval and consent to participate

The study was conducted in accordance with the World Medical Association (WMA) Declaration of Helsinki (2013) and approved by the the Research Ethics Committee of Santiago-Lugo (protocol No. 2022/18). All participants provided written consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Influence of the soft tissue thickness and the levels of vitamin D3 as important factors in bone preservation around the dental implant platform: A retrospective observational study

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Abstract

Background. Most dental implants are made of titanium (Ti) or, more recently, ceramic. Both of these materials are safe and biocompatible. Maintaining healthy bone around the dental implant platform is a key component of any modern implant therapy. Understanding the impact of various external factors on marginal bone preservation is essential for accurate diagnosis before any planned procedure.

Objectives. The aim of the present study was to assess the preservation of the alveolar bone near the implant platform with regard to varying parameters of gingival thickness over the implant site and different serum vitamin D3 levels.

Material and methods. The study analyzed the records of 72 patients who had a total of 115 dental implants inserted with the simultaneous preoperative determination of vitamin D3 levels and the soft tissue thickness between January 2022 and February 2023 at a private medical facility.

Results. Patients with vitamin D deficiency showed significantly higher levels of bone loss around implants as compared to those with normal vitamin D levels. Moreover, the thickness of the gingival tissue over the implant site had a significant effect on the preservation of the alveolar bone around the implant platform, regardless of the vitamin D3 status.

Conclusions. Monitoring the concentration of 25-hydroxycholecalciferol (25(OH)D), along with the radiological assessment of the gingival tissue thickness, may be a simple and reliable method to achieve predictable outcomes in dental implantology.

Keywords: crestal bone loss, vitamin D3 deficiency, thin mucosal tissue, thick mucosal tissue

Highlights

- Dental implants are considered the standard treatment for dental deficiencies.
- Bone loss around implants is influenced by the gingival height and vitamin D3 levels.
- Monitoring these factors could reliably predict outcomes in dental implantology.

Introduction

Successful osseointegration is a key criterion for successful implantological outcomes, and it is achieved through functional ankylosis. Titanium (Ti), which is used for dental implants, fuses with bone to form a functional unit known as the initial bone–implant contact (BIC).[1,2] Depending on its concentration, vitamin D can enhance[3] or slow down[4,5] new bone formation.

Vitamin D is essential for maintaining bone mineralization by regulating Ca^{2+} and PO_4^{3-} ion concentrations in blood.[6] Vitamin D deficiency in both children and adults leads to decreased intestinal absorption of calcium (Ca), resulting in temporary decreases in the ionized Ca concentration in blood.[5]

Vitamin D exists in 2 forms: vitamin D2, referred to as ergocalciferol, found in plant and fungal organisms; and vitamin D3, referred to as cholecalciferol, synthesized by animal organisms, including humans.[7]

Under the influence of ultraviolet B (UVB) radiation at a wavelength of 290–315 nm (characteristic of sunlight) and in the presence of the appropriate reductase, the B-ring of 7-dehydrocholesterol opens, and a non-permanent compound – pre-vitamin D3 – is formed. This, in turn, isomerizes to cholecalciferol at skin temperature (about 25°C), which then enters the bloodstream.[7,8] In the liver, 25-hydroxylase influences the formation of 25-hydroxycholecalciferol (25(OH)D, known as calcidiol). The 2nd stage takes place in the kidneys, where 1α-hydroxylase catalyzes the conversion of the compound to its active form, 1α,25-dihydroxycholecalciferol (1α,25(OH)2D, called calcitriol) (Fig. 1).[7]

Calcitriol plays an important role in maintaining the health of the craniofacial bones and teeth. Calcitriol deficiency can lead to oral diseases, such as periodontitis, early tooth loss, osteoporotic fractures, impaired healing of wounds or fractures, and disrupted bone formation around dental implants.[10]

A sufficient level of vitamin D3 is crucial on the day of surgery, as it modulates the immune system, and influences the production of cathelicidin and defensin.[9,10] It also reduces the expression of proinflammatory cytokines and positively affects bone metabolism through osteosuppression by influencing osteoblasts and osteoclasts, as well as regulating continuous bone remodeling around implants after prosthetic restoration.[10]

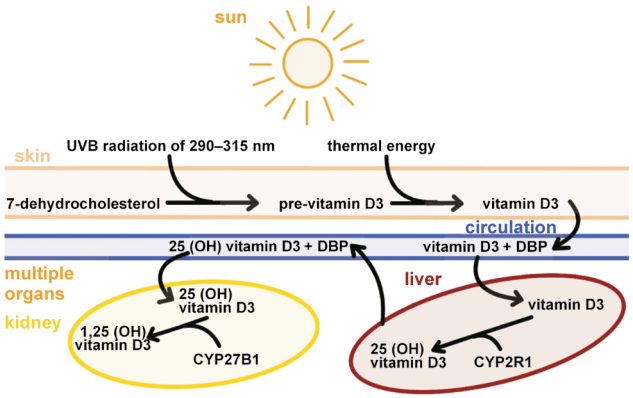

Fig. 1. Endogenous synthesis of 1,25(OH) vitamin D3

UVB – ultraviolet B; DBP – vitamin D-binding protein; CYP2R1 – vitamin D 25-hydroxylase; CYP27B1 – 1α-hydroxylase.
Reprinted from reference[9] under the CC-BY 4.0 license.

The body supply of vitamin D can be assessed through laboratory tests based on the serum concentration of 25(OH)D (Table 1). This measurement indicates the available vitamin D in the bloodstream and is an established marker of the vitamin D status.

Another important factor in maintaining bone tissue health around the neck of the implant is the amount of gingival soft tissue. It has been suggested that a minimum of 3 mm of peri-implant mucosa is required for a stable epithelial connective tissue attachment to be formed.[11]

The transition from alveolar mucosa to peri-implant soft tissue after implant placement is a difficult and complex process. Płudowski et al. suggested that if insufficient gingival tissue is available, bone loss may occur to ensure the development of a proper biological width.[12]

The assessment of the gingival height can be invasive when using a needle, an endodontic tool or a periodontal probe, or non-invasive, including ultrasound or optical coherence tomography (OCT). Radiological methods, such as the paralleling technique with radiovisiography (RVG) or cone-beam computed tomography (CBCT) can also be used (Fig. 2).

This retrospective study aimed to investigate the impact of dental implant procedures on the bone tissue under various complex combinations of factors, such as insufficient vitamin D levels with a thick soft tissue biotype or recommended vitamin D levels with a thin soft tissue biotype.

Table 1. Assessment of the vitamin D status based on the serum 25-hydroxycholecalciferol (25(OH)D) concentration for all age groups[21,22]

25(OH)D concentration	Category	Action to consider
0–20 ng/mL, 0–50 nmol/L	deficiency	should be treated medically
20–30 ng/mL, 50–75 nmol/L	suboptimal vitamin D status	calls for a moderate increase of a vitamin D daily dose
30–50 ng/mL, 75–125 nmol/L	adequate vitamin D status	the supplementation scheme and dose should be maintained
50–100 ng/mL, 125–250 nmol/L	high vitamin D supply	the supplementation dose can be maintained for lower concentrations of this range or moderately decreased for higher concentrations
>100 ng/mL, >250 nmol/L	risky for overall health outcomes	the reduction/cessation of vitamin D supplementation until obtaining the target 25(OH)D concentration
>200 ng/mL, >500 nmol/L	toxic levels	the cessation of vitamin D supplementation until obtaining the target 25(OH)D concentration; such people may need a specific medical intervention to correct toxic effects

Material and methods

Population

The study analyzed records from the treatment histories of 72 patients who received a total of 115 dental implants between January 2022 and February 2023 at a private medical facility in Krakow, Poland. The mean age of the patients was 44.7 ±11.21 years.

Eligibility for the study

All eligible patients were adults in good general health, with no history of allergic reactions to drugs or dental materials, including Ti. Patient data was included if patients had completed all the required treatment and control steps. Criteria for inclusion in the study group embraced the absence of the need for simultaneous bone augmentation and the complete healing of the alveolar ridge with a clearly defined cortical plate, suitable for accurate measurements. Patients were included if the dental implant was placed at the bone level with the simultaneous preoperative assessment of vitamin D3 levels, and if healing occurred without complications. All 72 patients received Nobel Biocare Replace Select™ CC implants with the TiUnite surface (Nobel Biocare, Kloten, Switzerland). Restorations were screw-retained with various suprastructures (a stock metal abutment with a lithium disilicate supracrestal or zirconia framework, and lithium disilicate supracrestal restorations supported with a Ti base). All analyzed cases included interdental and partial deficiencies, with reconstruction types ranging from three-point bridges to single implant crowns. In all cases, the time interval between implant placement surgery and the exposure day was 4 months for the maxilla and 3 months for the mandible. In all cases, the emergence profile of the abutment maintained a consistent distance from the alveolar bone surrounding the implant platform.

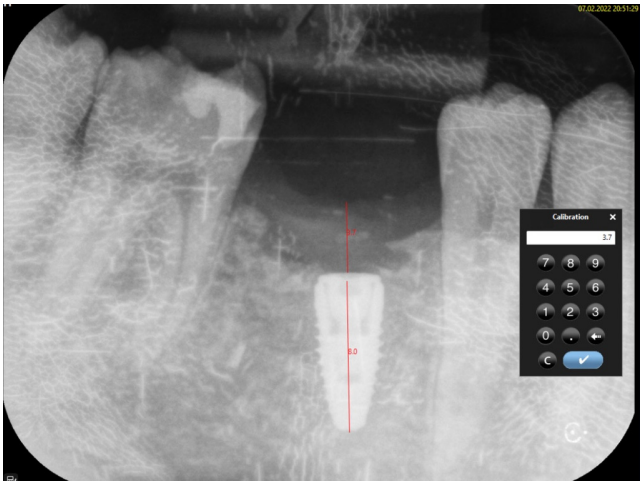

Fig. 2. Measurement of the mucosal thickness at the time of implant placement on the intraoral radiographs taken using the paralleling technique (calibration was performed with the use of dedicated software)

Radiological evaluation of the bone tissue level around implants

Bone loss was assessed using software designed for digital RVG analysis. Each evaluated image was calibrated against the implant length on the RVG and from medical records. The calculated value represented the arithmetic mean of the measurements taken on both sides of the implant, which were visible on the two-dimensional (2D) image. The calculations were made starting from the platform of the implant along its long axis (Fig. 3 and 4).

Bone loss was calculated as an average using the following formula (Equation 1):

$$z = (zL + zR) : 2 \quad (1)$$

where:
z – bone atrophy [mm];
zL – bone atrophy on the left side [mm]; and
zR – bone atrophy on the right side [mm].

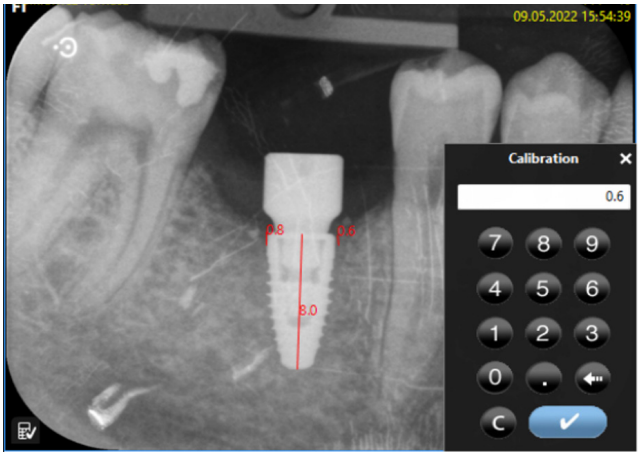

Fig. 3. Measurement of bone loss during the installation of the healing screw on the intraoral radiographs taken using the paralleling technique (calibration was performed with the use of dedicated software)

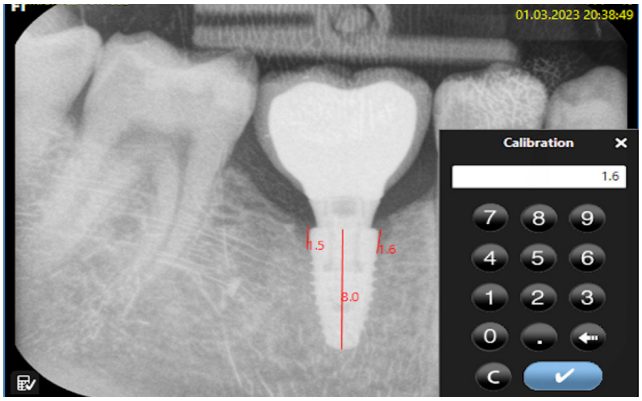

Fig. 4. Measurement of bone loss during the installation of the crown on the implant on the intraoral radiographs taken using the paralleling technique (calibration was performed with the use of dedicated software)

Statistical analysis

The groups were compared using the Mann–Whitney *U* test after rejecting the assumptions for normality and homogeneity of variance. The significance level was set at 0.05. Statistical analysis was conducted using the R software, v.1.2.5042 (https://www.r-project.org).

Results

The cases (N = 115) were divided into 2 groups: group A (n = 55) were implants inserted with 25(OH)D levels below 30 ng/mL in blood serum, consisting of 17 cases with a gingival height of 3 mm or more and 38 cases with a gingival height of less than 3 mm; and group B (n = 60) were implants inserted with 25(OH)D levels equal to or above 30 ng/mL in blood serum, consisting of 27 cases with a gingival height of 3 mm or more and 33 cases with a gingival height of less than 3 mm (Fig. 5).

On the day of implant exposure, in group A (vitamin D3 deficiency), 8 cases of crestal bone loss around the implant platform were observed with a gingival thickness over the implant site of less than 3 mm, and no cases with a gingival thickness of 3 mm or more (p = 0.040). However, in group B (vitamin D3 in the normal range), only 1 case of crestal bone loss around the implant platform was observed with a gingival thickness over the implant site of less than 3 mm, and no cases with a gingival thickness of 3 mm or more (p = 0.366) (Fig. 6).

Interestingly, on the day of prosthetic crown placement, in group A, 14 cases of bone atrophy around the implant platform were observed with a gingival thickness over the implant site of less than 3 mm, and 4 cases with a gingival thickness equal to or greater than 3 mm (p = 0.213). On the other hand, in group B, 9 cases of bone atrophy around the implant platform were observed with a gingival thickness over the implant site of less than 3 mm, and no cases with a gingival thickness equal to or greater than 3 mm (p < 0.001) (Fig. 6).

Regardless of the day, no differences were detected based on gender (p = 0.173 and p = 0.390, respectively) or the implant region (p = 0.725 and p = 0.244, respectively).

Discussion

The stability of the crestal bone is one of the most desired features of successful implant treatment. Several factors contribute to bone loss, including improper bone healing around the implant, smoking, infection, inadequate keratinized gingiva in relation to the polished implant collar, overload, and a micro-gap.[13]

Among these factors, Berglundh and Lindhe highlighted the initial mucosal tissue thickness as a cause of crestal bone loss.[14] In an animal experiment, they found that thin

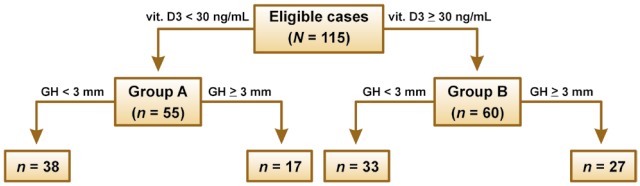

Fig. 5. Flowchart illustrating the assignment of cases based on the 25-hydroxycholecalciferol (25(OH)D) concentration and the gingival height (GH)

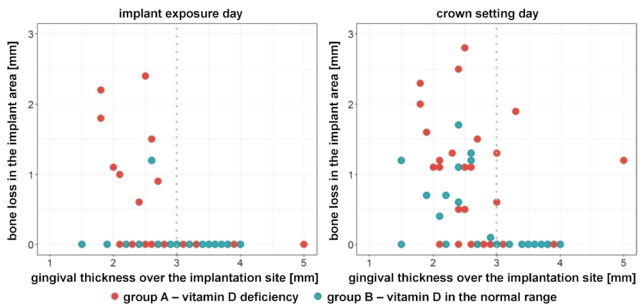

Fig. 6. Bone loss in the implant area with regard to the level of vitamin D and the gingival thickness over the implantation site

tissue could lead to crestal bone loss during the formation of the peri-implant seal. The implants installed in thick tissue were associated with significantly less bone loss.[14] In another study, a mucosal tissue thickness of 2 mm or less was associated with bone loss of 1.38 mm, whereas implants placed in thicker tissue were associated with significantly less bone loss at 0.25 mm.[15]

Additionally, Linkevicius et al. examined the effect of the mucosal thickness on marginal bone loss in 19 patients with 46 implants in 2009.[16] They placed 23 implants 2 mm supracrestally and 23 at the bone level. The mucosal thickness was classified as thin, medium or thick. Crestal bone changes were measured at the end of a 1-year follow-up. On average, thin, medium and thick mucosa showed bone loss of 1.35 mm, 0.32 mm and 0.12 mm, respectively.[16] Thus, the pre-implantation thickness of the mucosa covering the edentulous alveolar ridge is very important for the subsequent stability of the peri-implant crestal bone and significantly determines the preservation of the bone tissue of the alveolar process surrounding the implant platform.

Another important factor is a vitamin D level in blood serum, which affects various stages of osteointegration. The vitamin D level has become an important field of knowledge in periodontology and implantology due to its role in the metabolism of the bone tissue and the immune system function.

Vitamin D levels typically decline with age and in individuals with periodontal diseases.[17] Lower vitamin D3 levels have been associated with recurrent aphthous stomatitis in comparison with healthy individuals.[18]

Our study demonstrated a significant difference in the bone level at implant sites between the groups. We observed significantly greater bone loss around implants in vitamin D deficient patients as compared to those with normal levels, both on the implant exposure and crown setting days (p = 0.010 and p = 0.009, respectively), particularly when the gingival thickness was less than 3 mm. Similar findings were reported in a prospective study in the Indian population by Bhandage et al., showing that every increase by 1 ng/mL in vitamin D levels resulted in a 0.48-unit increase in the implant stability quotient (ISQ) value at 3 months and an increase by 0.62 units at 6 months.[19]

These results align with conclusions from a systematic review, which showed that vitamin D deficiency seemed to have an impact on marginal bone loss.[20] It was also reported to negatively affect osseointegration in humans and animals.[10] However, there is still little evidence that vitamin D supplementation enhances osseointegration in humans.[10,13,21]

Given the high prevalence of vitamin D deficiency, it seems appropriate to determine the blood levels of 25(OH) D before implantation.[22] However, further research is needed to establish the beneficial effects of vitamin D supplementation on dental implant osseointegration.

Limitations

The height of the abutment and the diameter of the abutment were not measured, and the implant diameter was not taken into consideration. Vitamin D levels were measured only preoperatively.

Conclusions

We recommend that monitoring of the 25(OH)D concentration and the radiological assessment of the gingival tissue thickness is included in a short checklist when planning every dental implant procedure.

By adhering to the principles of thorough diagnosis, precise surgical procedures, appropriate material selection, and diligent postoperative care of dental restorations, along with assessing the soft tissue thickness and vitamin D3 levels, we anticipate more predictable long-term outcomes and treatment effectiveness.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jagiellonian University Medical College, Krakow, Poland (No. of approval: 118.6120.160.2023). All patients involved in the study provided written informed consent for processing their data and using it for scientific research purposes.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Analysis of collagen gel degradation in an experimental cell culture model using fibroblasts isolated from peri-implantitis lesions

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Conflict of interest
None declared

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Abstract

Background. Periodontitis and peri-implantitis are chronic inflammatory diseases that lead to progressive connective tissue degradation and alveolar bone resorption. The presence of characteristic periodontitis-associated fibroblasts (PAFs) that display a remarkably high capacity for extracellular matrix (ECM) degradation was previously reported in periodontitis lesions.

Objectives. The aim of the study was to analyze collagen gel degradation in an experimental cell culture model using fibroblasts isolated from peri-implantitis lesions.

Material and methods. A patient-derived experimental cell culture model of periodontitis was developed. Gingival tissues were obtained during peri-implant, periodontal, and tooth extraction surgeries. Fibroblasts isolated from tissues affected by peri-implantitis, mixed in a three-dimensional (3D) collagen gel, were co-cultured with gingival epithelial cells. The degree of collagen gel degradation was analyzed using gel contraction, and a histologic examination was performed.

Results. In 5 examined cases, gel contraction was observed in the 3D co-culture model to considerably different degrees, which may demonstrate the presence of peri-implantitis-associated fibroblasts (PIAFs) that display a high capacity for collagen degradation. Histologically, the collagen gels with PIAFs showed numerous vacuoles adjacent to the cells when compared to gels with normal fibroblasts. The PIAFs from one case showed a rapid and significantly elevated level of collagen gel degradation in comparison to the PIAFs from the other cases.

Conclusions. The study revealed the presence of PIAFs among the fibroblasts isolated from peri-implantitis lesions, displaying a capacity for collagen degradation. Further detailed studies are required to clarify the characteristics of PIAFs as well as their role in the occurrence and progression of peri-implantitis.

Keywords: periodontitis, fibroblasts, peri-implantitis, epithelial cells, primary cells

Highlights

- Peri-implantitis-associated fibroblasts (PIAFs) isolated from peri-implantitis lesions demonstrated collagen-degrading activity.
- The degree of contraction of the gel including PIAFs in a 3D culture model varied considerably among cases.
- Collagen gels with PIAFs showed numerous vacuoles near cells, unlike gels with normal fibroblasts.
- PIAFs may constitute a key component in the pathogenesis and progression of peri-implantitis.

Introduction

Implant therapy is a well-established method for the replacement of absent or lost teeth. Its popularity can be attributed to its high rates of fundamental long-term survival.[1] Various studies have been conducted on the development of implant materials[2–4] and treatment procedures for implant placement.[5–7] However, the occurrence of peri-implantitis has become an increasingly prominent concern, affecting the stability of surrounding tissues and, consequently, the longevity of implants.[8,9] Definitive effective therapies have not been established, largely due to the difficulty of treatment procedures associated with the complicated structures of dental implants.[10–13] The pathological nature of peri-implantitis is similar to that of periodontitis, being closely associated with the accumulation of bacterial biofilm.[14] However, the mechanism of the occurrence and progression of peri-implantitis remains unclear.

Recently, Ohshima et al. used an experimental three-dimensional (3D) culture model of periodontitis to report the presence of characteristic periodontitis-associated fibroblasts (PAFs) in the periodontitis lesion.[15–17] These PAFs were found to highly degrade collagen gel. During connective tissue metabolism, activated fibroblasts are known to produce extracellular matrix (ECM) components as well as proteolytic enzymes, thereby contributing to ECM remodeling and degradation.[18,19] Ohshima et al. have isolated PAFs from the gingival tissue of periodontitis lesions in patients and consecutively characterized significantly different gene expression signatures of these PAFs compared to normal gingival fibroblasts derived from healthy individuals.[15–17]

In order to develop an experimental model of periodontitis, primary cultured gingival epithelial cells and periodontally-affected fibroblasts were co-cultured in collagen gels, which appeared to recapitulate epithelial cell–fibroblast interactions in the gingival connective tissue.[15,16,20] A series of previous reports have demonstrated that PAFs display a remarkably higher capacity for ECM degradation compared to normal fibroblasts.[15–17]

Based on the findings gained through the identification and study of PAFs, we speculated that there would exist peri-implantitis-associated fibroblasts (PIAFs) that display a capacity for collagen degradation, similar to PAFs, in the peri-implantitis lesions. Thus, the purpose of the present study was to analyze the presence of fibroblasts with a high capacity for collagen degradation, isolated from peri-implantitis lesions. In the present study, we collected peri-implant tissues during peri-implantitis surgery in the clinic, isolated fibroblasts from the tissues, and investigated the collagen gel contraction in the experimental 3D cell co-culture model.

Material and methods

Cell culture

Gingival tissues were obtained during peri-implant, periodontal, and tooth extraction surgeries at the Department of Periodontics, Institute of Science Tokyo Hospital (formerly Tokyo Medical and Dental University Hospital) and at Dental Hospital, School of Dentistry, Nihon University (Tokyo, Japan). Informed consent was obtained from all patients. The patients with peri-implantitis who were included in the study were indicated for surgical treatment and exhibited moderate to severe peri-implantitis, with probing pocket depth ≥6 mm, bleeding on probing (BOP) and bone resorption. Exclusion criteria encompassed current smokers, patients taking medications that induce bone necrosis after periodontal surgery (such as bisphosphonates), individuals with mobile dental implants, patients with systemic/local diseases undergoing treatment, and pregnant women. The protocol of the study was approved by the Ethics Committee of Nihon University School of Dentistry (approval No. 2013-1, EP18D018) and the Faculty of Dentistry, Institute of Science Tokyo (approval No. D2014-141-02).

During peri-implant surgery, excessive marginal gingival tissues, diseased connective tissues of the inner surface of the gingival flaps, and/or diseased connective tissues from within the bone defects around implants were obtained.

Gingival fibroblasts and epithelial cells were isolated from the gingival and connective tissues. Cell cultures were performed as previously described.[21–23] With regard to the PIAFs, briefly, the collected tissues were cut into small pieces and placed into 6-well plates. Fibroblast populations were established from each well, and subsequently pooled to form a single population.

Collagen gel co-culture assay

The experimental model, previously used for periodontitis, was employed in this study. The model involved the 3D co-culture of gingival fibroblasts (isolated from periodontitis lesions) and gingival epithelial cells.[15,16,20]

Briefly, as presented in Fig. 1, collagen gels were prepared by mixing 0.5 mL of fibroblast cell suspension (2.5×10^5 cells) in fetal bovine serum (FBS), 2.3 mL of type I collagen (Cellmatrix Type I-A; Nitta Gelatin Inc., Osaka, Japan), 670 µL of 5× Dulbecco's Modified Eagle Medium (DMEM), and 330 µL of reconstitution buffer, in accordance with the manufacturer's instructions. The mixed solution (3.5 mL) was placed into each well of a 6-well plate and allowed to gelatinize in an incubator (MCO-18AC-PJ; Panasonic Corp., Ora, Japan) set at 37°C for 30 min. Subsequently, gingival epithelial cells (2.5×10^5/2 mL) were seeded onto the surface of each gel. The co-culture medium consisted of a mixture of 0.5 mL of DMEM supplemented with 10% FBS and 1.5 mL of EpiLife™ medium with Supplement S7 (Thermo Fisher Scientific, Waltham, USA).

The gels were cultured overnight and carefully separated from the edge of each well to generate a floating gel culture. The floated gels were then cultured in the medium for 5 days and evaluated.

Gel contraction was observed and photographed for area measurement. The surface areas of gels were measured using the ImageJ software (NIH, Bethesda, USA), and the gel size rate (GSR) of each sample was expressed as a ratio of the individual sample gel area (with fibroblasts derived from a peri-implantitis lesion) to the average control gel area (with normal fibroblasts). The degree of gel contraction was determined using the GSR, as follows: none (–): GSR ≥ 80%; mild (+): 80% > GSR ≥ 50%; moderate (++): 50% > GSR ≥ 20%; and severe (+++): GSR < 20%.

For histological analysis, some gels were cultured at the air-liquid interface to induce stratified epithelium.[24] Cell inserts (Falcon® Cell Strainer; Corning, New York, USA) were adjusted and placed upside down in the wells. The floated gels were cultured on the turned-over inserts for an additional 5 days. After this period, the gels were fixed in 10% neutral buffered formalin solution and embedded in paraffin. Vertical sections were stained with hematoxylin and eosin (H&E) and examined under an optical microscope (ECLIPSE Ni-L; Nikon Corp., Tokyo, Japan) (Fig. 1).

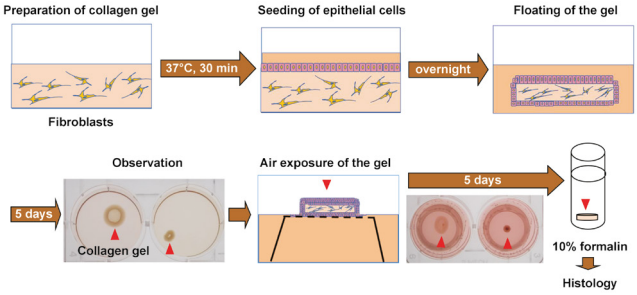

Fig. 1. Three-dimensional (3D) co-culture of gingival fibroblasts and epithelial cells

Results

Case demographics

The peri-implant tissues were obtained from peri-implantitis regions of 5 patients at the Department of Periodontics, Institute of Science Tokyo Hospital. The study sample included 2 males and 3 females with ages ranging from 56 to 83 years (mean (*M*): 69.2 ±10.6 years). The number of implants with peri-implantitis and the total number of existing implants were as follows: 4 and 7 for case 1, 1 and 1 for case 2, 5 and 8 for case 3, 3 and 5 for case 4, and 3 and 5 for case 5. Each patient exhibited localized peri-implantitis in the absence of generalized peri-implantitis. None of the patients had a history of implant loss. Cases 2 and 5 had hypertension, with case 2 receiving a calcium channel blocker medication. One patient (case 4) was being monitored without medication for aortic valve dysfunction. One individual (case 5) exhibited a thoracic aortic aneurysm, necessitating the administration of an anticoagulant.

The demographic information of the participants and the characteristics/locations of the treated implants are presented in Table 1. The levels of peri-implantitis for those implants ranged from moderate to severe (4.2–8.7 mm of bone defect depth) in terms of bone resorption, with 6–10 mm of probing pocket depth (PPD), and BOP observed at all sites. Figure 2 presents the clinical photographs and radiographs of 2 cases (case 1 and case 4).

Affected fibroblasts were isolated from the peri-implant tissues obtained from the participants. Epithelial cells were obtained from 5 patients (systemically healthy females, aged 23–76 years) during peri-implant, periodontal and extraction surgeries. The control group consisted of normal fibroblasts obtained from 3 patients (2 males and 1 female, aged 23–27 years) during tooth extraction (FDI tooth positions 34, 38, 48). These patients were non-smokers, systemically healthy, and had no periodontal inflammation around the teeth. The above epithelial cells and normal fibroblasts were obtained from the Dental Hospital, School of Dentistry, Nihon University.

PIAF detection

Fibroblast growth was observed to be optimal in 4 cases and poor in 1 case. The 5 cases showed gel contraction to varying extents (Table 2, Fig. 3), which may indicate the presence of PIAFs that display the capacity for collagen degradation. Severe (+++) gel contraction was detected in 3 cases (1, 4, 5) for which good cell growth was observed.

Table 1. Peri-implantitis regions and the demographic characteristics of the patients

Case	Age [years]	Sex	Implant site (FDI)	Deepest PPD [mm]	BOP	Bone defect depth [mm]	Mobility [degree]
1*	68	F	13	7	+	8.7	0
2	63	M	11	6	+	4.2	0
3	56	M	15 (14–16)	8	+	6.4	0
4	76	F	46	10	+	5.1	0
5	83	F	15 (14–16)	8	+	5.3	0

F – female; M – male; PPD – probing pocket depth; BOP – bleeding on probing; *observation of collagen gel contraction was performed earlier (on days 2–4), compared to the other cases (day 5).

In 2 of those cases (1 and 4), a significant number of populations showed severe (+++) gel contractions, with case 1 being associated with the most severe gel contractions. Interestingly, the gel contraction observed in case 1 was notably aggressive, with the fastest contraction speed noted in this study, surpassing the rates recorded in our prior investigations employing PAFs. Consequently, the gel evaluation for case 1 was conducted earlier, on days 2–4.

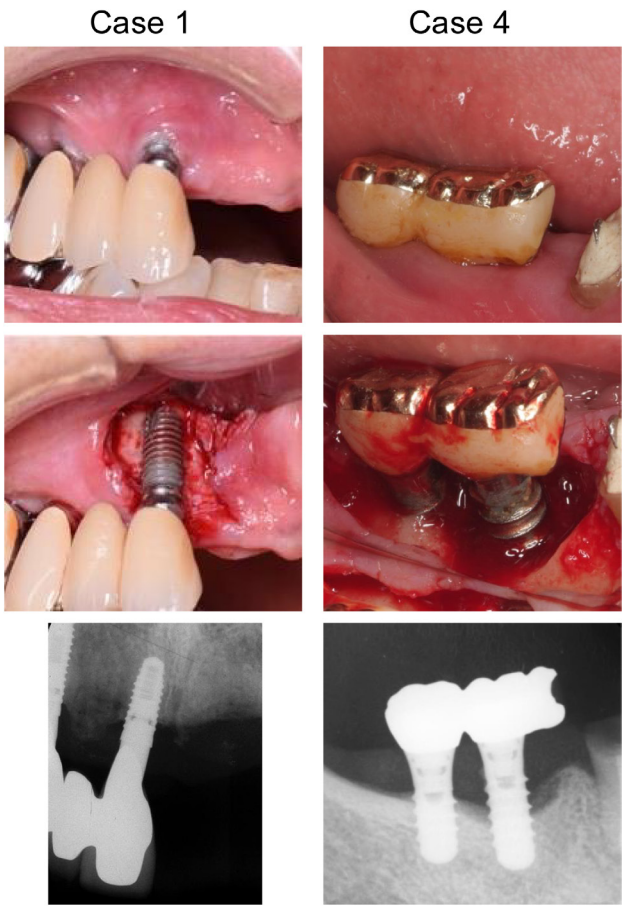

Fig. 2. Peri-implantitis cases with severe collagen gel degradation

Case 1 is a 68-year-old female patient. The 13 (FDI) implant showed severe peri-implantitis with 7-mm probing pocket depth (PPD) and bleeding on probing (BOP). After flap reflection, the buccal site of the implant was completely exposed to the apex, possibly due to an original dehiscence and subsequent bone resorption caused by peri-implantitis. Case 4 is a 76-year-old female patient. The 46 (FDI) implant showed severe peri-implantitis with 10-mm PPD and BOP. After flap reflection, the implant site exhibited a severe circumferential bone defect, as delineated in the radiograph.

Histological observation of the gels

Figure 4 shows representative photomicrographs of gels with normal fibroblasts (Fig. 4A,C,E) and PIAFs (case 4) (Fig. 4B,D,F). The collagen gels with normal fibroblasts showed plain and weak staining with few cells (Fig. 4C,E), while the gels with PIAFs demonstrated a significant contraction with a substantial cellular presence. This contraction can be attributed to the shrinkage of the gel and the presence of numerous vacuoles adjacent to the cells (Fig. 4D,F), possibly occurring due to the degradation of the collagen matrix. The cell nuclei were considerably different, with spindle-shaped nuclei characteristic of normal fibroblasts and round-shaped nuclei predominating in PIAFs.

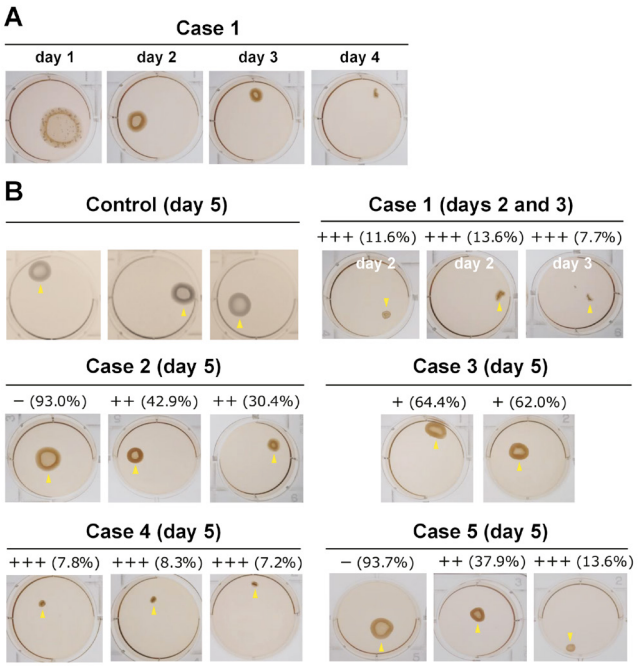

Fig. 3. Degree of collagen gel contraction after three-dimensional (3D) co-culture of fibroblasts (derived from peri-implantitis lesions) and gingival epithelial cells

A. Representative gel contraction over time (case 1); B. Representative distributions of gel size in the control group (normal fibroblasts) and cases 1–5, on day 5, and days 2 and 3 for case 1. In each case, 3 representative populations are presented, with the exception of case 3, where only 2 populations were obtained following cell culturing. The degree of contraction was determined using the gel size rate (GSR), as follows: none (–): GSR ≥ 80%; mild (+): 80% > GSR ≥ 50%; moderate (++): 50% > GSR ≥ 20%; and severe (+++): GSR < 20%.

Table 2. Degrees of collagen gel contraction after three-dimensional (3D) co-culture of fibroblasts (derived from peri-implantitis lesions) and gingival epithelial cells

Case	Sample tissues	Cell growth	Degree of gel contraction				Populations, *n*
			none (–) (GSR ≥ 80%)	mild (+) (80% > GSR ≥ 50%)	moderate (++) (50% > GSR ≥ 20%)	severe (+++) (GSR < 20%)	
1	marginal gingival tissue, diseased connective tissue	good	0 (0%)	1 (10%)	3 (30%)	6 (60%)	10
2	diseased connective tissue	good	2 (20%)	0 (0%)	8 (80%)	0 (0%)	10
3	diseased connective tissue	poor	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2
4	marginal gingival tissue, diseased connective tissue	good	2 (17%)	2 (17%)	3 (25%)	5 (42%)	12
5	diseased connective tissue	good	2 (11%)	14 (78%)	1 (6%)	1 (6%)	18

GSR – gel size rate.

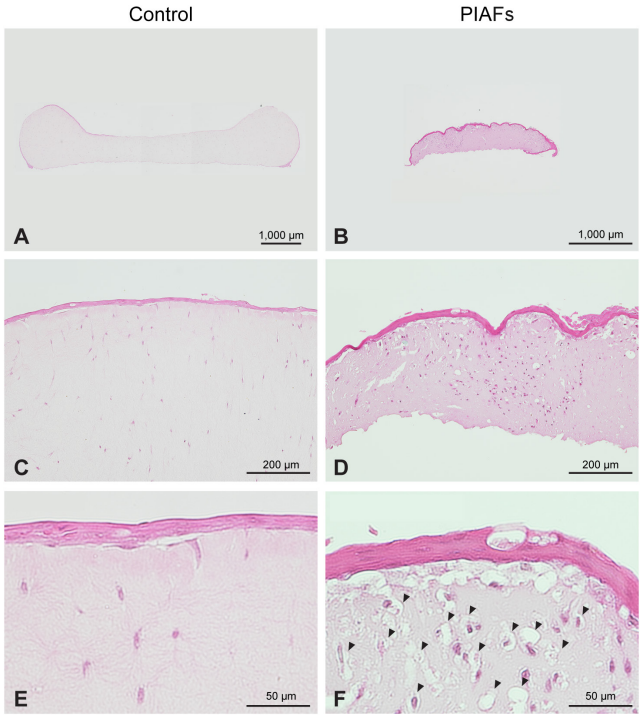

Fig. 4. Representative photomicrographs of gels with normal fibroblasts (control) and peri-implantitis-associated fibroblasts (PIAFs) (case 4)

Compared to the control gel (A), the gel containing PIAFs (case 4) (B) showed significant contraction. The control collagen gel with normal fibroblasts (C,E) exhibited plain staining with a reduced number of cells, while the gel with PIAFs (D,F) displayed numerous vacuoles (black arrowheads) adjacent to cells (due to collagen matrix degradation), as well as an increased cell count (due to gel shrinkage).

Discussion

In the present study, we analyzed fibroblasts isolated from peri-implantitis lesions in vitro using a 3D experimental collagen gel model co-cultured with epithelial cells. In the context of moderate to severe peri-implantitis regions, a range of collagen gel contraction degrees was observed. This may be attributable to the presence of characteristic fibroblasts that highly degrade collagen in peri-implantitis lesions. We have proposed to name these peri-implantitis lesion cells "peri-implantitis-associated fibroblasts" (PIAFs), similar to "periodontitis-associated fibroblasts" (PAFs) that we previously detected and reported in the periodontitis lesion.[15,16]

Periodontitis-associated fibroblasts are frequently detected in severe periodontitis lesions.[15–17,20] These cells have a unique ability to significantly degrade collagen gel in a 3D experimental model co-cultured with epithelial cells. In order to induce collagen degradation with fibroblasts, it was determined that the interaction with primary gingival epithelial cells and primary gingival fibroblasts is important and necessary. Thus, this 3D experimental co-culture model was suitable for inducing interaction, and was employed using both clinical samples in the present study.

Enzymatic degradation is necessary for gel degradation.[25] In our PAF study, we reported that the gene expression of matrix-degrading enzymes, such as matrix metalloproteinase (MMP)-1 and -3, was upregulated.[15] The upregulation of transforming growth factor-beta (TGF-β) responsive genes, such as *Smad7* and *type I collagen α1 chain*, confirmed the activity of TGF-β during the contraction of the gel. In addition, it was demonstrated that vascular endothelial growth factor (VGFT) receptor-1 (Flt-1) and hepatocyte growth factor (HGF) are involved in matrix degradation in the 3D culture system. Furthermore, PAFs were revealed to be important components in the pathogenesis of periodontitis.[16,20] Gel degradation was significantly suppressed by serine protease inhibition (aprotinin) and hydroxamate/MMP inhibition (marimastat),[15] which is in line with previous reports.[26]

In addition, we investigated genes related to cancer-associated fibroblasts (CAFs),[27,28] since the migration of gingival epithelial cells to the apical region (epithelial downgrowth) during the progression of periodontitis exhibits similarities to the invasive process of cancer cells.[29] Among the CAF-associated genes, *HGF*, *COX-2*, *LAMA2*, *SFRP-1*, *SFRP-2*, *CXCL12*, *ITGA11*, and *ANGPTL2* were unregulated during the process of gel degradation in co-cultures. However, the expression levels of some genes such as *MMP-2*, *PAI-I*, *CCL5*, and *syndecan-1* remained unaltered,[15,16,30,31] indicating the possibility of similar characteristics between CAFs and PAFs.

In the present study, the extent of the gel contraction exhibited significant variation among the cases, possibly due to the presence of different numbers of PIAFs, as well as differently affected PIAFs in each case. Case 2 received a calcium channel blocker, which may have affected gingival overgrowth, but the patient did not show generalized peri-implantitis, and the level of collagen gel contraction was generally not severe. Cases 1 and 4 demonstrated a high degree of collagen gel degradation potential. Notably, PIAFs in case 1 demonstrated the most significant propensity to rapidly and aggressively degrade collagen gel among all cases, even when compared to the levels that had been previously observed in studies using PAFs. In the present study, case 1 was systemically healthy. A thorough examination of the sampling sites in these 2 cases suggests the potential existence of PIAFs within the inner surface of periodontal flaps rather than in the diseased connective tissue within the bone defects. In preliminary experiments, the application of anti-HGF neutralizing antibodies, which have an inhibitory effect on collagen degradation in PAFs, to the co-culture with case 1 PIAFs, failed to inhibit collagen degradation. However, imatinib (anticancer drug) inhibited collagen degradation, suggesting that PIAFs in case 1 may differ from the typical PAFs. The aggressive nature of PIAFs in case 1 may be associated with the advanced status of peri-implantitis observed in that patient.

Initially, PAFs and PIAFs occur around the cervical area after the interaction of the inflammatory sulcular epithelium and gingival fibroblasts. Then, PAFs may gradually affect the periodontal ligament, resulting in attachment loss and disease progression in periodontitis. However, in the case of peri-implantitis, there is no connective tissue between implants and bone. Thus, if PIAFs are associated with the progression of peri-implantitis, PAFs/PIAFs may also directly affect bone. Further investigation is necessary to clarify the influence of PAFs/PIAFs on periodontal and peri-implant bone.

In the context of peri-implantitis, different characteristics of implant surfaces can elicit different biological responses in peri-implant fibroblasts and bone cells.[32] Furthermore, in contrast to the case of periodontitis, the release of toxic titanium particles due to mechanical wear, chemical corrosion, microbial contamination, and implant surface treatment may impact peri-implant tissues.[33] Consequently, titanium might contribute to collagen degradation associated with PIAFs, as compared to PAFs. Recently, other complications with dental implants, including the occurrence of cancer around implants, have been reported.[34–36] It is conceivable that the aggressive characteristics of PIAFs might be associated with cancer occurrence.

The effective treatment of peri-implantitis represents a major clinical challenge.[13] This is partially due to the lack of detailed information and understanding regarding the molecular and cellular pathogenesis of peri-implantitis, as well as its occurrence mechanism. This study demonstrated the presence of PIAFs with the capability of matrix degradation in peri-implantitis lesions, a finding which indicates that PIAFs may constitute a key component in the pathogenesis and progression of peri-implantitis. If the capacity for collagen degradation in PIAFs can be controlled, a new treatment strategy may be developed in peri-implant disease therapy. These points support the clinical relevance of the present study within the context of peri-implantitis treatment.

Limitations

There are some limitations to the present study. These include the small sample size, the presence of comorbidities, the disparity in ages between normal fibroblasts and PIAFs, the absence of immune cells in the experimental cell culture model, the assumption that collagen contraction is caused exclusively by degradation provoked by the cells, and the lack of more accurate assays to evaluate the collagen degradation. These points should be investigated through detailed studies. In this preliminary report, we conducted a semi-quantitative analysis of gel area measurement. In subsequent studies, analyses such as collagen zymography, gene expression, collagenase/MMP detection, and proteomics for collagen matrix degradation should be considered.

Conclusions

The fibroblasts isolated from peri-implantitis lesions showed various degrees of collagen gel contraction when co-cultured with epithelial cells using a 3D collagen gel model. This observation suggests the potential presence of PIAFs, which are capable of collagen degradation. In particular, aggressive types of PIAFs were detected, the characteristics of which may differ from those of PAFs. Further detailed studies are required to clarify the characteristics of PIAFs as well as their role in the occurrence and progression of peri-implantitis.

Ethics approval and consent to participate

The protocol of the study was approved by the Ethics Committee of Nihon University School of Dentistry (approval No. 2013-1, EP18D018) and the Faculty of Dentistry, Institute of Science Tokyo (approval No. D2014-141-02). Informed consent was obtained from all patients.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Sex differences in a self-report questionnaire related to obstructive sleep apnea: An online cross-sectional survey

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Abstract

Background. Obstructive sleep apnea (OSA) is associated with an increased likelihood of health issues, such as hypertension, cardiovascular disease and stroke. Screening is typically performed through self-report questionnaires related to OSA symptoms.

Objectives. The present study aimed to evaluate sex differences in the commonly used questionnaires for the evaluation of OSA symptoms in order to determine whether different OSA screening tools should be considered in males and females.

Material and methods. The data was collected from the general population ($N = 622$, 66% female) through an online cross-sectional survey. The survey incorporated the STOP-Bang Questionnaire, the Epworth Sleepiness Scale (ESS), the Patient Health Questionnaire-4 (PHQ-4), the Fatigue Assessment Scale (FAS), and sleep bruxism (SB) questionnaires.

Results. Female subjects exhibited elevated levels of anxiety and fatigue ($p < 0.001$ for both) and the potential presence of SB ($p < 0.005$). The logistic regression analysis revealed that the odds of moderate to severe OSA increased by 5–8% for age and sleepiness, were higher for subjects exhibiting SB (an increase of 82%), and were particularly high for males (male sex increased the odds of moderate to severe OSA by over 5 times). Despite higher fatigue scores among females, the effect of fatigue on the probability of moderate to severe OSA in females was non-significant. While male subjects demonstrated lower fatigue scores, these levels were significantly associated with the risk of moderate to severe OSA. Daytime sleepiness did not influence the OSA risk for either sex.

Conclusions. The impact of reported fatigue on the prevalence of OSA is substantial among males but non-significant among females. The efficacy of daytime sleepiness scales in evaluating OSA is poor. The fatigue scale may be more effective in the screening of OSA, at least in males. Limitations of the study include potential response bias due to participant anonymity and the use of the STOP-Bang Questionnaire instead of polysomnography, the gold standard for OSA diagnosis.

Keywords: sex, STOP-Bang, obstructive sleep apnea (OSA), Epworth Sleepiness Scale (ESS), Fatigue Assessment Scale (FAS)

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Highlights

- Male sex increased the odds of moderate to severe obstructive sleep apnea (OSA) by more than 5 times, while higher levels of anxiety and fatigue in females were not predictors of the OSA risk.
- Fatigue scores effectively predicted the OSA risk in males, whereas daytime sleepiness measures were ineffective for both sexes.
- Sleep bruxism increased the odds of OSA by 82%, independent of sex.
- Sex-specific OSA screening strategies are recommended due to distinct symptom patterns in males and females.

Introduction

Obstructive sleep apnea (OSA) belongs to a broad list of conditions referred to as sleep-disordered breathing, which range from snoring to OSA.[1] Obstructive sleep apnea is characterized by a repetitive collapse of the upper airway during sleep, frequently associated with oxygen desaturation and/or arousal from sleep.[2] Although patients with nocturnal snoring tend to have smaller airway dimensions, their pharyngeal measurements do not differ from those of non-snoring individuals.[3] The only parameter found to relate to upper airway volume is vertical skeletal dimension.[4]

Obstructive sleep apnea is closely linked to arterial hypertension. A study on patients with comorbid arterial hypertension and OSA revealed notable associations between sleep fragmentation and the levels of calcium, uric acid and magnesium in their blood samples.[5] Additionally, OSA has been associated with the disruption of the circadian rhythm and metabolic dysregulation of circadian clock proteins.[6]

The prevalence of OSA is higher in males than females, even after correcting for age and body mass index (BMI).[1,7] The clinical presentation of OSA differs between the sexes.[8,9] These differences may originate from hormonal influences and/or anatomic differences, such as the shape of the upper airways, distribution of body fat, and craniofacial morphology. Generally, females are more symptomatic, experience prolonged partial upper airway obstruction, and report insomnia as a symptom of OSA more frequently than men.[10] Males tend to report sleepiness, snoring and apnea, while females report fatigue, initial insomnia, depression, and morning headaches.[8,11] As a result, women are less likely to be diagnosed and treated for OSA than men.[10,12] In some cases, women with OSA may be misdiagnosed and treated for other conditions, such as depression, insomnia and hypothyroidism.[13] A comparison of males and females with similar OSA severity reveals that women utilize healthcare resources to a greater extent than males due to atypical symptoms, a poor perception of health and the overuse of psychoactive medication.[14]

The gold standard for OSA diagnosis is polysomnography,[10,15] because it monitors multiple physiological parameters during sleep, ensuring an accurate and objective diagnosis. However, initial screening is usually performed using self-report questionnaires related to OSA symptoms, whose predictive performance has been widely evaluated.[16,17] Several such screening tools include the Epworth Sleepiness Scale (ESS),[18] the STOP and STOP-Bang Questionnaires,[19] the Gait Outcomes Assessment List (GOAL) Questionnaire,[20] the Berlin Questionnaire,[20] the Athens Insomnia Scale (AIS),[21] and the Fatigue Assessment Scale (FAS).[22] However, it is not yet clear whether there is a sex-specific influence on the predictive performance of the different questionnaires. Pataka et al. evaluated possible sex differences in various questionnaires used for OSA prediction and concluded that sex-specific evaluation of questionnaires is necessary to prevent OSA underdiagnosis.[23]

This study utilized an online questionnaire to effectively identify issues related to OSA. This method enables access to a broad spectrum of the population, including men and women who might be unaware of their condition or have been underdiagnosed with OSA.

Few questionnaires were assessed to determine the most effective tool for each subgroup (based on sex and age).

The present study aimed to examine sex differences in the commonly used questionnaires for the evaluation of OSA symptoms (ESS,[18] STOP-Bang Questionnaire,[24] FAS,[22] and Patient Health Questionnaire-4 (PHQ-4))[25] in order to determine whether different OSA screening tools should be considered for males and females.

Material and methods

The study was conducted as a cross-sectional online survey through the administration of anonymous questionnaires. Participation in the survey was voluntary. The survey was posted on WhatsApp groups and billboards from March 2023 to October 2023. The data for the present study was collected through Google Forms (https://docs.google.com/forms/d/1ZDd74tBezP5D-xsj0VxBj23yKZ_YnI8SaNOLkF8CDyk/edit).

The minimum required sample size was determined using a sample size calculator (https://www.calculator.net/sample-size-calculator.html). The following parameters were used: a population size of 9,000,000 (representing

the population size in Israel); a confidence level of 95%; a maximum error of 5%; and a population proportion of 20%.[26] Based on these calculations, a minimum sample size of 246 individuals was determined.

The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee at Tel Aviv University, Israel, approved all study procedures (approval No. 0005883-2 15/1/2023).

Instruments and study variables

Demographic information

The following information was collected: age; sex; personal status (single, married, divorced, widowed); number of children and the age of the youngest child; pregnancy (yes/no); use of pharmacological drugs to improve sleep (including melatonin) (yes/no); treatment for disruptive sleep (e.g., continuous positive airway pressure (CPAP) machine, oral appliances) (yes/no); having a job (yes/no) and the type of work (daytime, night-time, shifts).

Risk of OSA

The Hebrew version of the STOP-Bang Questionnaire, developed and validated by Chung et al. as a screening tool for OSA,[24] was used in the present study. The questionnaire showed high sensitivity, especially among patients with moderate to severe OSA.

The first part of the questionnaire consists of 4 self-report yes/no questions concerning Snoring, Tiredness during daytime, Observed apneas, and high blood Pressure (STOP). The total score on the STOP questionnaire ranges from 0 to 4.

The second part of the questionnaire (Bang) refers to BMI, Age, Neck circumference, and Gender (BANG). In the present study, data concerning neck circumference and height (necessary for the calculation of BMI) was not collected.

As the aim of the present study was to identify subjects with moderate to severe risk of OSA who should be referred for further evaluation, 2 categories of OSA were defined, as suggested by Chung et al.[24]:
– low risk: score of 0–2 on the self-report questions of the STOP part;
– moderate to severe risk: a combination of 2 items from the STOP part and 1 from the BANG part, as follows: STOP score of 3–4; or STOP ≥ 2 + BANG ≥ 1; or STOP ≥ 2 + male sex; or STOP ≥ 2 + age >50 years.

Daytime sleepiness (ESS)

Daytime sleepiness was assessed using the ESS.[18] The questionnaire measures the subject's general level of daytime sleepiness. The questionnaire consists of 8 items, which are scored on a scale from 1 to 4. The scale assesses daytime sleepiness through asking the subjects about their propensity to fall asleep or doze off during common daily activities (e.g., reading, watching TV, talking). The final scores for daytime sleepiness are as follows:
– 0–5: low normal;
– 6–10: high normal;
– 11–12: mildly excessive;
– 13–15: moderately excessive;
– 16–24: severely excessive.

Patient Health Questionnaire-4 (PHQ-4)

The Patient Health Questionnaire-4 is used for screening anxiety and depression.[25] It is a validated ultra brief tool consisting of 2 questions about anxiety and 2 questions about depression.

Respondents rate the frequency with which they had been bothered by specific problems over the past 2 weeks on a scale from 0 (not at all) to 3 (nearly every day). The total score ranges from 0 to 12, and the conditions are usually evaluated using the following cut-off scores[27]:
– 0–2: normal;
– 3–5: mild;
– 6–8: moderate;
– 9–12: severe.

The questionnaire also enables to perform a separate evaluation for anxiety and depression. A total score of at least 3 for the first 2 questions indicates the presence of anxiety. A total score of at least 3 for the last 2 questions is indicative of depression.

Fatigue Assessment Scale (FAS)

The Fatigue Assessment Scale is a self-report questionnaire[22,27] designed to evaluate symptoms of chronic fatigue, both physical and mental. It is comprised of 10 questions, each rated on a 1–5 scale. The total score ranges from 10 to 50 points, as follows:
– 10–21: normal;
– 22–34: fatigue;
– ≥35: extreme fatigue.

Possible sleep bruxism

Possible sleep bruxism (SB) was evaluated according to the Oral Behaviors Checklist (component of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), official Hebrew version).[28] The question posed to participants was as follows: "Based on the past month, how often have you clenched or ground your teeth while asleep?". Sleep bruxism was diagnosed when subjects reported clenching or grinding their teeth at a frequency of at least 1–3 nights per week. This methodology is considered valid for the screening of bruxism among large populations.[29,30] It adheres to the definition of "possible" bruxism, as defined by the consensus papers on bruxism.[31]

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). The χ^2 test was used to compare categorical variables. The logistic regression analysis evaluated the multivariate effect of the study variables on OSA.

Finally, the Hayes PROCESS model for SPSS v. 4.1[32] was used to analyze the relationship between the risk of OSA and FAS and between the risk of OSA and ESS, while taking into account the effect of sex.

Results

A total of 836 subjects provided responses to the questionnaire. Given the potential impact of certain factors on nighttime sleep, the following subjects were excluded from the analyses: participants who have a child younger than 1 year (n = 45); pregnant individuals (n = 25); those working night shifts (n = 81); individuals who use sleeping medications and/or melatonin (n = 41); and subjects treated for OSA or using CPAP (n = 48). Some participants responded positively to more than one of the above categories.

The final number of subjects included in the study was 622 (74.4% of the original sample, with 66% being female). Female subjects were significantly older than males (mean age: 46.3 ±15.4 years vs. 37.8 ±7.4 years, respectively; $p < 0.001$) (Fig. 1).

OSA

About 20% of the female respondents were classified as having a moderate to severe OSA risk, while approx. 46% of the male respondents were identified as having a moderate to severe OSA risk (significantly higher percentage than that in females; $\chi^2 = 113.869$, degrees of freedom (df) = 2, $p < 0.001$) (Table 1).

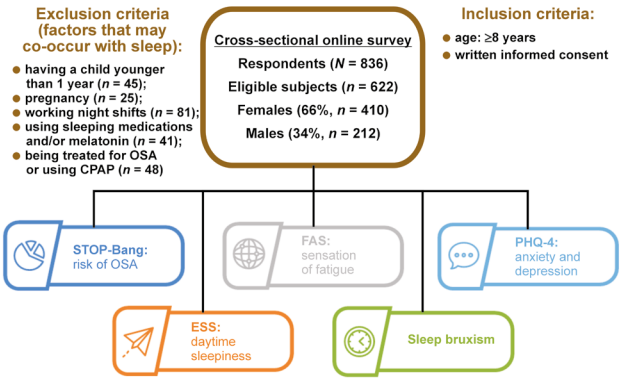

Fig. 1. Flowchart of the study

OSA – obstructive sleep apnea; CPAP – continuous positive airway pressure; FAS – Fatigue Assessment Scale; PHQ-4 – Patient Health Questionnaire-4; ESS – Epworth Sleepiness Scale.

PHQ-4

A greater proportion of females than males were categorized as mild, moderate and severe based on their total PHQ-4 score (χ^2 = 19.215, df = 3, $p < 0.001$). Similarly, more females than males were classified as suffering from anxiety (χ^2 = 10.903, df = 10, $p < 0.001$) (Table 1). The differences between males and females in the depression scale were borderline significant (13.4% vs. 18.5%, respectively; χ^2 = 2.523, df = 1, p = 0.069).

FAS

Significant differences were observed between males and females in terms of their level of fatigue, with more females reporting fatigue and extreme fatigue compared to males (χ^2 = 15.173, df = 1, $p < 0.001$) (Table 1).

ESS

No differences between sexes could be detected with regard to ESS categories (χ^2 = 2.070, df = 2, p = 0.558).

Possible SB

Significant differences were identified between males and females with respect to possible SB, with females exhibiting a significantly higher prevalence of possible SB compared to males (χ^2 = 10.450, df = 1, $p < 0.005$) (Table 1).

Table 1. Comparison between male and female study participants

Variable		Males	Females
OSA risk	low	106 (53.8)	312 (81.3)
	moderate	82 (41.6)	23 (6.0)
	severe	9 (4.6)	49 (12.8)
	total	197 (100.0)	384 (100.0)
PHQ-4	normal	127 (61.4)	172 (43.2)
	mild	62 (30.0)	162 (40.7)
	moderate	14 (6.8)	44 (11.1)
	severe	4 (1.9)	20 (5.0)
	total	207 (100)	398 (100.0)
Anxiety	no	177 (85.5)	295 (73.8)
	yes	30 (14.5)	105 (26.3)
	total	207 (100.0)	400 (100.0)
FAS	normal	122 (59.8)	169 (43.0)
	fatigue	79 (38.7)	207 (52.7)
	extreme fatigue	3 (1.5)	17 (4.3)
	total	204 (100.0)	393 (100.0)
SB	no SB	184 (89.3)	315 (78.8)
	possible SB	22 (10.7)	85 (21.3)
	total	206 (100.0)	400 (100.0)

Data presented as number (percentage) (n (%)); OSA – obstructive sleep apnea; PHQ-4 – Patient Health Questionnaire-4; FAS – Fatigue Assessment Scale; SB – sleep bruxism.

Multivariate analyses

The logistic regression analysis was used to evaluate the relationship between age, sex, ESS, FAS, SB, anxiety, FAS and sex interaction, and OSA. As delineated in the Material and methods section, OSA was categorized as low risk vs. moderate/severe risk (Nagelkerke $R^2 = 0.202$) (Table 2).

The Hayes PROCESS model was used to analyze the relationship between OSA and FAS, while taking into account the effect of sex (Fig. 2). The conditional effect of FAS on the values of the male sex was as follows:

Table 2. Odds ratio for moderate to severe risk of obstructive sleep apnea (OSA)

Variable	*OR*	95% *CI*	
		lower	upper
Age	1.050	1.028	1.072
Sex (male)	5.607	3.201	9.823
ESS	1.084	1.032	1.139
FAS	1.029	0.967	1.095
SB	1.822	1.014	3.273
Anxiety	0.496	0.255	0.966
FAS*male	1.134	1.029	1.249

ESS – Epworth Sleepiness Scale; *OR* – odds ratio; *CI* – confidence interval.

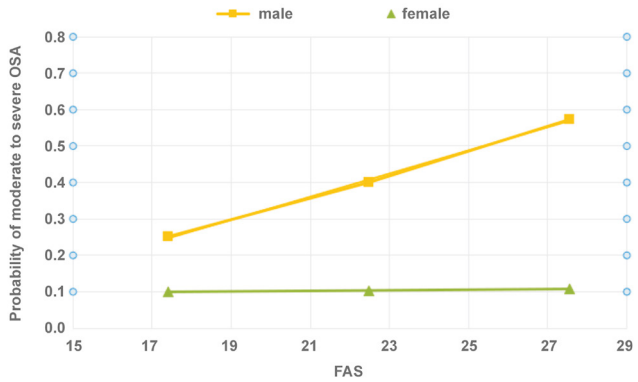

Fig. 2. Conditional effect of the Fatigue Assessment Scale (FAS) on the probability of moderate to severe obstructive sleep apnea (OSA) (with sex as a moderator)

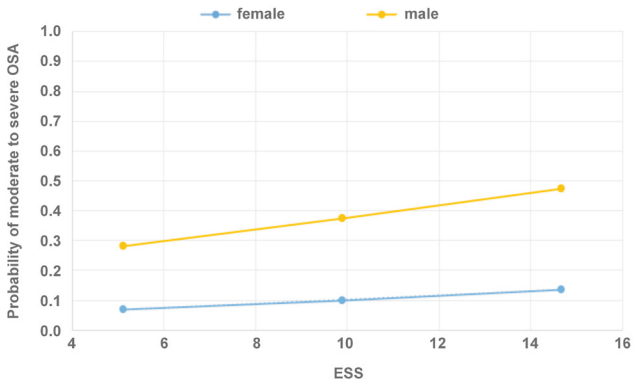

Fig. 3. Conditional effect of the Epworth Sleepiness Scale (ESS) on the probability of moderate to severe obstructive sleep apnea (OSA) (with sex as a moderator)

effect size = 0.1539, $p = 0.003$ (95% confidence interval (*CI*): 0.0709–0.2368). The conditional effect of FAS on the values of female sex was not observed.

The Hayes PROCESS model was used to analyze the relationship between OSA and ESS, while taking into account the effect of sex (Fig. 3). The investigation revealed no conditional effect of ESS on values of either sex.

Discussion

Obstructive sleep apnea is associated with an increased likelihood of developing hypertension, cardiovascular disease, stroke, daytime sleepiness, motor vehicle accidents, and diminished quality of life.[33]

The initial screening for OSA is usually conducted through the administration of self-report questionnaires. One of the most common tools for the initial screening of OSA is the STOP-Bang Questionnaire.[24] Numerous studies have demonstrated its efficacy in the screening of moderate-to-severe OSA, exhibiting excellent diagnostic performance.[34–36] Additionally, the tool demonstrates a high sensitivity in predicting moderate-to-severe OSA and severe OSA, when compared to the ESS.

In 2022, the United States Preventive Services Task Force (USPSTF) published a systematic review addressing the topic of screening for OSA in adults.[36] The authors noted the absence of sufficient evidence to support the accuracy of screening questionnaires in identifying adults within the general population who are at an increased risk for OSA.[36]

Sex plays a significant role in OSA, with more males being affected by OSA compared to females, even after correcting for age and BMI.[7,23] Today, the medical community is increasingly aware that males and females may react differently to medical conditions, and that sex-specific approaches should be applied to many of the commonly used diagnostic and treatment protocols.[37]

In the present study, the STOP tool was used to screen for subjects who should be referred for further evaluation (namely, subjects who present moderate to severe risk of OSA). Due to the mode of the study (online survey), not all of the Bang data was collected, specifically the neck circumference and BMI. Nevertheless, the collected information enabled the accurate differentiation between subjects who do not require further intervention (low OSA risk) and those requiring referral for professional evaluation (moderate to severe risk of OSA).

As expected, there were significant differences between males and females in the OSA, PHQ-4, SB, FAS, and anxiety scores. While males showed higher rates of OSA, females exhibited heightened levels of PHQ-4, anxiety and SB. These results align with the findings reported in recent literature on the subject.[7,38–41] While there were no significant differences in daytime sleepiness (ESS) scores between males and females, notable differences emerged in the levels of fatigue (FAS) and their correlation with OSA.

The logistic regression analysis demonstrated that the odds of OSA increased for age, male sex, FAS, SB, and the FAS and sex (male) interaction. The likelihood of moderate to severe OSA increased by 5–8% for age and ESS. This increase was particularly pronounced in subjects who exhibited SB behavior (an increase of 82%), and was especially high among males, as being male was associated with an over fivefold increase in the risk of moderate to severe OSA compared to females.

Sleep bruxism and OSA are closely associated. The connections between OSA and SB and OSA and sex are widely reported.[42] For example, a higher prevalence of OSA has been reported in males compared to females,[43] and severe OSA has been identified as a risk factor for SB.[44] Interestingly, females tend to be symptomatic at lower apnea–hypopnea index (AHI) scores due to the long-term consequences of disrupted rapid eye movement (REM) sleep or more episodes of upper airway resistance during sleep, which can lead to symptoms such as daytime fatigue.[43]

In the present survey, female subjects exhibited higher levels of fatigue than males. However, no significant differences were observed between the sexes in terms of daytime sleepiness scores. This finding aligns with the conclusions of previous studies.[10,23] Despite the higher fatigue scores observed among females, the effect of fatigue on the probability of moderate to severe OSA in this population was not statistically significant. On the other hand, males exhibited lower fatigue scores; yet, their fatigue level had a significant effect on the risk of moderate to severe OSA. The impact of reported fatigue on the risk of OSA is significant for males and non-significant for females, despite the relatively high levels of fatigue reported by females.

There were no statistically significant differences in ESS scores between the sexes. This instrument is often used to assess subjective sleepiness.[18] Although the ESS is widely used as a screening tool for OSA, its diagnostic reliability has been questioned. Kum et al. found that ESS scores increased as AHI thresholds increased, but some studies have found a weak or no correlation between ESS scores and OSA severity, suggesting that ESS may not aid in OSA diagnosis.[45,46] The results of the present study are in agreement with those by Miller et al.[47] and Ghandeharioun et al.,[48] suggesting that ESS is not efficient in predicting moderate-to-severe OSA. Similarly, Hamilton and Chai-Coetzer demonstrated that ESS is a poor marker of OSA.[49,50] Apparently, for AHI ≥ 5/h, the sensitivity of ESS is rather low (approx. 50%).[49] The present results suggest that while the daytime sleepiness scale exhibits poor OSA screening abilities, the fatigue scale may be more effective, at least for male subjects.

The results concerning anxiety are somewhat puzzling. The presence of anxiety led to an odds ratio (*OR*) of less than 1, meaning that the predicted probability of OSA decreases as anxiety increases. Chen et al. demonstrated that patients with OSA had significantly higher comorbidity rates for anxiety disorders.[51] Additionally, a cross-sectional exploratory study which analyzed data from patients suffering from treatment-resistant depression showed that daytime fatigue may have the potential to mask an underlying sleep disorder in women.[52] An extensive study on 2,251 participants from the Netherlands Sleep Registry showed that anxiety can serve as a bridge factor of self-reported SB not only to insomnia but also to depression, OSA risk, age, and sex.[53] However, nothing in the present results suggests such findings. The PHQ-4 is a brief screening questionnaire whose definition of anxiety and depression relies on merely 2 questions for each condition. It would be too pretentious to draw any conclusions concerning OSA and anxiety/depression from the present results. The role of anxiety and depression in OSA is more complex than initially assumed.[54]

The USPSTF has indicated a need for more accurate studies on screening tools in a general adult primary care population, especially in persons with unrecognized or mild OSA symptoms.[35] The inclusion of screening modes for fatigue, rather than for daytime sleepiness, could enable better screening for moderate to severe OSA, at least in male subjects.

Limitations

The data was collected from the public via an anonymous Internet survey. Subjects who chose to participate do not represent the general population. It is possible that subjects with a special interest in OSA constituted the majority of the participants, a factor that could have led to response bias. Secondly, the risk of moderate to severe OSA was determined through the STOP-Bang Questionnaire rather than through polysomnography, which is the gold standard for OSA diagnosis. Further studies, incorporating additional screening questionnaires (such as GOAL and/or Berlin) and encompassing larger populations, should be performed to better define sex-specific screening tools for OSA.

Conclusions

Fatigue, rather than daytime sleepiness, can serve as an effective indicator of the risk for moderate to severe OSA in males.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee at Tel Aviv University, Israel, approved all study procedures (approval No. 0005883-2 15/1/2023). Informed consent was obtained from all study participants.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Accuracy of electronic apex locators in the determination of the working length in teeth with natural apical root resorption in the presence of different irrigation solutions

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Abstract

Background. Root resorption (RR) is usually a consequence of dental trauma, pulpal infection, primary occlusal pressure, or orthodontic tooth movement, leading to the loss of anatomical root formation. As a result of apical RR, the apical constriction is destroyed, and the determination of the working length (WL) may become difficult.

Objectives. The purpose of the present study was to evaluate the accuracy of 4 different electronic apex locators (EALs) – ProPex® II, Propex Pixi®, DentaPort ZX, and DTE DPEX V – in the determination of WL in teeth with natural apical RR in the presence of different irrigation solutions (ISs).

Material and methods. A total of 36 teeth with natural apical RR were included in the study. The actual length (AL) was determined under a stereomicroscope at ×15 magnification. Afterward, the electronic length (EL) was established by using 4 different EALs in the presence of different ISs. The ISs used in the measurements were freshly prepared, and 6 groups were organized based on the solution used. The control group was marked as group 1. For groups 2–6, 1% sodium hypochlorite (NaOCl), 2.5% NaOCl, saline, 2% chlorhexidine (CHX), and 17% ethylenediaminetetraacetic acid (EDTA) were used, respectively. After each measurement, the roots were washed with 5 mL of distilled water and dried with paper points before the same teeth were used in the subsequent group. The absolute length was subtracted from EL for each tooth to calculate the difference. The data was analyzed statistically.

Results. For each device, there were no significant differences in the success rates between the ISs used. In group 1, there were no significant differences among the 4 EALs. In group 2, DentaPort ZX was significantly more successful than DTE DPEX V ($p = 0.037$). There were no significant differences among EALs in groups 3–6.

Conclusions. The difference between DentaPort ZX and DTE DPEX V was statistically significant in group 2 (1% NaOCl).

Keywords: electronic apex locators, irrigation solution, natural root resorption

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Highlights

- This research is the first to evaluate the accuracy of electronic apex locators (EALs) in the determination of the working length (WL) in teeth with naturally occurring apical root resorption in the presence of different irrigation solutions.
- Common endodontic irrigants (NaOCl, saline, CHX, EDTA) did not significantly impact the performance of EALs.
- DentaPort ZX showed superior accuracy as compared to DTE DPEX V in the presence of 1% NaOCl.
- All 4 tested EALs provided acceptable WL measurements within ±1 mm tolerance despite apical resorption.
- The study offers valuable clinical insights for root canal procedures involving resorptive apical conditions.

Introduction

Root resorption (RR) is a pathological process that concludes with the destruction of dental hard tissues, such as cement and dentin. In primary teeth, RR is usually physiological, but in permanent teeth, it can be pathological – it may occur at the inner surface of the root canal space (internal RR), the outer surface of the root (external RR), or both sides of the root together.[1]

External RR is often a consequence of severe dental trauma, pulpal infection, primary occlusal pressure, or orthodontic tooth movement leading to the loss of anatomical root formation. External RR is usually diagnosed through clinical and radiographical examinations, without any clinical symptoms.[2,3] Although external RR is common, there is no generally accepted treatment protocol. The treatment process varies according to the patient's symptoms. Root canal treatment (RCT) is accepted in symptomatic patients, while follow-up is sufficient in asymptomatic patients.

Working length (WL) determination is of utmost importance in RCT. The working length is expressed as either 0.5–2.0 mm short of the radiographic apex or the extension of the apical constriction.[4] An adequate WL ensures the disinfection of the whole root canal and prevents damage to the periapical tissues.[5] The measurement of WL can be acquired in various ways, e.g., by using an electronic apex locator (EAL).[6] Among the common methods of WL determination is the radiographic method. However, it has several disadvantages, such as distortion and the superposition of anatomical structures.[7]

The determination of WL with EALs is a well-known technique employed in permanent teeth. The accuracy of EALs is affected by various factors. The most important ones are the moisture content in root canals, irrigation solutions (ISs) and the diameter of the apical foramen.[8] Irrigation solutions are used to clean and disinfect the canal and are vital in RCT. To clean the root canal of a tooth, many materials have been used, the most common being sodium hypochlorite (NaOCl), chlorhexidine (CHX) and ethylenediaminetetraacetic acid (EDTA).[9] In addition, a recent study showed that most of dental professionals preferred the use of the full-strength NaOCI concentration as the main IS.[10] However, the presence of ISs in the root canal space may impact the performance of EALs.[7–9,11]

Although there are studies in the literature showing the accuracy of EALs in the determination of WL in primary teeth with apical RR and permanent teeth with artificial RR,[12,13] no study has investigated the accuracy of EALs in the determination of WL in permanent teeth with natural RR.

The present study evaluated the effects of different ISs on the accuracy of 4 different EALs in the determination of WL in extracted teeth with natural apical RR. The null hypothesis was that there would be no differences with regard to different ISs between EAL measurements during RR.

Material and methods

The Clinical Research Ethics Committee of Akdeniz University, Antalya, Turkey, reviewed and approved the study design (No. of approval: KAEK-476).

The G*Power program, v. 3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower) was used to determine the sample size. The total sample size was calculated as 36 for each EAL, with an alpha value of 0.05, power of 80% and an effect size of 0.6.

The inclusion criteria were as follows: permanent single- or multi-rooted teeth with external RR in the apical third of the root; the single-rooted teeth extracted due to periodontal or orthodontic reasons; the multi-rooted teeth extracted, as they could not be restored. After the inspection of the teeth under a stereomicroscope (Stemi; Carl Zeiss, Jena, Germany), teeth after RCT, or with cracks and fracture lines were excluded from the study. Five teeth were excluded because of the fractured roots. The remnants of hard and soft tissues on the teeth were removed with the help of a scaler. To prevent the teeth from drying, they were stored in saline solution during the study period.

Determination of the actual length

The incisal edges or cusps of the included teeth were flattened to create a stable and reliable coronal reference point after the endodontic access cavities were prepared in all teeth. Using a stereomicroscope at ×15 magnification, a #20 K-type file was advanced until it could be seen apically, at the start of the resorption area. The first time the file was noticed, a rubber stopper was attached to the

incisal edge of the tooth, and then the length between the tip of the file and the rubber stopper was measured with an endometer and recorded as the actual length (AL). This procedure was repeated 3 times for each tooth to prevent operator failures.

Determination of the electronic length

Four different EALs were used: ProPex® II (Dentsply Maillefer, Ballaigues, Switzerland); Propex Pixi® (Dentsply Maillefer); DentaPort ZX (J. Morita Tokyo, Saitama, Japan); and DTE DPEX V (Guilin Woodpecker Medical Instruments, Guilin, China).

Alginate was prepared according to the manufacturer's instructions. The lip clip of the each EAL was placed in the alginate and the teeth were embedded in the alginate impression model at the cementoenamel junction (CEJ). The ISs were freshly prepared, and 6 groups were organized based on the solution used:

– group 1 (control, no solution): After the root canals were dried with paper points, EL was determined by using the 4 different EALs. A #20 K-file was proceeded throughout the canal until the signals 'Apex' for ProPex II, '0.0' for Propex Pixi, '0.0' for DentaPort ZX, and '0.0' for DTE DPEX V occurred on the EALs to confine the apical resorption area. The rubber stopper was attached when the signals were persistently perceived on the screen of the device for 5 s, and then the length between the tip of the file and the rubber stopper was measured with an endometer and recorded as the electronic length (EL). This procedure was repeated 3 times for each tooth;
– group 2 (1% NaOCl): For each tooth, 5 mL of freshly prepared 1% NaOCl was used as IS. The EL values were determined using the same electronic measurement method as in group 1;
– group 3 (2.5% NaOCl): For each tooth, 5 mL of freshly prepared 2.5% NaOCl was used as IS. The EL values were determined using the same electronic measurement method as in group 1;
– group 4 (saline): Saline in the amount of 5 mL was used as IS. The EL values were determined using the same electronic measurement method as in group 1;
– group 5 (2% CHX): For each tooth, 5 mL of freshly prepared 2% CHX was used as IS. The EL values were determined using the same electronic measurement method as in group 1; and
– group 6 (17% EDTA): For each tooth, 5 mL of freshly prepared 17% EDTA was used as IS. The EL values were determined using the same electronic measurement method as in group 1.

The same teeth were used in all groups. After using each IS, the root canals were irrigated with 5 mL of distilled water to prevent the interaction of fluids and dried with a paper point before using the next IS. The procedures were performed on separate days for each group. For all 6 groups, the procedures were performed by a single operator.

Statistical analysis

The deviation of WL was calculated by subtracting AL from EL for each tooth (EL – AL). The difference was negative (–) when the electronic measurement was shorter than AL and positive (+) when the electronic measurement was longer than AL, whilst (0) indicated coinciding measurements. Statistical analysis was conducted to evaluate the deviation between the recorded AL values and the EL values, how much the obtained WLs deviated from the resorption area (*0 points), and whether this deviation was significant. The accuracy of WL determination methods was assessed with a tolerance of ±1 mm. The possible differences between the acceptable measurements obtained by means of EALs were analyzed with the χ^2 test. To control for type I errors, Bonferroni's correction was used in pairwise comparisons, using standard statistical software – IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA). The significance level was set at 5% for all tests.

Results

In this study, 36 teeth with natural RR were evaluated. The accuracy of 4 EALs depending on the IS used within the tolerance range of ±1 mm is shown in detail in Table 1.

Table 1. Comparison of the acceptable working length (WL) measurements (a range of tolerance of ±1 mm) between the study groups according to different electronic apex locators (EALs)

Group	ProPex II	Propex Pixi	DentaPort ZX	DTE DPEX V	*p*-value
Group 1	25 (69.4)[a]	22 (61.1)[a]	23 (63.9)[a]	21 (58.3)[a]	0.790
Group 2	18 (50.0)[a,b]	23 (63.9)[a,b]	27 (75.0)[b]	16 (44.4)[a]	0.037*
Group 3	22 (61.1)[a]	22 (61.1)[a]	25 (69.4)[a]	21 (58.3)[a]	0.785
Group 4	25 (69.4)[a]	22 (61.1)[a]	26 (72.2)[a]	22 (61.1)[a]	0.664
Group 5	21 (58.3)[a]	21 (58.3)[a]	22 (61.1)[a]	17 (47.2)[a]	0.645
Group 6	25 (69.4)[a]	28 (77.8)[a]	29 (80.6)[a]	23 (63.9)[a]	0.362

Data presented as number (percentage) (*n* (%)).
Groups: 1 – no solutions (control); 2 – 1% NaOCl; 3 – 2.5% NaOCl; 4 – saline; 5 – 2% CHX; 6 – 17% EDTA. * statistically significant; different letters indicate differences between the columns.

In group 1, there were no significant differences among the 4 EALs and the most successful results were obtained by Propex II. In group 2, DentaPort ZX was significantly more successful than DTE DPEX V (p = 0.037). There were no significant differences among EALs in groups 3–6. The success rates for each EAL in the study groups are shown in Table 2. Additionally, there were no significant differences in the success rates among the ISs for each device (Table 3).

Discussion

The determination of WL is an important step in RCT. An accurate WL measurement enables the adequate and complete instrumentation and filling of root canals. Although radiographic images are used for assessing WL in RCT, EALs are considered effective tools for determining the WL of the root canal.[14] There are various in vitro and in vivo studies in the literature that show the efficacy and accuracy of EALs in primary and permanent teeth.[15–17]

In this study, alginate was used to create an environment to simulate the clinical conditions during the EAL measurements. Although, in some studies, various materials such as gelatin, agar, floral sponge, and saline have been used,[18,19] the performance of alginate has been found to be superior in mimicking the tooth and surrounding tissues due to its colloidal consistency. In addition, the good electroconductive properties, low cost, availability, and easy preparation of alginate made it the preferred medium in this in vitro study.

Studies examining the accuracy of EALs provided different clinically acceptable margins of error in locating the apical foramen.[20–22] Goldberg et al. investigated the accuracy of Root ZX in teeth with simulated resorption, adopting a tolerance range of ±0.5 mm, ±1 mm and ±1.5 mm, and the success rates of Root ZX were 62.7%, 94.0% and 100.0%, respectively.[21] Thus far, a tolerance range of ±0.5 mm has been accepted in some studies.[6,22] However, a tolerance range of ±1.0 mm has been found clinically more appropriate due to the wide anatomical variation in the apical region.[20] As a result of apical RR, apical constriction disappears, and thus, the determination of WL becomes difficult in teeth with apical RR. In the present study, the tolerance range was determined as ±1.0 mm.

Many studies in the literature have shown the accuracy of EALs in determining WL.[23–25] Somma et al. used 3 EALs (DentaPort ZX, Raypex 5 and ProPex II) to evaluate their accuracy in vivo.[23] Although the best results were acquired with DentaPort ZX, there was no significant difference among the devices.[23] Oliveira et al. assessed Root ZX II, Raypex 6, Apex ID, ProPex II, and Propex Pixi in their study, and found no differences between the devices, as well.[24] These results are consistent with our study. In our study, a significant difference was found only in group 2 (1% NaOCl), and the most accurate measurements were obtained with DentaPort ZX in this group. Furthermore, the success rate of DentaPort ZX was higher in comparison with all other devices. Since there were statistically significant differences between EAL measurements with regard to different ISs, the null hypothesis was rejected.

Table 2. Accurate measurement rates of the electronic apex locators (EALs) relative to the irrigation solutions (ISs)

Group	ProPex II			Propex Pixi			DentaPort ZX			DTE DPEX V		
	–	0	+	–	0	+	–	0	+	–	0	+
Group 1	18 (50.0)	12 (33.3)	6 (16.7)	24 (66.7)	5 (13.9)	7 (19.4)	26 (72.2)	2 (5.6)	8 (22.2)	25 (69.4)	6 (16.7)	5 (13.9)
Group 2	26 (72.2)	5 (13.9)	5 (13.9)	29 (80.6)	4 (11.1)	3 (8.3)	23 (63.9)	8 (22.2)	5 (13.9)	30 (83.3)	3 (8.3)	3 (8.3)
Group 3	24 (66.7)	4 (11.1)	8 (22.2)	27 (75.0)	4 (11.1)	5 (13.9)	21 (58.3)	12 (33.3)	3 (8.3)	30 (83.3)	5 (13.9)	1 (2.8)
Group 4	23 (63.9)	9 (25.0)	4 (11.1)	27 (75.0)	6 (16.7)	3 (8.3)	19 (52.8)	8 (22.2)	9 (25.0)	27 (75.0)	6 (16.7)	3 (8.3)
Group 5	24 (66.7)	6 (16.7)	6 (16.7)	27 (75.0)	5 (13.9)	4 (11.1)	26 (72.2)	5 (13.9)	5 (13.9)	26 (72.2)	4 (11.1)	6 (16.7)
Group 6	26 (72.2)	5 (13.9)	5 (13.9)	24 (66.7)	5 (13.9)	7 (19.4)	22 (61.1)	7 (19.4)	7 (19.4)	27 (75.0)	5 (13.9)	4 (11.1)

Data presented as *n* (%).
(–): AL > EL; (0): AL = EL; (+) : AL < EL (AL – actual length, EL – electronic length).

Table 3. Distribution of the accurate working length (WL) measurements (a range of tolerance of ±1 mm) with regard to the irrigation solutions (ISs) used and different electronic apex locators (EALs)

EAL	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	*p*-value
ProPex II	25 (69.4)[a]	18 (50.0)[a]	22 (61.1)[a]	25 (69.4)[a]	21 (58.3)[a]	25 (69.4)[a]	0.420
Propex Pixi	22 (61.1)[a]	23 (63.9)[a]	22 (61.1)[a]	22 (61.1)[a]	21 (58.3)[a]	28 (77.8)[a]	0.570
DentaPort ZX	23 (63.9)[a]	27 (75.0)[a]	25 (69.4)[a]	26 (72.2)[a]	22 (61.1)[a]	29 (80.6)[a]	0.480
DTE DPEX V	21 (58.3)[a]	16 (44.4)[a]	21 (58.3)[a]	22 (61.1)[a]	17 (47.2)[a]	23 (63.9)[a]	0.470

Data presented as *n* (%).
The same letters indicate no differences between the columns.

There is no consensus on the accuracy of EAL measurements regarding the apical foramen diameter and the file size. Shacham et al. indicated that more accurate results could be obtained with EAL when the difference between the size of the file used for measurement and the apical foramen diameter of the canal was reduced.[6] Also, it was reported that an apical foramen diameter greater than 0.6 mm led to erroneous EAL results.[6] In addition, Kolanu et al. indicated that the accuracy of Propex Pixi decreased with an increased apical foramen diameter.[26] Akisue et al. also showed in their study that a larger apical foramen diameter caused a lower accuracy of EAL.[25] To obtain more accurate results, they recommended the use of a file suitable for the diameter of the apical foramen.[25] In this study, to attain more accurate results, electronic measurements were made using a #20 K-type file, since the apical foramen was enlarged due to RR.

The chemomechanical preparation of root canals is an important step in RCT. Removing the infected pulp, bacteria and microbial products from the canals cannot be achieved by preparation with endodontic instruments only. Thus, ISs play a vital role in the complete disinfection of the root canal space. In clinical practice, the most often used IS is NaOCl at different concentrations, such as 1%, 2.5% or 5%. In routine RCT, CHX at a concentration of 2% and EDTA at a concentration of 17% are the other commonly used ISs.[27] In this study, 1% NaOCl, 2.5% NaOCl, 0.9% saline, 2% CHX, and 17% EDTA were used as ISs during EAL measurements, as these ISs are used more frequently in daily clinical practice.

Prasad et al. investigated the accuracy of electronic measurements made with EALs (Root ZX and iRoot) in the presence of saline, NaOCl, CHX, and EDTA.[28] They showed that the presence of ISs in the root canal marginally affected the accuracy of the EALs, with the difference being non-significant.[28]

Baruah et al. used 0.1% octenidine dihydrochloride (OCT), 2% CHX, and heated and unheated 5% NaOCl as ISs in a study that compared Root ZX Mini and ProPex II.[29] It was revealed that the presence of ISs in the canal increased the reliability of EALs. Root ZX Mini was more consistent than ProPex II in the presence of various ISs. Nonetheless, no significant difference was found between the EALs.[29] In the present study, the success rate of DentaPort ZX was higher than Propex II in the presence of all irrigants, as well.

Since RR develops physiologically in primary teeth, it is very difficult to determine WL accurately when performing RCT in primary teeth. In order to prevent damage to permanent teeth, the WL of primary teeth should be carefully determined. An in vitro study by Tosun et al. investigated the accuracy of EALs with a tolerance range of ±1 mm in primary teeth with and without apical RR, and indicated that the presence of apical RR affected the performance of EALs.[17] Goldberg et al. evaluated the accuracy of Root ZX in permanent single-rooted teeth with simulated apical RR; the roots of the teeth were irrigated with normal saline solution.[21] The researchers reported that the accuracy of Root ZX was 94.0% within 1 mm of the direct visual measurement.[21] To make a consistent comparison with previous studies, the tolerance range was set as ±1 mm in the present study, as well.

Limitations

This in vitro study has various limitations, such as the absence of oral fluids and tissues. Also, as it is difficult to find teeth with natural RR, and single- or multi-rooted teeth were not differentiated in this study. Thus, the results of this study should be verified by clinical studies.

Conclusions

DentaPort ZX was more successful than DTE DPEX V in group 2 (1% NaOCl). In addition, none of the ISs affected the performance of EALs.

Ethics approval and consent to participate

The Clinical Research Ethics Committee of Akdeniz University, Antalya, Turkey, reviewed and approved the study design (No. of approval: KAEK-476).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

How does artificial aging affect the mechanical properties of occlusal splint materials processed via various technologies?

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Abstract

Background. The mechanical reliability of occlusal splints and their long-term behavior are significant factors determining the clinical outcome of temporomandibular disorder (TMD) therapy. However, improvements are still needed in this area.

Objectives. This in vitro study aimed to (1) compare the hardness and flexural properties of materials manufactured using 3 techniques for occlusal splint fabrication (conventional heat curing, thermoforming followed by light curing, and three-dimensional (3D) printing) and (2) analyze the effect of artificial aging on the properties of the materials.

Material and methods. A total of 120 disc-shaped specimens were manufactured for the Shore D hardness evaluation, and 120 bar-shaped specimens were fabricated for the flexural properties evaluation ($n = 15$ for each group). Each material was tested in 2 groups of specimens, non-aged and artificially aged (stored for 90 days in water at 37°C). Statistical differences were assessed using one-way or two-way parametric analysis of variance (ANOVA) with Tukey's or Šídák's post-hoc test, or the non-parametric Kruskal–Wallis test with Dunn's post-hoc test. A p-value of less than 0.05 was considered statistically significant.

Results. The mechanical properties of the materials varied significantly. Among the non-aged materials, the 3D-printed resin exhibited the highest Shore D hardness (85.3D), but it decreased significantly after 90 days of water storage (80.4D, $p < 0.0001$). The unpolished heat curing acrylic showed the highest resistance to artificial aging ($p = 0.0436$). However, its hardness decreased significantly after polishing (81.0D vs. 83.4D, $p = 0.0015$). The conventional heat curing material also exhibited superior flexural properties ($\sigma = 89.63$ MPa, $E = 2616$ MPa). All tested materials were susceptible to deterioration due to aging.

Conclusions. The conventional method of occlusal splint fabrication remains the optimal choice, particularly for long-term use. However, it is still necessary to develop materials that are resistant to aging in order to ensure successful clinical performance.

Keywords: polymer, thermoforming, dental materials, 3D printing, oral splint

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Introduction

In accordance with the Glossary of Prosthodontics Terms, an occlusal splint is "any removable artificial occlusal surface affecting the relationship of the mandible to the maxillae used for diagnosis or therapy; uses of this device may include, but are not limited to, occlusal stabilization for treatment of temporomandibular disorders, diagnostic overlay prior to extensive intervention, radiation therapy, occlusal positioning, and prevention of wear of the dentition or damage to brittle restorative materials such as dental porcelain."[1] In particular, occlusal splints are a critical component of the non-invasive management of bruxism and temporomandibular disorders (TMD). They can be used for occlusal positioning or stabilization and for preventing tooth wear, which is a multifactorial condition. Studies reported the beneficial effect of occlusal splints in reducing masticatory muscle activity.[2–6] However, the effectiveness of occlusal splints in managing orofacial myalgia and myofascial pain still requires investigation.[7] Similarly, various alternative treatment modalities are being validated for their ability to improve the quality of life of patients with TMD.[8–10]

Due to the high prevalence of TMD and the increasing popularity of occlusal splints, there is a need to search for new, reliable and convenient device manufacturing methods. Thermoplastics for vacuum forming or pressure forming offer an easier and faster fabrication process compared to heat-cured and self-cured (auto-polymerizing) acrylic resins, which were the first materials used to construct custom-made splints. Nekora et al. demonstrated that patients had no preference between vacuum-formed and heat-cured acrylic splints.[11] In addition to conventional laboratory-made devices, digitally fabricated milled splints represent a viable alternative.[12]

Modern dentistry frequently employs computer-aided design/computer-aided manufacturing (CAD/CAM) techniques, which have led to the development of subtractive and additive techniques. As a result, three-dimensional (3D) printing is becoming increasingly popular among prosthodontists worldwide. The technology is used to produce prosthetic restorations, dental models, implants, surgical guides, custom trays, orthodontic appliances, and occlusal splints.[13–17] Furthermore, it provides a simple means of manufacturing complex, custom-designed objects, reducing material waste and working time.[18,19] Despite its many advantages, there are several limitations to 3D printing that require comprehensive research into the materials and techniques used in the fabrication of oral appliances.[20–22]

The fabrication of occlusal splints may be simplified by the introduction of 3D printing or thermoforming technologies, provided that the materials used are mechanically reliable, biologically safe and long-lasting. This is necessary to ensure the clinical success of occlusal splint therapy.[23,24] Several in vitro studies have evaluated the mechanical behavior of occlusal splint materials processed using various methods,[25–30] while others have demonstrated that post-curing affects the results.[31,32] However, most of the research conducted to date has focused on evaluating the initial parameters of splint materials. Few studies have addressed the long-term behavior of such devices.[22,27,33–40] The problem of poor occlusal splint longevity, mainly due to fracturing or deforming over time, still needs to be solved.[29,34,38]

Flexural strength and hardness are important mechanical properties that determine a material's capacity to resist bending and indentation, which are the main causes of splint damage. Hence, this in vitro study aimed to compare the hardness and flexural properties of specimens manufactured using 3 techniques for occlusal splint fabrication (conventional heat curing, thermoforming followed by light curing, and 3D printing). The study examined the impact of 90 days of water storage on material properties. The research hypotheses state that there are no significant differences in the selected material properties between (1) specimens manufactured using different techniques and (2) specimens subjected to artificial aging and non-aged ones.

Material and methods

Material

The materials selected for this in vitro study could be used to fabricate occlusal splints using 3 different manufacturing techniques, as listed in Table 1: a conventional hand-processed heat-curing acrylic resin (polymethyl methacrylate (PMMA), Villacryl H Plus 0; Everall7, Warsaw, Poland); a plastic (polyethylenterephthalat+glycol (PET-G)) sheet processed via thermoforming (DURAN®; Scheu-Dental GmbH, Iserlohn, Germany) and adjusted with a build-up made of a light-cured (LC) mixture of acrylic resins, fillers and initiators (Durasplint LC; Scheu-Dental GmbH); a photopolymer resin for 3D printing via the stereolithography (SLA) method (Dental LT Clear; Vertex Dental, Soesterberg, Netherlands).

Specimen preparation

The materials were processed according to the manufacturer's instructions to prepare disc-shaped and bar-shaped specimens that comply with the relevant International Organization for Standardization (ISO) standards. To limit intragroup variance, a single investigator was involved in specimen preparation. A total of 120 disc-shaped specimens (for the Shore D hardness evaluation) and 120 bar-shaped specimens (for the flexural properties evaluation) were fabricated. Each material was tested in 2 groups of specimens, non-aged and artificially aged (stored for 90 days in water at 37°C). The overall study design is illustrated in Fig. 1.

Table 1. Description of the dental splint materials evaluated in the study

Study group	Material	Manufacturer	Type of material	Method of processing
Heat-cured	Villacryl H Plus 0	Everall7, Warsaw, Poland	acrylic resin (PMMA)	hand-processed heat curing
Thermoformed + light-cured	DURAN® + Durasplint LC	Scheu-Dental GmbH, Iserlohn, Germany	PET-G copolyester + a built-up made of a mixture of acrylic resins, fillers and initiators	thermoforming (DURAN®) + light curing (Durasplint LC)
3D-printed	Dental LT Clear	Vertex Dental, Soesterberg, Netherlands	photopolymer resin	3D printing (SLA technique) and UV light post-curing

PMMA – polymethyl methacrylate; PET-G – polyethylenterephthalat+glycol; SLA – stereolithography; UV – ultraviolet.

Heat curing

The powder was mixed with the liquid (Villacryl H Plus 0), and when the material reached a dough-like consistency, it was introduced into the mold in a polymerization flask. Then, it was pressed under 8.6 bar using a P-400 hydraulic press (Sirio Dental, Meldola, Italy) and polymerized under short-term conditions (heating the water from 60°C to 100°C for 30 min and then boiling it for additional 30 min) in an ISP-1 polymerization unit (InterSonic, Olsztyn, Poland).

Thermoforming and light curing

The bases of the specimens were made of 1.5-mm DURAN (Scheu-Dental GmbH) thermoformed over a die of the appropriate size using the Ministar S® pressure molding unit (Scheu-Dental GmbH). Then, the surface of the material was sandblasted with aluminum oxide (Al_2O_3, 110 µm) (Ardent, Wrocław, Poland) in a sandblasting unit (Basic Eco; Renfert, Hilzingen, Germany) and dried. A thin layer of LC-Primer (Scheu-Dental GmbH) was applied to the upper surface of DURAN and polymerized for 5 min in an LC-6 Light Oven (Scheu-Dental GmbH). Then, Durasplint LC (Scheu-Dental GmbH) was adapted to the pressure-molded DURAN base. The final specimen was cured twice for 10 min using the LC-6 Light Oven.

Three-dimensional printing

The 3D printing objects were designed using Meshmixer v. 3.5.474 (Autodesk Inc., San Francisco, USA). The .stl files were created and uploaded to PreForm software, v. 3.28.1 (Formlabs Inc., Somerville, USA), for the addition of supports and the setting of printing parameters. The specimens made of the Dental LT Clear resin (Vertex Dental) were printed in a Form 2 printer (Formlabs Inc.) at a 90° angle to the building platform (edgewise orientation of the specimens) in 100-µm layers with supporting structures. The printed specimens were placed in the Cleaning and Finish Kit (Formlabs Inc.), washed twice for 10 min in 99% isopropanol (PPH Stanlab, Lublin, Poland), and left to air dry at room temperature for 30 min. They were then post-cured in a Form Cure (Formlabs Inc.) equipped with 405-nm multi-directional light emitting diodes (LEDs). Two sets of curing parameters were tested, namely 80°C for 20 min (as recommended by the manufacturer) and 60°C for 30 min (the alternative method).

Finishing and polishing

All specimens were finished using sandpaper (grit P500, P1000 and P1200; P.S. Trading, Ożarow Mazowiecki, Poland) and 0.6-mm pumice stone powder (Everall7). Finally, the upper side of each specimen was polished with polishing paste for resin and metals (Everall7)

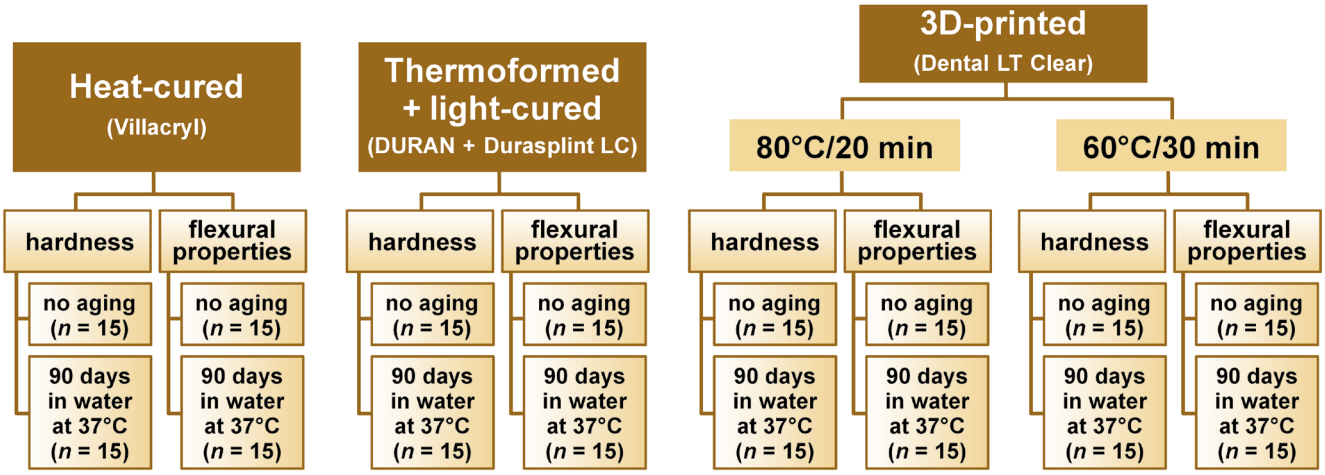

Fig. 1. Study design

using a Poliret Mini unit (REITEL Feinwerktechnik GmbH, Bad Essen, Germany). For the specimens made of thermoformed and LC materials, the side made of DURAN was left unpolished, while the side made of Durasplint LC was polished, following standard clinical practice.

Artificial aging

The specimens were stored in distilled water (chemPUR, Karlsruhe, Germany) at 37°C for 90 days in the CLN 15 Smart incubator (Pol-Eko sp. k., Wodzisław Śląski, Poland). The water was changed weekly and a constant temperature was maintained.

Shore D hardness evaluation

The tests were conducted by a single investigator in accordance with the PN-EN ISO 868:2005 guidelines.[41] Before testing, the disc-shaped specimens with a diameter of 30 mm and a height of 5 mm were conditioned for 88 h in a standard atmosphere (23/50). The Shore D hardness value was measured for each specimen at 5 points (at a distance of at least 9 mm away from the specimen's edges and 6 mm away from each other) using an HBA 100-1 Shore durometer (Sauter AG, Basel, Switzerland). The polished and unpolished sides of the specimens were tested separately. The value was read 15 s after pressing the durometer foot against the specimen.

Flexural properties evaluation

The tests were conducted by a single investigator in accordance with the PN-EN ISO 20795-1:2013 guidelines.[42] Before testing, the bar-shaped specimens (64 mm × 10.0 (±0.2) mm × 3.3 (±0.2) mm) were conditioned in distilled water at 37°C for 50 h. Then, the height and width of each specimen were measured at 5 points using a Magnusson digital caliper (150 mm) (Limit, Alingsås, Sweden), and the mean cross-sectional area was calculated immediately before testing. The three-point bending test was performed using the Universal Testing Machine (Z10-X700; AML Instruments Ltd, Lincoln, UK) at a constant displacement rate of 5 mm/min and a span length of 50 mm between the supports.

Flexural strength (σ [MPa]) was calculated using the following formula (Equation 1):

$$\sigma = 3Fl/2bh^2 \quad (1)$$

where:

F – maximum load [N];

l – distance between the supports [mm] (±0.01 mm);

b – width of the specimen [mm]; and

h – height of the specimen [mm].

Flexural modulus (E [MPa]) was determined using the following formula (Equation 2):

$$E = (F/d)(l^3/[4bh^3]) \quad (2)$$

where:

load (F) divided by displacement (d) is the slope in the linear elastic region of the load/displacement curve; l, b and h are as defined above.

Statistical analysis

The results were analyzed using GraphPad Prism software, v. 9.1.2. (GraphPad Software, San Diego, USA). All measurements were carried out for $n = 15$ specimens in each group. The sample size was calculated using G*Power software, v. 3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), for an effect size of 0.4, $\alpha = 0.05$ and power of 0.8. The results were presented as box plots, which provide a visual representation of the five-number summary of a dataset (minimum, first quartile, median, third quartile, and maximum). Data normality was tested with a Shapiro–Wilk test. The majority of data passed the normality test, with the exception of the results for the flexural modulus evaluation and the flexural strength after aging. These results were analyzed using the non-parametric Kruskal–Wallis test.

Differences between the hardness of the materials were tested using two-way parametric analysis of variance (ANOVA) for multiple comparisons with Tukey's (for comparisons of materials within polished or unpolished groups) or Šídák's (for comparisons between polished and unpolished groups within each material and for comparison between artificially-aged and non-aged groups) post-hoc multiple comparisons test. The study tested the differences in flexural properties of the materials using either one-way parametric ANOVA with Tukey's post-hoc multiple comparisons test or the non-parametric Kruskal–Wallis ANOVA with post-hoc Dunn's test. Differences in the flexural properties between artificially aged and non-aged groups were compared using two-way ANOVA with Šídák's post-hoc multiple comparisons test. Differences between the groups were considered statistically significant at $p < 0.05$.

Results

Shore D hardness

The Shore D hardness of the materials varied between the groups (Fig. 2). The two-way ANOVA showed significant differences in hardness between specimens manufactured using different techniques and between specimens subjected to artificial aging and non-aged ones (all $p < 0.0001$). On this basis, both null hypotheses were rejected, and detailed multiple comparisons were conducted. When unpolished non-aged specimens were analyzed, the lowest value was found for those made of DURAN and processed via thermoforming ($p < 0.0001$ for all comparisons), while the high-

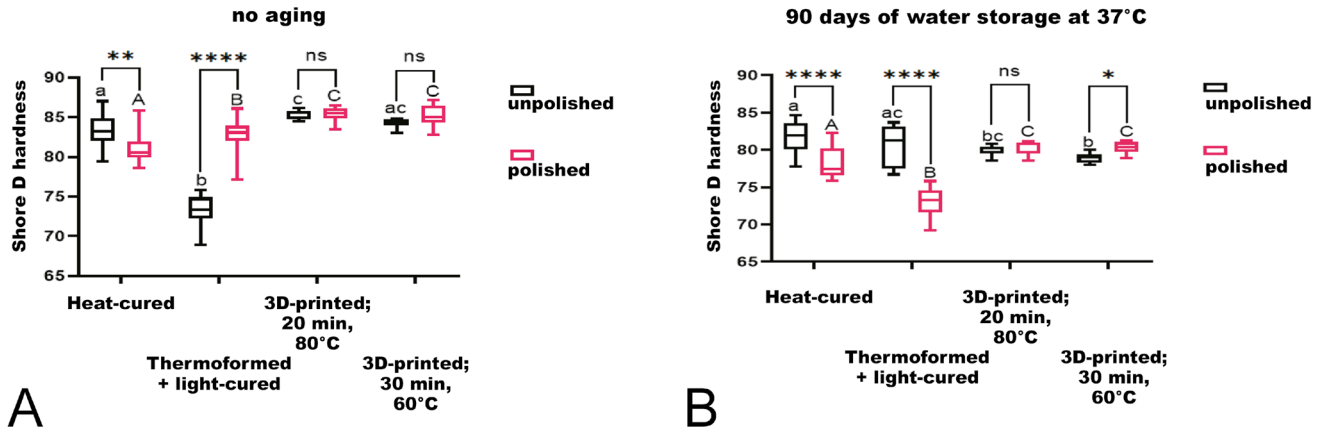

Fig. 2. Comparison of Shore D hardness of dental splint materials (heat-cured (Villacryl), thermoformed + light-cured (DURAN + Durasplint LC), and 3D-printed resin (Dental LT Clear) post-cured at 2 sets of parameters (20 min/80°C and 30 min/60°C))

A. Analysis of unpolished and polished specimens without aging; B. Analysis of unpolished and polished specimens after artificial aging. Different letters indicate significant differences between materials within the unpolished/polished group (small letters/capital letters). Asterisks indicate significant differences between unpolished and polished specimens; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$; ns – not significant (two-way analysis of variance (ANOVA))

est value was found for specimens made of a 3D-printable Dental LT resin cured for 20 min at 80°C ($p = 0.0054$ when compared to the heat-cured PMMA and $p = 0.2171$ when compared to the resin cured for 30 min at 60°C) (Fig. 2A). Additionally, polishing significantly reduced the Shore D hardness of the heat-cured PMMA ($p = 0.0015$). There was a significant difference between the 2 sides of thermoformed LC specimens. The side made of DURAN (unpolished) had a significantly lower Shore D hardness than the side made of Durasplint LC (polished) ($p < 0.0001$). No significant differences were observed between unpolished and polished specimens for both types of post-curing. The resin used for 3D printing exhibited the highest Shore D hardness value among all polished materials, regardless of the final post-curing parameters applied.

The analysis of the specimens subjected to artificial aging revealed interesting results (Fig. 2B). When specimens without polishing were compared, the heat-cured PMMA and thermoformed DURAN specimens showed the highest Shore D hardness. However, among the polished specimens, the 3D-printed resins had the highest Shore D hardness, while the Durasplint LT specimens had the lowest value (all $p < 0.0001$).

A comparison of the non-aged and aged specimens revealed that the Shore D hardness of the 3D-printable Dental LT resin was significantly reduced among the unpolished materials after 90 days of water storage ($p < 0.0001$). Conversely, the Shore D hardness of DURAN significantly increased after artificial aging ($p < 0.0001$). Only the conventional heat-cured PMMA demonstrated greater resistance to changes in hardness; however, the difference between aged and non-aged specimens was still significant ($p = 0.0436$). When comparing the polished specimens, prolonged water storage resulted in a significant reduction of the Shore D hardness for all analyzed materials (all $p < 0.0001$) (Table 2).

Flexural properties

The materials differed in terms of ultimate flexural strength and flexural modulus (Fig. 3). Statistical tests, including the one-way ANOVA, Kruskal–Wallis test and two-way ANOVA revealed significant differences in flexural properties between specimens manufactured using different techniques and between specimens subjected to artificial aging and non-aged ones (all $p < 0.0001$). On this basis, both null hypotheses were rejected, and de-

Table 2. Differences in the Shore D hardness between non-aged and artificially aged materials

Comparison	Group	Mean difference	*SE*	95% *CI*	*t*	*df*	*p*-value
Unpolished, non-aged vs. artificially aged	heat-cured	1.578	0.6111	0.03160–3.125	2.583	114.0	0.0436*
	thermoformed + light-cured	–7.250	0.5579	–8.662–5.838	13.00	114.0	<0.0001*
	3D-printed; 20 min, 80°C	5.400	0.5762	3.942–6.858	9.372	114.0	<0.0001*
	3D-printed; 30 min, 60°C	5.371	0.5517	3.975–6.767	9.736	114.0	<0.0001*
Polished, non-aged vs. artificially aged	heat-cured	2.713	0.6093	1.171–4.255	4.453	114.0	<0.0001*
	thermoformed + light-cured	10.08	0.5562	8.667–11.48	18.11	114.0	<0.0001*
	3D-printed; 20 min, 80°C	4.900	0.5745	3.446–6.354	8.530	114.0	<0.0001*
	3D-printed; 30 min, 60°C	4.916	0.5500	3.524–6.308	8.937	114.0	<0.0001*

SE – standard error; *CI* – confidence interval; *t* – test statistic; *df* – degrees of freedom; * statistically significant ($p < 0.05$, Šídák's multiple comparisons test).

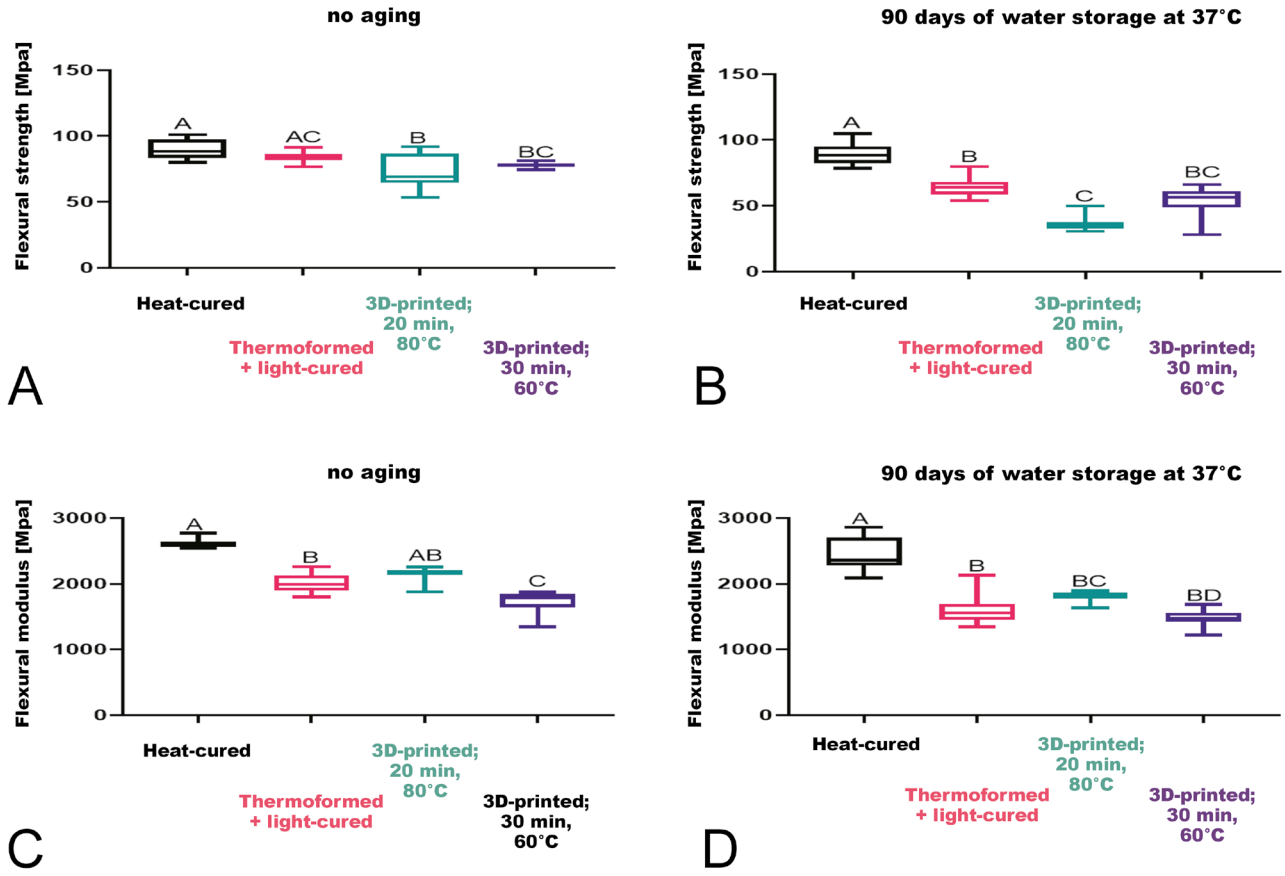

Fig. 3. Comparison of flexural properties of dental splint materials (heat-cured (Villacryl), thermoformed + light-cured (DURAN + Durasplint LC), and 3D-printed resin (Dental LT Clear) post-cured at 2 sets of parameters (20 min/80°C and 30 min/60°C))

A. Flexural strength of specimens without aging; B. Flexural strength of specimens after artificial aging; C. Flexural modulus of specimens without aging; D. Flexural modulus of specimens after artificial aging. Different letters indicate significant differences between materials ($p < 0.05$); parametric one-way ANOVA with post hoc Tukey's multiple comparisons test (A,C) or the nonparametric Kruskal–Wallis ANOVA with post hoc Dunn's test (B,D).

tailed multiple comparisons were performed. When non-aged specimens were compared, the highest values were observed for the conventional heat-cured PMMA, while specimens made of the 3D-printable resin had significantly lower flexural strength values. The material post-cured for 20 min at 80°C had the lowest value, but the difference between the 2 types of post-curing was not statistically significant ($p = 0.2629$). The comparison of flexural modulus revealed that the lowest modulus was obtained for the 3D-printable resin post-cured for 30 min at 60°C (Fig. 3C). Upon analysis of artificially aged specimens, the materials were ranked in terms of flexural strength or modulus in the same manner as non-aged specimens. However, the differences between the materials were more pronounced (Fig. 3). The comparison of flexural strength differences between non-aged and artificially aged materials revealed that long-term exposure to increased temperatures and humidity significantly influenced their mechanical properties (Table 3). For materials processed by thermoforming and light-curing and by 3D printing,

Table 3. Differences in flexural strength and modulus between non-aged and artificially aged materials

Property	Group	Mean difference	*SE*	95% *CI*	*t*	*df*	*p*-value
Flexural strength	heat-cured	0.6510	3.065	–7.103–8.405	0.2124	117.0	0.9992
	thermoformed + light-cured	19.23	2.615	12.62–25.85	7.354	117.0	<0.0001*
	3D-printed; 20 min, 80°C	36.70	2.845	29.50–43.89	12.90	117.0	<0.0001*
	3D-printed; 30 min, 60°C	23.92	2.655	17.21–30.64	9.013	117.0	<0.0001*
Flexural modulus	heat-cured	174.4	65.38	8.852–339.9	2.667	113.0	0.0347*
	thermoformed + light-cured	393.3	55.64	252.5–534.1	7.069	113.0	<0.0001*
	3D-printed; 20 min, 80°C	350.3	59.63	199.3–501.2	5.874	113.0	<0.0001*
	3D-printed; 30 min, 60°C	250.4	55.64	109.5–391.2	4.500	113.0	<0.0001*

* statistically significant ($p < 0.05$, Šídák's multiple comparisons test).

water storage significantly reduced their flexural strength and modulus (all $p < 0.0001$). In the case of heat-cured acrylic resin, artificial aging did not affect its ability to withstand bending forces, as there was no significant decrease in ultimate flexural strength observed ($p = 0.9992$). However, the flexural modulus was significantly reduced ($p = 0.0347$).

Discussion

The 3D printing technology has the potential to revolutionize the fabrication of occlusal splints. This technology enables the precise and cost-effective production of complex parts, while reducing material waste and environmental impact. However, the benefits of this technology must be accompanied by clinical success, which is strongly dependent on the mechanical reliability, biological safety and longevity of the materials used.

Occlusal splints must be mechanically resistant due to the excessive occlusal forces they are often subjected to.[6] Numerous research studies have compared the mechanical properties of 3D-printed splints and conventionally fabricated appliances, although an indisputable conclusion has not been reached. Similarly, studies on thermoplastic foils, in particular those with a build-up made of an LC resin, are scarce.[14] A study by Patzelt et al. revealed that a digital workflow was more time-efficient, and the fit of the digitally-fabricated splints was better than that of the conventionally-fabricated ones. The wear of the 2 different materials showed comparable results.[19] In contrast, Lutz et al. demonstrated that 3D-printed splint materials had lower wear and fracture resistance compared to those that were milled or conventionally fabricated.[29] Furthermore, a comparison between thermoformed DURAN clear dental aligners and the 3D-printed Dental LT clear resin revealed that 3D-printed material was more accurate and able to withstand higher compressive loads.[28]

In the current study, we evaluated 2 mechanical properties of materials that determine the clinical performance of occlusal splints and are closely related to the resistance to localized deformation. Splints are exposed to flexural stress during teeth clenching and grinding, which can cause deformation, crack formation and, ultimately, fracture. The basic qualities that can be used to describe the resistance of a material to such processes are Shore D hardness, which measures its resistance to indentation, and flexural strength and modulus, which measure its resistance to bending. To the best of the authors' knowledge, this is the first comparative study of material properties used in 3 types of splint fabrication techniques, including thermoforming and LC. The study accounts for differences between polished and non-polished specimens and complies with the relevant standards. The results indicated that 3D-printed materials had the highest Shore D hardness, while the conventional heat-cured PMMA demonstrated superior flexural properties. The hardness and elastic modulus of occlusal splint materials manufactured using 4 different methods (heat curing, CAD-milling, 3D printing, and vacuum forming) were also evaluated by Grymak et al., revealing that both the processing method and the printing angle had an influence on the hardness and elastic modulus of the materials.[26] The same group revealed that vacuum-formed occlusal splints showed the highest wear,[43] while 3D-printed materials exhibited significantly lower wear resistance compared to CAD-milled and heat-cured materials.[27]

A significant strength of the present study is the use of artificial aging, which is crucial for simulating the impact of long-term usage in the oral environment. Several methods of artificial aging are used for the in vitro evaluation of dental materials, including thermal degradation (simulated via thermocycling), physical and chemical degradation in various environments (storage in water, artificial saliva, ethanol, and sodium hydroxide solutions), as well as mechanical loading under varying conditions (static and dynamic tests and simulated chewing or tooth brushing). As occlusal splints are specific oral devices that are removed before eating or drinking, they are not exposed to food, beverages or temperature changes resulting from contact with hot or cold meals. For this reason, instead of thermocycling or immersion in more aggressive media, artificial aging in water at 37°C was performed. An interesting continuation of the preliminary research would be the simulation of mechanical degradation (e.g., dynamic loading).

The effects of 90-day aging on dental splints were analyzed, as their longevity can vary from 6 to 8 weeks to several years, depending on the severity of the disorders. To simulate degradation upon exposure to factors present in the oral cavity, the specimens were immersed in water at elevated temperatures. Several studies on this issue have provided inconsistent conclusions. Antonopoulou et al. showed that 3D-printed occlusal devices were dimensionally stable after applying various storage methods and concluded that splints could be stored with or without water and light, without any clinically detectable effect on fitting accuracy.[40] On the other hand, Berli et al. revealed that 3D-printable resins had significantly lower flexural strength and hardness, as well as higher water sorption and solubility, compared to pressed or milled materials. Moreover, 3D-printed materials were found to be more susceptible to thermal aging.[34] Similarly, Reymus and Stawarczyk found that 3D-printed materials were more susceptible to artificial aging than the conventionally milled materials.[38] In the research presented in this paper, the authors confirmed that all materials tested were prone to a decrease in hardness and flexural properties due to artificial aging. The significant decrease in the mechanical properties of both 3D-printable and conventional materials may result from the plasticization of the polymer structure due to water sorption.[33]

Limitations

The main limitation of the current study is the limited number of materials and brands compared, the narrow range of properties investigated, and the laboratory nature of the research. For example, analyzing wear resistance through two-body wear tests using a chewing simulator would be a very valuable continuation of the study. Moreover, the clinical success of splint therapy is contingent upon several properties, including the degree of conversion, biocompatibility and microbial adhesion, which are closely related to surface roughness.[23,24,44,45]Addit ionally, it would be beneficial to investigate the influence of various cleaning or disinfecting techniques on the properties of splints. Finally, the results of comprehensive in vitro tests could suggest methods to improve the materials and techniques used, paving the way for the fabrication of splints with optimal clinical performance.

Conclusions

Both research hypotheses should be rejected in the light of the results obtained in this paper. The 3D printing resin exhibited the highest Shore D hardness among all polished and artificially aged materials, while the conventional heat-cured PMMA revealed superior flexural properties. All materials were susceptible to the deterioration of mechanical properties due to artificial aging, but the conventional PMMA demonstrated the highest resistance to changes after storage in water at 37°C.

There is still a need to develop new materials and techniques used for occlusal splint production in order to improve resistance to deformation or fracture. Currently, the conventional method still remains the optimal choice for successful clinical performance, especially for long-term splint use.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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Original paper

Effect of dental implant macrogeometry on the probability of survival and strain distribution of an implant–abutment set

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Conflict of interest
None declared

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Abstract

Background. The effect of the macrogeometry of dental implants with double trapezoidal threads on the probability of survival and the long-term success of oral rehabilitation is unclear.

Objectives. The purpose of this in vitro study was to evaluate the effect of dental implant macrogeometry on the probability of survival, failure mode and strain distribution of an implant–abutment set.

Material and methods. Dental implants were divided into 2 groups according to their macrogeometry ($n = 21$ per group): trapezoidal thread (control group); and double trapezoidal thread (test group). The macrogeometry analysis was performed with the use of computed microtomography ($n = 1$). The specimens were subjected to single load to failure (SLF) ($n = 3$), which permitted the step-stress profiles for design-based step-stress accelerated life testing (SSALT) ($n = 18$). The probability of survival and reliability for a mission of 50,000 cycles were calculated at 100 N and 150 N. The scanning electron microscope (SEM) was used to analyze the failure mode of the implant–abutment set. The digital image correlation (DIC) ($n = 3$) was performed using the implant–abutment set embedded in a polyurethane resin subjected to a static load of 250 N in axial and non-axial positions.

Results. No statistically significant differences were observed between the groups with respect to the probability of survival. All groups showed a reliability level higher than 95% at 100 N, while a decrease in reliability was observed at 150 N. The Weibull modulus and characteristic resistance exhibited no significant differences between the groups. The β mean values (control = 0.66, test = 0.33) indicated that failures were dictated by material strength. The SEM revealed an abutment and implant body fracture, characterized by fracture initiation on the lingual surface that subsequently propagated to the opposing buccal side. In the context of non-axial loading, the test group exhibited a higher concentration of tensile strain in the cervical region (152.05 μs), while the control group exhibited a predominance of compression strain (−800.00 μs).

Conclusions. The macrogeometry of dental implants did not influence the failure mode and probability of survival, but modified the strain distribution of the implant–abutment set.

Keywords: dental implants, macrogeometry, probability of survival, strain distribution

Highlights

- The macrogeometry of dental implants, including parallel and double trapezoidal threads, did not affect the failure mode or survival probability under clinically relevant loads for anterior teeth.
- Both implant designs exhibited high reliability (95–98%) at 100 N, but reliability decreased at higher loads (150 N).
- Double trapezoidal threads caused higher tensile strain in the cervical region under non-axial loading, potentially increasing fracture risk in the implant–abutment assembly.

Introduction

The predictability and long-term success rate of dental implant treatment have been related to several factors, including material biocompatibility, management of surgical technique, bone quality, loading conditions, surface treatments, and implant geometry.[1,2] Implant geometry refers to the three-dimensional implant structure, and may be categorized into 2 modalities, namely macrogeometry and microgeometry. Macrogeometry refers to the prosthetic connection, implant body and thread design, while microgeometry is related to the surface treatment, surface morphology and implant material.[3,4] The macrogeometry of dental implants comprises factors such as implant diameter, length, surface characteristics, and thread design.

New dental implant macrogeometries, which include models with deep threads and a smaller thread pitch, were developed to increase primary implant stability and improve strain distribution to the peri-implant bone, especially in cases of low bone quality (III and IV bone type), implant placement after extraction, and immediate loading protocols.[3–5] The macrogeometry of implants is related to the longevity of treatment, because it determines bone-to-implant contact, masticatory strain distribution and the mechanical strength of the implant.[3–5]

Mechanical complications are frequent in implant dentistry. Preclinical studies can simulate the conditions present in the oral environment and predict clinical performance. One of the mechanical complications is implant fracture. An inadequate implant diameter and length, especially in cases of compromised bone quality or high occlusal forces, can increase the risk of implant fracture, compromising the overall implant stability and longevity. Additionally, the lack of a suitable thread design and surface characteristics can further exacerbate this risk. Another complication associated with macrogeometry is screw loosening. An insufficient implant diameter, thread pitch or inadequate surface roughness can result in ineffective retention of the abutment screw. This can lead to improper load distribution, compromised esthetics and implant failure over time.[6–10] While static testing can be helpful in ranking different implant systems' load to failure performance, structural failure is time-dependent as a function of the implant–abutment set. Higher complication rates have been observed with longer usage times. Therefore, the fatigue test is considered a clinically relevant predictor of material strength.[11] Some authors have evaluated the influence of macrogeometry on the fatigue behavior of dental implants,[12–18] and most of them found that the diameter of the implant influences the fatigue behavior,[14–16] whereas the influence of the characteristics of the implant thread on the fatigue behavior of the implant–abutment set is uncertain.[19]

Digital image correlation (DIC) is a non-contact optical method used to evaluate the strain distribution on the surface of a material during a mechanical test. To that end, several images are captured using a camera and analyzed by specialized software, which calculates the displacement of the surface points.[20,21] A number of studies have compared the results of DIC with those of photoelasticity[22] and finite element analysis (FEA).[23,24] The authors found that the distribution of surface deformation is comparable, thereby validating the model and visualizing the strain distribution of dental implants and implant-supported protheses.[22–28] Therefore, the purpose of this study was to evaluate the failure mode and probability of survival of the implant–abutment set, composed of dental implants with internal conical connection and different microgeometries. These implants were subjected to step-stress accelerated life testing (SSALT) and strain distribution using DIC. The null hypothesis stated that the macrogeometry of dental implants with internal conical connection does not influence the failure mode, probability of survival and strain distribution of the implant–abutment set.

Material and methods

Titanium dental implants with a conical shape and internal conical connection were divided into 2 groups (n = 21 per group), according to their macrogeometry: a control group, represented by implants with trapezoidal threads (CM implants; Singular Implants, Parnamirim, Brazil); and a test group, represented by implants with double trapezoidal threads (Go Direct CM; Singular Implants) (Fig. 1, Table 1).

Table 1. Characteristics of dental implants used in the study

Characteristic	Control group	Test group
Commercial name	CM implants (Singular Implants, Parnamirim, Brazil)	Cone Morse Go Direct (Singular Implants, Parnamirim, Brazil)
Diameter [mm]	3.5	3.5
Length [mm]	11.5	11.5
Indication	III and IV bone types	I, II, III, and IV bone types
Cervical zone	presents at 0.4 mm between the platform and the threads	presents at 1.0 mm with pyramidal microthreads, improving secondary stability and preventing bone loss
Body	cylindrical with a conical apex	the body diameter is larger than the platform diameter, and it has a conical apex with a 0.6-mm chamfer
Active apex	soft, rounded, small tip and 3 flutes	soft, rounded, small tip and 1 helicoidal flute
External threads	trapezoidal	double trapezoidal
Thread pitch [mm]	0.80	1.00
Thread depth [mm]	0.40	0.40–0.55

The probability of survival was determined using SSALT.[14,15,17–19,25] The implants were positioned in a 30° inclination matrix according to ISO 14801:2016, and embedded in a polyurethane resin with the implant platform positioned 3 mm above the resin level, simulating 3 mm of bone resorption. The prefabricated universal abutments (Singular Implants) were then connected to the implants, tightened using a digital torque gauge (TQ-680; Instrutherm, São Paulo, Brazil) (32 N·cm), and covered with a stainless steel hemispherical loading member. Three specimens from each group were subjected to single load to fracture (SLF) in a universal testing machine (Biopdi, São Paulo, Brazil), with a 1000-kgf load cell and a displacement of 1 mm/min. Three loading profiles were designed for SSALT (n = 18) (Fig. 2) and labeled as light (n = 9), moderate (n = 6) or aggressive (n = 3), according to the ratio distribution of 3:2:1.[11,15,17–19,25] The force used in each loading profile ranged between 20% and 60% of the SLF mean value.[11] Step-stress accelerated life testing was performed using a fatigue test equipment (Biopdi), with an isometric loading protocol, 4 Hz, in water at 37°C (Fig. 3). The specimens were cycled until failure or until reaching the loading limit. At the end of each cycle, they were analyzed to verify the presence of any deformations and/or fractures.

The failure mode analysis (n = 3) was performed qualitatively using a scanning electron microscope (SEM) (EVO MA10; Zeiss, Oberkochen, Germany) under ×90 and ×500 magnification to identify the origin and direction of crack propagation.

A qualitative analysis was conducted by means of computed microtomography (n = 1) (SkyScan 1176; Bruker, Kontich, Belgium) using the following scanning parameters: 90 kV; 272 mA; a copper (Cu) filter with an exposure time of 81 ms per image in 360°; 9-µm isotropic voxel; and a frame of 4. The images were obtained before SSALT and reconstructed using the NRecon v.1.6.9.18 software (Bruker). The image of the implant with the best quality was selected. The analysis of implant macrogeometry (wall thickness, depth and thread pitch) was performed using the linear measurement tool of the CTan software, v.1.14.4.1+ (Bruker).

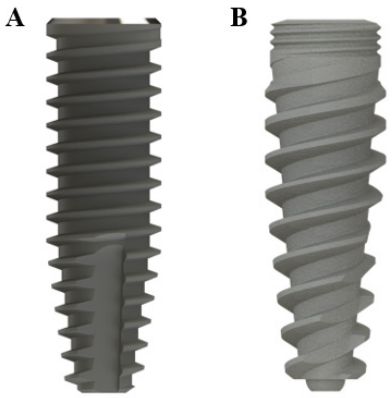

Fig. 1. Dental implants evaluated in the study

A. Implant with a trapezoidal thread (control group); B. Implant with a double trapezoidal thread (test group).

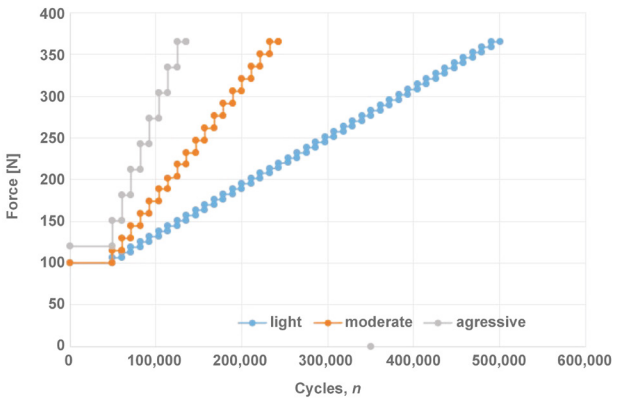

Fig. 2. Graphic representation of loading profiles designed for step-stress accelerated life testing (SSALT)

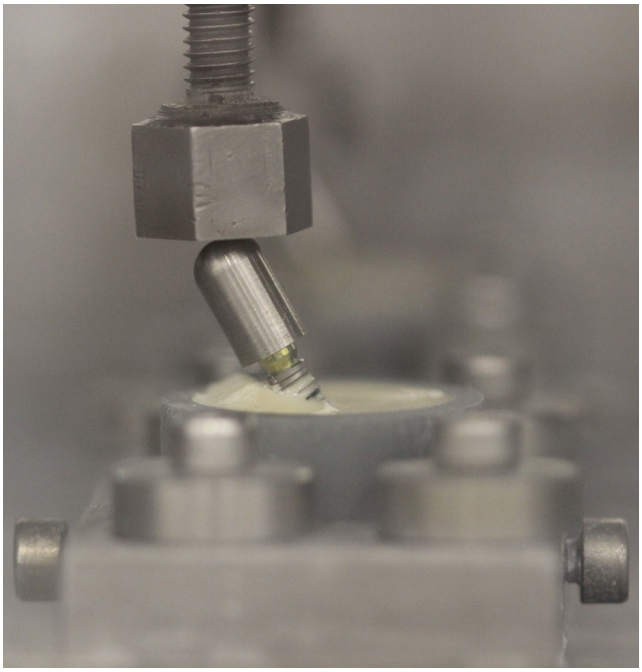

Fig. 3. Implant–abutment hemispherical loading member positioned for SSALT

The distribution of strain around the implant was analyzed using DIC (n = 3).[21–24,26–28] The master model of polymethylmethacrylate (PMMA) was made with dimensions of 55 mm × 30 mm × 14 mm (length, height and depth, respectively), and the implant–abutment set was fixed with cyanoacrylate glue (Super Bonder; Loctite, São Paulo, Brazil). After 24 h, an impression of the PMMA master model was obtained using silicone (Silikon; Odontomega, Ribeirão Preto, Brazil). The implant–abutment set was embedded in polyurethane resin (F16; Axson Technologies, Cergy, France), thereby capturing the predefined position of the implant. The surface of each model was coated with a thin layer of white paint (Colorgin Premium; Colorgin, Taboão da Serra, Brazil) and with small black spray dots (Colorgin) (Fig. 4).[22,26,27] A random surface pattern was applied to all models with speckles simultaneously, and it was calibrated using a plate with black dots provided by the manufacturer.

The digital image correlation complete system (StrainMaster; Polytec GmbH, Waldbronn, Germany) included 2 charge-coupled device (CCD) digital cameras (Imager E-lite 2M, 1101132; LaVision GmbH, Göttingen, Germany), with a resolution of 1,039 × 1,395 pixels, which were used to capture images of the model under loading. The DaVis 8.0 software (LaVision GmbH) was employed for image analysis and strain calculation. Two types of load application were used: axial (model positioned horizontally); and non-axial (model positioned on an acrylic base with an angle of 30°). Static loads were applied using a universal testing machine (Biopdi) with a load cell of 50 kgf, a test speed of 1 mm/min, and up to a load of 250 N.[22,26,27] During load application, point dislodgement was tracked by the software in order to calculate the strain on the model surface. The qualitative

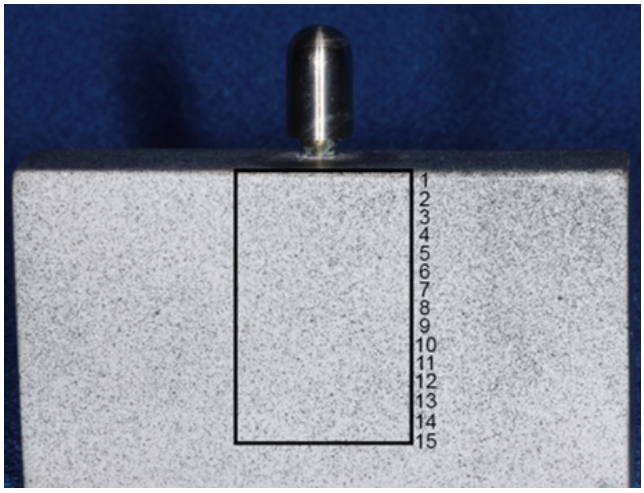

Fig. 4. Polyurethane model with small black dots showing the region analyzed using digital image correlation (DIC) with vertical distance measurement along the implant

analysis of the images was based on a color scale, with positive values (ranging from yellow to red) denoting tensile strain and negative values (ranging from green to blue) indicating compressive strain. To ensure the repeatability and reliability of the DIC, 3 loadings were performed on each model, and the results were analyzed.

Statistical analysis

For SSALT, the use level probability Weibull curve (probability of failure vs. the number of cycles) was calculated (Alta Pro 9; ReliaSoft, Tucson, USA) using as a parameter 60% of the maximum load found in SLF and a bilateral 90% confidence interval (*CI*). The reliability was calculated for a mission of 50,000 cycles at 100 N and 150 N, and the differences between these missions were identified through the implementation of the Weibull calculation with a two-way 90% *CI*.

Results

The mean values obtained in SLF were 539.86 N for the control group and 676.12 N for the test group. The mean of the 2 groups (607.99 N) was used to establish the applied load on the SSALT light, moderate and aggressive loading profiles. The β mean values derived from the use level probability Weibull plot (two-way 90% *CI*) were 0.66 and 0.33 for the control and test groups, respectively. These findings indicate that failures were dictated by material strength, which is associated with premature failures and not with fatigue damage accumulation. The results of the Weibull distribution (Weibull modulus (m) and characteristic resistance (η)) demonstrated no differences between the groups, considering the overlap of *CI*s (Fig. 5).

The reliability of the mission (50,000 cycles) at 100 N and 150 N showed no significant differences between the groups (Table 2). The probability of survival decreased for

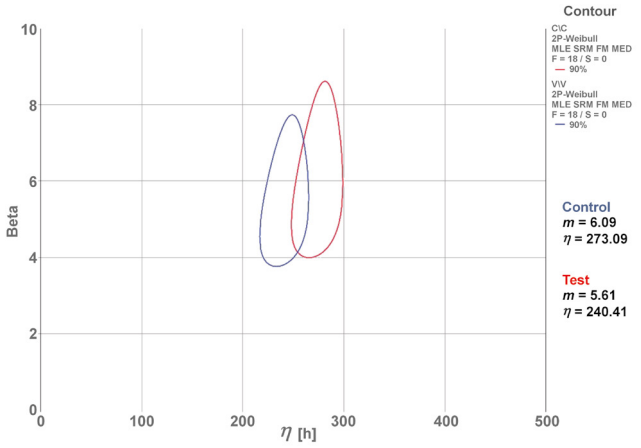

Fig. 5. Weibull contour plot

MLE – maximum likelihood estimation; SRM – standard regression method; FM – Fisher matrix; MED – median ranks; F – probability of failure; S – probability of survival; C/C – control group; V/V – test group.

both groups at 150 N, indicating that cumulative damage at higher loadings is associated with lower survival, especially in the test group (57%).

Table 2. Reliability calculated for a mission of 50,000 cycles according to the applied load

Variable	Control group		Test group	
	100 N	150 N	100 N	150 N
Upper limit	1.00	0.90	0.98	0.71
Reliability	0.98	0.79	0.95	0.57
Lower limit	0.92	0.59	0.87	0.39
β	0.66		0.33	

All specimens failed after SSALT, and the failure was restricted to fractures in the abutment and implant, specifically between the region of the third thread of the implant and the first thread of the abutment. The SEM micrographs demonstrated the failure mode of fractured specimens. The fracture's initiation was attributed to tensile strain on the lingual surface, with propagation to the buccal surface. A representative image of the SEM revealed the fracture origin, crack propagation direction (Fig. 6A,B) and the presence of longitudinal cracks on the internal walls of the hexagon vertex (Fig. 6C,D).

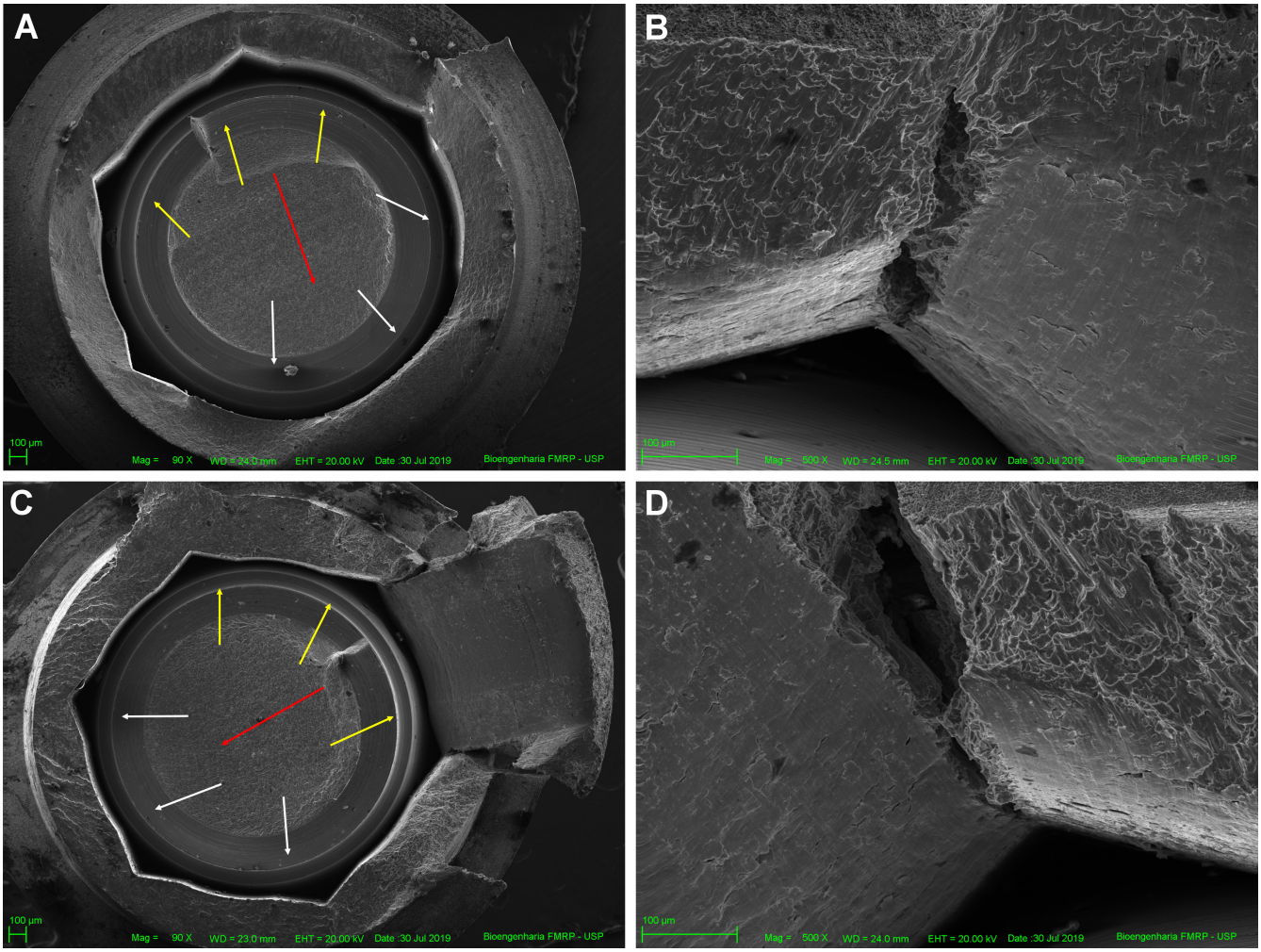

Fig. 6. Scanning electron micrographs of the fractured implant–abutment set after SSALT

A,B. Control group; C,D. Test group; WD – working distance; EHT – extra high tension. The yellow arrows denote the fracture origin, the red arrows indicate the direction of crack propagation, and the white arrows represent compressive strain.

The two-dimensional qualitative analysis with computed microtomography revealed differences in the macrogeometry of the groups, including thread pitch (control = 0.805 mm, test = 1.696 mm), thread depth (control = 0.381 mm, test = 0.402 mm) and wall thickness (control = 0.294 mm, test = 0.304 mm). Furthermore, a difference was observed in the cross-section of the 2 implants (control – trapezoidal, test – double trapezoidal).

The strain generated under axial and non-axial loading is illustrated in Fig. 7 and 8, respectively. The qualitative analysis of DIC showed a predominance of tensile strain in the middle and apical regions, and compressive strain in the cervical zone in both groups exposed to axial loading. The application of non-axial loading resulted in strain concentration in the middle and apical regions of the control group, and a substantial tensile strain concentration in the cervical zone of the test group. Figure 9 presents the distribution of strain generated along the implant. All groups showed similar behavior under axial loading. In non-axial loading, the control group demonstrated a strain distribution that was analogous to that observed in axial loading. Conversely, the test group exhibited a predominance of tensile strain concentration, especially in the cervical zone.

Discussion

The knowledge about the effect of dental implant macrogeometry on the fatigue behavior of the implant–abutment set is important due to its potential to predict clinical complications.[5,11] Therefore, this study evaluated

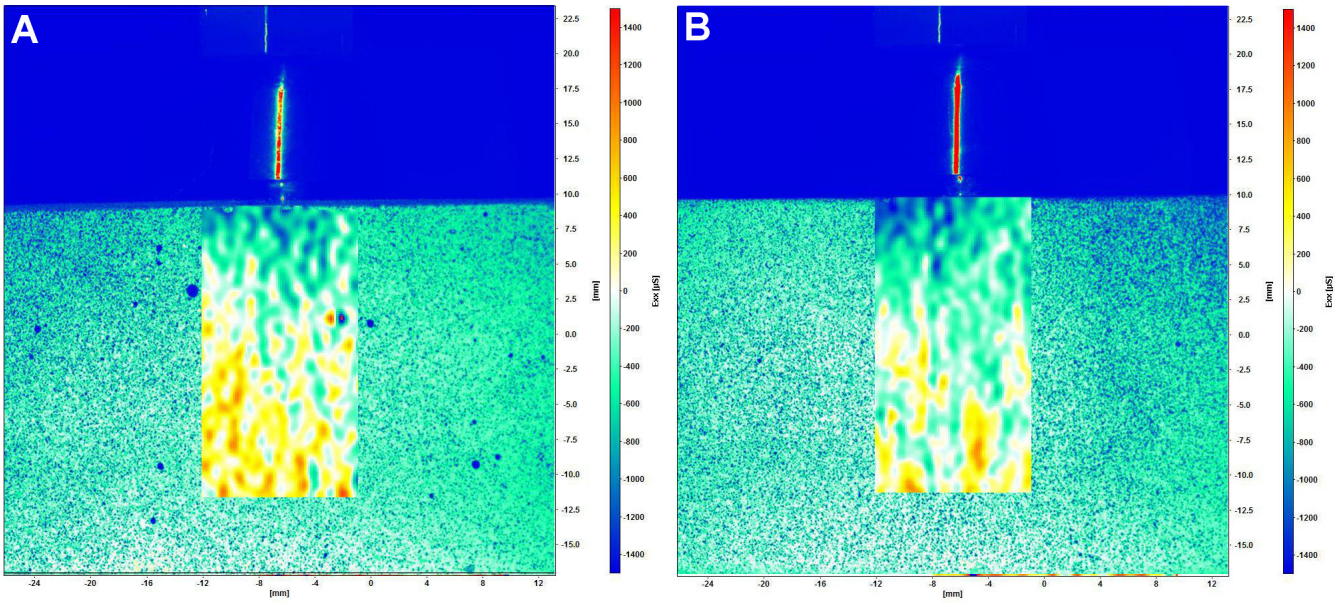

Fig. 7. Surface strain under axial loading at 250 N
A. Control group; B. Test group.

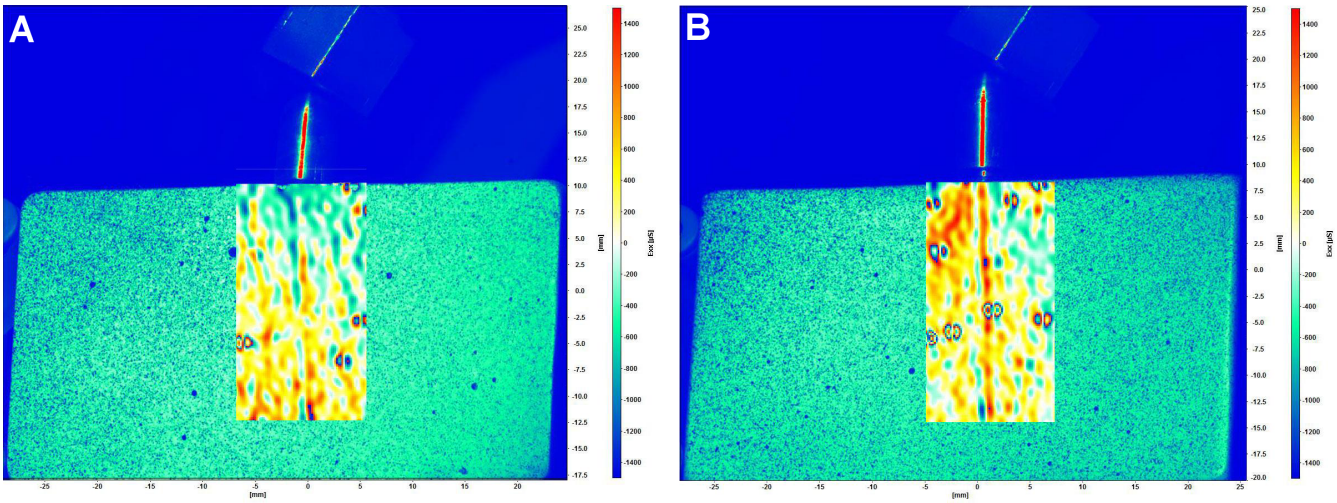

Fig. 8. Surface strain under non-axial loading at 250 N
A. Control group; B. Test group.

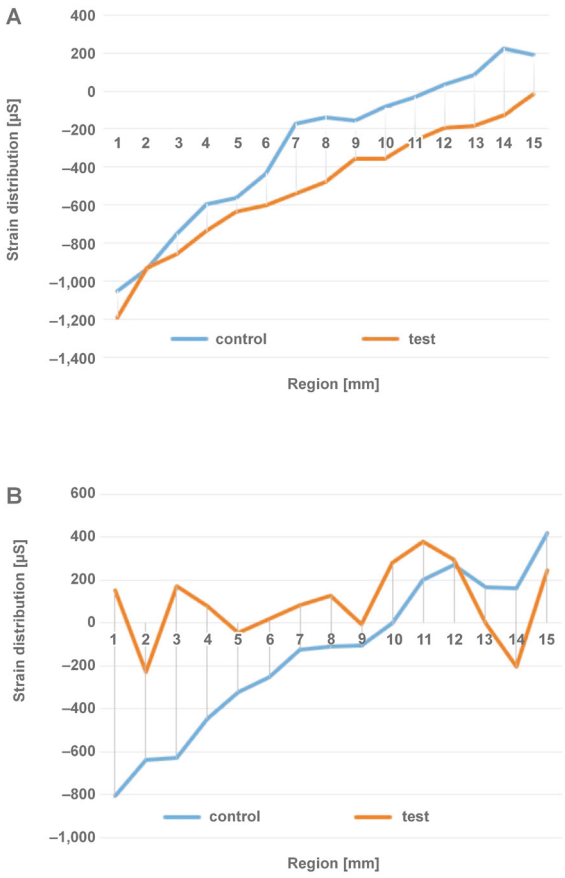

Fig. 9. Strain distribution around the implant after axial (A) and non-axial loading (B)

The horizontal axis represents the vertical distance measurements along the implant, as presented in Fig. 4.

the failure mode and probability of survival of dental implants with internal conical connection and different macrogeometries, including differences in the shape, pitch and depth of threads. The null hypothesis was partially accepted. The implant macrogeometry had no influence on the failure mode or probability of survival; however, there was a difference in stress transmission between the groups.

The specimens were subjected to cyclic loading during SSALT. The load levels increased successively until failure or suspension to reproduce the failure modes found clinically.[11] The results suggest that all implant macrogeometries presented high reliability (95–98%), simulating maximum bite forces found in the anterior region (100 N). Thus, they can be considered a reliable option for incisors.[29,30] In the posterior regions, the load-bearing capacity ranged between 300 N and 800 N.[31] However, the results of the present study should only be compared to the load-bearing capacity found in the anterior regions due to the positioning of the specimens at a 30° angle and the 3-mm exposure of the implant in the cervical region. This configuration simulates a worst-case scenario. On the other hand, loading in posterior crowns occurs in axial loading. A non-significant reduction in reliability was observed in both groups at 150 N. Bordin et al. found a similar probability of survival for narrow and extra-narrow dental implants with internal conical connection subjected to SSALT.[17] Both groups demonstrated high reliability at 50 N and 100 N, and there was a decrease in reliability at 150 N and 180 N, though no significant differences were observed between the groups.

The β value revealed by the SSALT data analysis is essential to understand the lifetime failure rate.[11,19] Both groups showed β value <1, indicating that failures of the implant–abutment set were dictated by the strength of the material, which is associated with premature failures. Failures attributable to the strength of the material were also reported by other authors.[12,17–19] Although the control group exhibited higher values of characteristic resistance and Weibull modulus than the test group, a statistical difference between the 2 groups was not identified. The Weibull modulus was used as an indicator of survival force and force distribution, predicting the presence of flaws in the material structure. The higher values observed in the control group indicated homogeneous failure distribution, low data dispersion, structural stability, and greater reliability.[11,32,33]

Previous studies have reported various failure modes of the implant–abutment set after SSALT, such as screw abutment fracture, abutment fracture,[12,13,15,17–19] and fracture of the implant body.[17] In the current study, the most prevalent failure mode was abutment fracture and implant body fracture, particularly in the region between the third thread of the implant and the first thread of the abutment. Scanning electron micrographs revealed fatigue and overload areas, corroborating the results of Weibull analysis, which stated that failures were caused by the strength of the material. The fracture originated when the strain exceeded the titanium strength, creating the deformation process and the formation of a plastic zone. This ultimately resulted in the fracture of the ductile implant body.[18]

In the present study, DIC illustrated tensile strain distribution under axial and non-axial loading conditions. Non-axial loading was performed using the same angulation as the specimens that were subjected to SSALT. The digital image correlation was used to analyze the surface strain of simulated bone models under a static load. This method offers the advantage of being easy to implement without disturbing the specimen, regardless of its type and size,[34,35] and to allow the precise full-field strain measurement. The DIC method offers the clear advantage of using real parts, which are very similar or even identical to those manufactured for patients in the clinic. This method ensures accuracy and repeatability, thereby facilitating analysis. Several studies[16–19,21–23] have indicated that DIC, despite manifesting tensions on the surface of models, corresponded accurately to findings derived from alternative test methods, such as finite element analysis.[30] Furthermore, these methodologies are not in competition with one another; rather, they are regarded as complementary approaches, allowing for a more profound

observation of the phenomena under study and facilitating the formulation of more substantiated conclusions. This, in turn, paves the way for novel studies aimed at advancing the knowledge available to clinicians.

Previous studies[21,34,35] have found that axial loading resulted in a better mechanical response, whereas non-axial loading exhibited the highest strain concentration in the cervical region of a dental implant.[21] The present study found high concentration of tensile strain in the cervical region of the implant in both groups, which contributed to abutment and implant body fracture. The test group exhibited a higher concentration of strain in the cervical region, demonstrating that dental implants with double trapezoidal threads presented a higher strain concentration in this region than implants with parallel threads. Accordingly, Freitas et al. evaluated the probability of survival and strain distribution of implants with internal conical connection and different microgeometries.[19] The researchers noted that dental implants with double trapezoidal threads presented a higher strain concentration in the implant and cortical bone.[19]

The present study demonstrated that the macrogeometry of dental implants had no effect on the probability of survival; however, it modified the strain distribution of the implant–abutment set. Dental implants with parallel and double trapezoidal threads presented comparable strain distribution during axial loading, but double trapezoidal threads resulted in a higher tensile strain concentration in the cervical region during non-axial loading than parallel ones. A limitation of this study is the construction of a crown, which was not modeled with the materials usually chosen in clinical settings. Thus, considering that materials used in the fabrication of abutments and crowns (metal alloys, zirconia, polyether ether ketone) can influence the distribution of deformations within the implant–abutment–crown assembly[26,36] due to shock absorption or not,[36] translating these results into clinical practice necessitates meticulous consideration. It is important to acknowledge the limitations of in vitro studies and to exercise caution when interpreting their results. The limitations of this study include the absence of an anatomical crown. Step-stress accelerated life testing did not assess complex factors found in the oral cavity, such as occlusal loading dynamics, neuromuscular forces and parafunctional habits. The models used for DIC were manufactured using a polyurethane resin that was solid, homogeneous, devoid of porosity, and isotropic. Despite these limitations, the results of this in vitro study demonstrated potential causes of implant–abutment set failures, establishing the foundation for future research. Our results must be validated by clinical trials that assess the influence of macrogeometry on treatment longevity.

Mechanical complications related to the macrogeometry of dental implants pose significant challenges to the long-term success of treatment. A comprehensive analysis and planning, encompassing the implant diameter, length, thread design, and surface characteristics, can help minimize these complications. Having achieved this objective, it is possible to guarantee optimal implant macrogeometry, enhancing implant longevity, patient satisfaction and oral health. Further research is required to develop advanced implant designs that effectively prevent these mechanical complications.

Conclusions

The results of this in vitro study demonstrated that the tested macrogeometries of dental implants exhibited a high probability of survival at loads that are clinically relevant for anterior teeth. Failure modes were restricted to abutment and implant body fracture. A higher concentration of tensile strain was observed in the cervical region of the dental implant when double trapezoidal threads were used in comparison to parallel threads.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Review

Oral care associated with a stay in an intensive care unit (ICU): A systematic review of clinical practice guidelines and scientific statements

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Abstract

The aim of the present systematic review was to critically evaluate the recommendations from evidence-based clinical practice guidelines (CPG) and scientific statements (SS), as well as expert consensus, related to the management of oral complications in intensive care unit (ICU) patients.

A search was made in the PubMed, Scopus, Ovid/Cochrane, and LILACS databases, following the CPG identification filters from the Canadian Agency for Drugs and Technologies in Health (CADTH). Both scientific repositories and document references were incorporated as well. The critical assessment was performed by means of the AGREE-II instrument (an ideal scenario) for CPG and SS, and using the AGREE-REX instrument for recommendations (ideal and local scenarios).

A total of 13 related recommendations from 4 SS were included. The mean score in AGREE-II was 58.25. The mean AGREE-REX scores were 45.82 and 39.07 for the ideal and local scenarios, respectively. The included recommendations focused on the oral care assessment, and the development of prevention and execution tools with regard to respiratory infections.

There is a lack of CPG following a rigorous methodology that would incorporate recommendations for oral care in ICU. Dentists are responsible for the development and improvement of recommendations from CPG and/or SS to mitigate oral complications in ICU patients.

Keywords: systematic review, intensive care unit, oral health, clinical practice guideline

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Highlights

- Patients remain at high risk for developing oral health complications after ICU discharge, despite adherence to current oral care guidelines.
- Current oral care protocols primarily aim to prevent respiratory infections, not long-term oral complications, highlighting a gap in comprehensive care.
- Enhancing guidelines to include long-term oral health and involving dentists in care planning is essential, while also accounting for healthcare system constraints and economic feasibility.

Introduction

The intensive care unit (ICU) treats critical patients who need immediate and prioritized healthcare.[1] Every day, in-patients are received in ICU because of several complications, among which the most common ones are arterial hypertension, diabetes mellitus, obesity, and chronic obstructive pulmonary disease (COPD).[2] However, it is important to highlight that other non-vital health aspects, such as oral health, could affect the quality of life (QoL) in the long term.[3] The goal of professional interdisciplinarity in ICU is to cooperate in the decision-making process based on the understanding of the in-patient's physiology, psychology and general health. It is necessary that ICU in-patients also receive oral care during their stay in hospital.[4] In the US, the number of ICU in-patients is estimated to be around 4.1 million per year.[5]

One of the most common health issues regards the mouth; for example, some patients show frustration for being unable to handle their thirst because of the dry mouth (xerostomia) associated with mechanical ventilation.[6] Some authors have reported the presence of plaque, tongue coating, dental caries, halitosis, mucosa lesions, periodontal disease, and residual fungal diseases within the oral cavity in in-patients.[3] The frequently observed dental caries might be related to the reduced salivary flow. Such a decrement of saliva is caused by changes in the oral microbiome, which is composed of microorganisms found in human body's ecosystems, including the oral cavity.[7] Similarly, patients with periodontitis could be at 3 times higher risk for nosocomial pneumonia than those without periodontal disease.[8] Indeed, elderly patients are those with the highest risk for periodontal colonization by pathogens.[9] The influence of oral bacteria on the occurrence of systemic complications has already been studied. Nevertheless, the importance and relevance of bacteria in the development of oral diseases during ICU in-patient stay, and the fact that the deterioration of oral health will affect the patient's QoL in future, have not been thoroughly discussed yet.

The lack of protocols for oral healthcare in critical care patients in ICU becomes the main reason for which they develop oral complications. Oral hygiene is important for all ICU in-patients. The appropriate oral hygiene is more complex in ventilated patients as compared to non-ventilated ones, who can practice self-care. However, there might be other health disorders that can constraint the the patient's movements, thus affecting the cleaning task, e.g., arthritis.[10–11] As reported by Kim et al., an oral hygienic care program proved to be successful in ICU patients due to the supervision of the dentist, reducing the plaque index scores and the *Candida albicans* colonization.[12]

An oral care assessment for ICU in-patients could determine their oral health status and point to possible actions, which could either prevent or treat oral diseases. Furthermore, an oral care assessment allows to establish protocols or clinical practice guidelines (CPG) for the ICU team, including oral health as a fundamental component of the in-patient's general health. There is a lack of CPG that would incorporate recommendations for oral care in ICU.

This work pursues to evaluate the reported recommendations within CPG and scientific statements (SS), as well as expert consensus, related to the management of oral complications in ICU patients. The aforementioned evaluation was performed by means of the AGREE-II and AGREE-REX tools.

Methods

Eligibility criteria

The PICAR format was used to define the inclusion criteria[13]:

– **P**opulation – ICU in-patients;
– **I**ntervention – any technique, method or strategy to prevent or treat oral sequelae due to stays in ICU;
– **C**omparator – any comparator;
– Guideline **A**ttribute – CPG or SS from national and international scientific societies or institutions in either English or Spanish; and
– **R**ecommendation – any either evidence-based or expert consensus recommendation of interest was included.

The exclusion criteria were not considered.

Information sources

Secondary database sources (PubMed, Scopus, Ovid/Cochrane, and LILACS), and the ICU-related CPG and SS in repositories from the Society of Critical Care Medicine (SCCM) were searched. The final database search was made on August 24, 2021. As additional sources, the references cited in the included documents and those in which the documents were cited were also reviewed.

Search strategy

The search for documents was made by using the CPG identification filters from the Canadian Agency for Drugs and Technologies in Health (CADTH),[14] together with oral health terms and ICU terms ("dental" OR "oral health" OR "oral hygiene" OR "oral care") AND ("critical care" OR "intensive care" OR "intensive unit" OR "ICU"). Other types of search fields, different from the CADTH ones, were not applied.

Selection process

The search results were sifted by 2 reviewers based on the title and abstract reading to exclude documents irrelevant with regard to the objective of this systematic review. The full text of the remainder of the results was read by the 2 reviewers to make the final decision about the inclusion, after reaching consensus in cases of disagreement.

Data collection process

Data extraction from the included documents was performed by 2 reviewers independently. To this end, an MS Excel file was used. In cases of disagreement, the full reading of the document was made, and the consensus was reached.

Data items

Information about the development process, for each included CPG or SS, was extracted taking as a reference the AGREE (Appraisal of Guidelines for Research and Evaluation) reporting checklist.[15] The extracted variables were objective, population, users toward whom the recommendations are oriented, development team conformation, and conflict of interest.

Synthesis methods

The CPG and SS were assessed by using the AGREE-II tool, which considers 6 different dimensions, i.e., scope and purpose, stakeholder involvement, rigor of development, clarity of presentation, applicability, editorial independence, and the overall assessment of the guidelines. A CPG is considered to have high methodological quality when its overall score is greater than or equal to 70 points.[16] The overall score for each CPG and SS was presented. In addition, an average score for each of the dimensions was also provided.

The different practice recommendations were assessed by means of the AGREE-REX tool, which considers 3 main domains: clinical applicability (evidence and applicability to target users and patients/populations); values and preferences (of target users, patients/populations, policy-/decision-makers, guideline developers), and implementability (purpose, and local application and adoption).[17] Each recommendation has an overall score in the ideal scenario, which is interpreted as the original scenario for the CPG and SS. Also, an assessment comprising the local scenario was made, corresponding to the Colombian context.

Each CPG was assessed by 2 reviewers independently, whereas all the recommendations were evaluated by the whole review team. The presented scores correspond to the mean value of the reviewers' scores. Neither CPG and SS nor recommendations were excluded because of their methodological quality.

Results

Study selection

A total of 2,303 articles were identified from the PubMed and Scopus databases. From these 2,303 articles, 307 were duplicated and thus removed from analysis, obtaining a total of 1,996 articles to be sifted. After following the established protocol consisting of title, objective, methodology, and full text review, 17 articles remained. Then, 2 articles could not be recovered, 11 were excluded given that they were neither CPG nor SS, and an additional one was excluded for not having oral-related recommendations. The final 3 articles were included in our study, from which 8 additional references were identified. After evaluating the additional 8 articles following the same protocol, 1 article was added to the study. Thus, the 4 articles obtained were discussed throughout the present study.[18–21] The flowchart of the study is presented in Fig. 1.

Study characteristics

The characterization and methodological quality of the included CPG and SS is summarized in Table 1.

The SS reported by Collins et al. evaluates several oral care aspects for adult ICU in-patients.[18] The practice for oral care together with its evidence was evaluated by a consensus committee from the British Association of Critical Care Nurses (BACCN). The assessment has been performed intending to improve the existing practices and protocols.

Table 1. Characterization and methodological quality (AGREE-II) of the CPG and SS including recommendations related to oral care in intensive care unit (ICU) in-patients

Study	Objective	Population	Users	Development team	AGREE-II score [%]
Collins et al.[18] 2021	To provide an evidence-based, BACCN-endorsed consensus paper for the best practice relating to implementing oral care, with the intention of promoting patient comfort, and reducing HAPand VAP in critically ill patients	adult ICU in-patients	ICU nurses	ICU nurses, a member of BACCN	100
Johnstone et al.[19] 2010	A survey was conducted among nurses to establish their baseline knowledge of oral hygiene and the current oral hygiene practices in PICU, which facilitated the development of an oral hygiene guideline for children in PICU	intubated and ventilated children in PICU	all registered PICU nurses	non-specified	50
Sedwick et al.[20] 2012	To develop a ventilator bundle and care practices for nurses in critical care units to reduce the rate of VAP	ICU in-patients	ICU nurses	nurses, physicians and respiratory therapists	50
Vollman et al.[21] 2015	ETT management and oral care performed to prevent buccal, oropharyngeal and tracheal trauma from the tube and the cuff, to provide oral hygiene, promote ventilation, and decrease the risk of VAP and HAP	ICU ventilated and non-ventilated patients	NA	non-specified	33

BACCN – British Association of Critical Care Nurses; HAP – hospital-acquired pneumonia; VAP – ventilator-associated pneumonia; PICU – pediatric intensive care unit; ETT – endotracheal tube; NA – data not available.

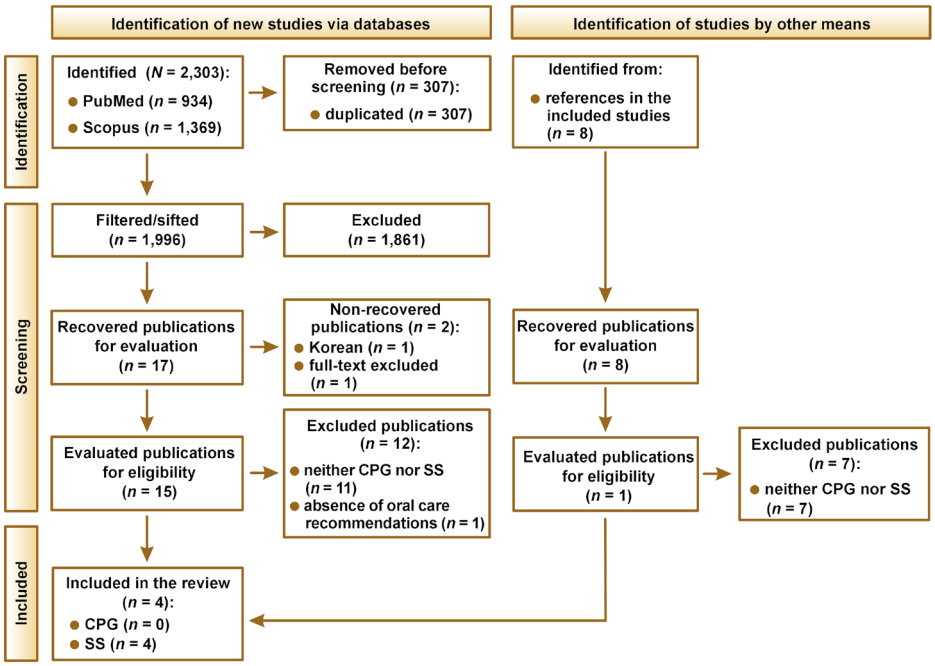

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the study
CPG – clinical practice guideline; SS – scientific statement.

Different factors, allowing to classify the quality of the recommendations as high and low, were established. In the study, in section 'Findings', 6 main recommendations are presented, i.e., the assessment and frequency of oral care, toothbrushing, oral care techniques and equipment, oral cleansing solution, toothpaste, and the technique for cleaning dentures.[18]

Guidelines for oral care and mouth hygiene for in-patients in pediatric intensive care units (PICU) were developed in a study by Johnstone et al.[19] The guidelines were proposed based on the evaluation of 14 articles of relevance, discussing oral care specifically in this pediatric population. The evidence used for the recommendation design shows that there exists a tight and direct relationship between poor oral hygiene and the increment of plaque, bacteria colonization and a high risk of developing nosocomial infections. Therefore, it is strongly emphasized that children in PICU require getting their mouths regularly cleaned and examined. The researchers promoted informal discussions with the nurses seeking to enhance the oral health standard for children in PICU.[19]

The SS presented by Sedgwick et al. comprised the set of recommendations reported by the U.S. Institute for Healthcare Improvement, extended by incorporating, among many other factors, some oral care protocols.[20] The added oral care procedures were designed in

cooperation with respiratory therapists. In fact, although the users of the recommendations were ICU nurses, the responsibility for oral care was impartially distributed among both respiratory therapists and nurses.[20] The previously mentioned cooperation reveals the need of taking into consideration multiple health disciplines in the ICU team.

Vollman et al. in their SS discussed the practices intended to provide oral hygiene and prevent oral trauma, including other aspects of the oral cavity.[21] These practices are oriented to ventilated and non-ventilated in-patients. This SS also presents the necessary knowledge for users and a detailed checklist of the required tools and equipment. Also, it proposes to involve non-ventilated patients and relatives in the procedures, providing useful information related to the recommendations.[21]

Results of individual studies

The CPG and SS were evaluated following the quality instrument AGREE-II, as presented in Table 1. A comparative diagram is shown in Fig. 2.

The SS by Collins et al. was assessed as having a high quality (100%).[18] The main limitation detected referred to the dimension corresponding to 'stakeholder involvement' (28%), showing that the population preferences were not taken into consideration in the development of the guidelines. Additionally, regarding the dimension corresponding to 'applicability' (42%), there is a lack of methods and strategies to make the implementation of recommendations easier. Nevertheless, regarding the dimension 'clarity of presentation' (100%), the recommendations are specific and non-ambiguous. Thus, one can find details concerning duration, tools and frequencies for the practices, in addition to a classification according to the population health status, i.e., ventilated and non-ventilated patients.[18]

The quality of the SS by Johnstone et al. was medium (50%).[19] The main strengths appeared in the 4th domain, corresponding to 'clarity of presentation' (100%), given that a flowchart diagram clearly shows, and in a specific manner, the practices to be followed according to children age subgroups, indicating times, frequencies and tools. On the other hand, the main limitation was identified in the 5th domain, corresponding to 'applicability' (8%). The SS does not present strategies to make the implementation accessible and easier nor does it discuss the possible obstacles that could interfere with the implementation. Additionally, advised or required resources to implement the recommendations are not presented.[19]

The quality of the SS by Sedwick et al. was determined to be medium (50%).[20] According to the evaluation results, the main limitation was detected in the 3rd domain, corresponding to 'rigor of development' (31%). Neither the search strategy nor systematic methods are presented for the evidence. On the other hand, the 1st domain is outstanding (89%), describing in detail the objective of the guidelines, the problem question and the population. Similarly, the 4th domain (83%), with the recommendations being precise and non-ambiguous.[20]

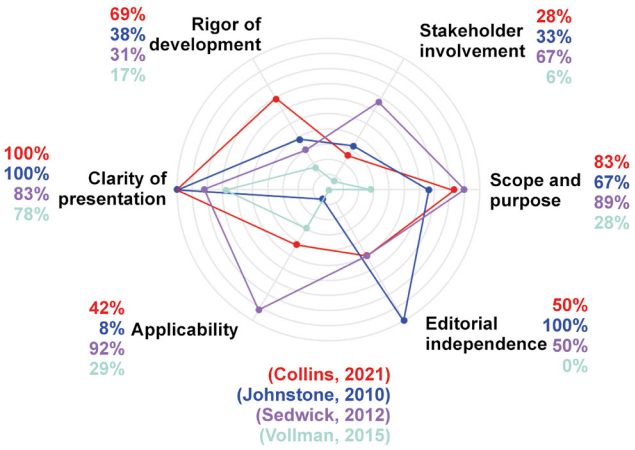

Fig. 2. Average scores for the clinical practice guidelines (CPG) and scientific statements (SS) with regard to the AGREE-II dimensions

The SS presented by Vollman et al. obtained scores showing a low quality (33%).[21] The main limitations were found in the domains number 2 (6%) and 6 (0%), corresponding to 'stakeholder involvement' and 'editorial independence', respectively. These low scores occurred, since the development team is not clearly mentioned, the preferences of the population were not considered in the development of the recommendations and the users of the guidelines are not presented. In contrast, the 4th domain, corresponding to the 'clarity of presentation' (78%), becomes the main strength of this SS given that the recommendations are presented in a very clear way, including step-by-step instructions, and avoiding ambiguity.[21]

Results of synthesis

The 4 included SS present a total of 13 recommendations, which are related to ICU in-patients' oral health (Table 2, Fig. 3).

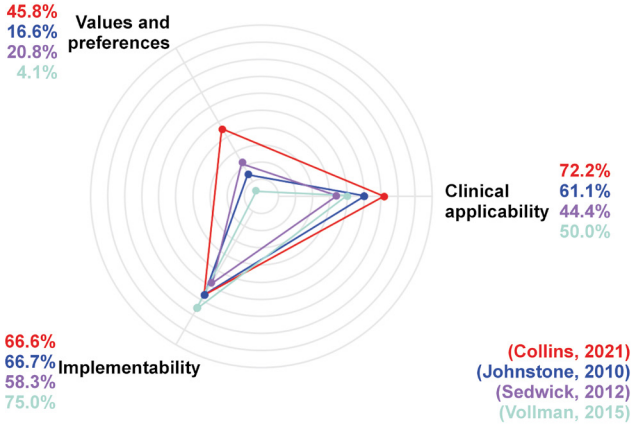

Fig. 3. Average scores for the recommendations with regard to the AGREE-REX dimensions in the ideal case

Table 2. Recommendation matrix for oral care in intensive care unit (ICU) in-patients and the AGREE-REX assessment of the recommendations

Study	No.	Recommendation	Orientation	Preference	AGREE-REX score [%]
Collins et al. BACCN	1.	– In a mechanically ventilated patient: Oral care assessment within 6 h of admission, using a standardized tool and documenting the results of the assessment. Assessment every 12 h is encouraged. – Non-ventilated patients: Oral assessment within 6 h of admission. – All critical care staff should be trained to perform the assessment.	assessment and frequency of oral care	oral assessment	59.3
	2.	– Ventilated patients: Brush the teeth twice daily for a minimum of 2 min. Oral cleansing with swabs, suctioning and moisturizing the mouth every 2–4 h. – Non-ventilated patients: the same oral care as for ventilated patients, with accommodations in the schedule, as needed, to the patient's condition, preferences (consent) and sleep patterns.	toothbrushing	prevention	
	3.	– Use a pediatric or soft small-headed toothbrush to remove plaque. Use suction to remove secretions and debris after brushing. – Clean and moisturize the oral cavity and teeth with a swab between episodes of brushing. – Foam swabs should not be stored in liquid. Each foam swab should be moistened immediately before use and the attachment of the head to the stick should be checked before use. – After cleaning, moisturize the oral mucosa throughout the oral cavity and the lips with artificial saliva/a lubricant.	oral care techniques and equipment	tools for the execution of the recommendations	
	4.	– Use an antiseptic oral rinse, such as chlorhexidine or cetylpyridinium chloride, after brushing. – Caution is advised with the routine use of chlorhexidine as part of an oral care program (the decision should be made in consultation with and agreement of a multi-professional team).	oral cleansing solution	prevention	
	5.	– Between episodes of brushing, consider using a debriding agent to assist in the removal of dental plaque.	toothpaste	prevention	
	6.	– Dentures: Daily cleaning using mechanical action with a toothbrush or a denture brush and an effective, non-abrasive denture cleanser. – Soaking dentures daily in a denture-cleansing solution. – Denture wearers should not keep their dentures in the mouth overnight unless there are specific reasons for keeping them in.	technique for cleaning dentures	prevention	
Johnstone et al. Oral hygiene in PICU	7.	– Evaluate bleeding, redness, ulceration, saliva, halitosis, external factors, debris, and the teeth (brushed teeth).	assessment tools	oral assessment	42.6
	8.	Neonates and infants with no teeth – Q2 hourly: Moisten the mouth with foam swabs or gauze wrapped around a gloved finger soaked in clean water or normal saline. – Q2 hourly/PRN: Coat the lips with Vaseline®.	mechanical and pharmacological intervention	prevention	
	9.	Infants and children with teeth (<6 years old) – Q12 hourly: Brush the teeth with a small, soft toothbrush and a smear of fluoride toothpaste. Suck out excess toothpaste, but do not rinse. – Q2 hourly: Moisten the mouth with foam swabs soaked in clean water or normal saline. – Q2 hourly/PRN: Coat the lips with Vaseline®.			
	10.	Children with teeth (>6 years old) – Q12 hourly: Brush the teeth with a small, soft toothbrush and a smear of fluoride toothpaste. Suck out excess toothpaste, but do not rinse. – Q12 hourly: Chlorhexidine mouth rinse 0.1% (10 mL of chlorhexidine 0.2% diluted with 10 mL of water). Irrigate with a syringe or wipe oral mucosa with a foam swab. Suck out excess solution, but do not rinse with water. Use at least 30 min after brushing the teeth with toothpaste. – Q2 hourly: Moisten the mouth with foam swabs soaked in clean water or normal saline. – Q2 hourly/PRN: Coat the lips with Vaseline®.			
Sedwick et al. Practice-based evidence	11.	– Perform mouth care every 2 h (or 12 times per day), alternating responsibility between respiratory therapists and nurses.	oral care	prevention	37.0
Vollman et al. Oral care practices	12.	Ventilated patient – If the patient is intubated orally, remove the bite block or the oropharyngeal airway (acting as a bite block) before proceeding with oral hygiene. – Initiate oral hygiene with a pediatric or adult (soft) toothbrush, at least twice a day. Suck out oropharyngeal secretions after brushing. Use toothpaste or a cleansing solution that assists in the breakdown of debris. – Use oral swabs with a 1.5% hydrogen peroxide solution to clean the mouth every 2–4 h. – Suck out oropharyngeal secretions after cleansing. Apply a mouth moisturizer to the oral mucosa and lips to keep the tissue moist. – Use suction in the oral cavity and pharynx at a minimal frequency of every 4 h. – Application of antiseptic oral rinses (chlorhexidine, cetylpyridinium chloride), added after brushing or in conjunction with comprehensive oral care.	mechanical and pharmacological intervention	prevention	44.4
	13.	Non-ventilated patients (independent self-care) – Instruct the patient to brush the teeth gently for 1–2 min and swish with an oral rinse. Moisturize the lips and mouth, as needed. – Encourage brushing 4 times a day (i.e., after each meal and before bedtime). Non-ventilated patients (dependent, unable to manage their own oral care or secretions safely) – Brush the teeth with a suction toothbrush and toothpaste or gel for 1–2 minutes, sucking frequently. Non-ventilated patients (edentulous, with dentures) – If no teeth or dentures, gently brush the gums and tongue 4 times a day (i.e., after each meal and before bedtime). If the patient is allowed nothing by mouth or is on tube feeding, oral care can be performed every 6 h. Apply an antiseptic oral rinse with a moistened swab and suction. Apply a moisturizer with a swab. – If the patient wears dentures, soak them at night in a denture cleanser. During the daytime, rinse or swab them with an antiseptic oral rinse after each meal and apply a moisturizer (PRN).			

The mean global scores in the ideal and local scenarios in the study by Collins et al., according to the AGREE-REX tool, were 59.3% and 40.7%, respectively, showing few weaknesses in the recommendations.[18] In the ideal scenario, the low score is mainly observed in the domain of 'values and preferences' (45.8%). since neither the objective users nor the patients' acceptability were considered within the recommendation design. Regarding the local scenario, the main weaknesses were identified in the domains 'values and preferences' (15.0%) and 'implementability' (41.6%). As part of the recommendations, professional team training is suggested, which necessarily implies the assignment of funds, making it difficult to implement in the local context. It is well known that there are several other priorities in the local healthcare system to assign resources. The strengths for the ideal scenario were identified in the domains 'clinical applicability' (72.2%) and 'implementability' (66.6%), highlighting the fact that the recommendations are exact and explained step by step, defining the population and the health issue it intends to solve. In the local scenario, the 'clinical applicability' got a score of 61.0% given that the recommendations are within the nurses' scope.[18]

The mean AGREE-REX scores for both the ideal and local scenarios in the study by Johnstone et al. showed a low value (approx. 16%) in the domain 'values and preferences' for both scenarios.[19] This low score is mainly related to the fact that the preferences of the population, users and patients, were not included in the SS. The main strength for the 2 scenarios (ideal and local) is focused in the 'implementability' domain (approx. 67%), with the alignment between the recommendations and the SS objective clear set, i.e., to improve the oral health of in-patient children in PICU. Moreover, the resources required for the implementation of the recommendations are considered, and the SS also establishes the training and knowledge needed by the users. Although the knowledge and training requirements can become a limitation due to the lack of personnel and equipment in some regions, the recommendations are equally aligned with the oral health objectives in the local context.[19]

For the SS reported by Sedwick et al., the mean global scores for the ideal and local scenarios, according to the tool AGREE-REX, were low, with values of 37.0% and 29.6%, respectively.[20] The main strength in the ideal context (the 'implementability' domain with 58.3%) is due to the impact of the recommendations on the patients' outcomes. Moreover, professional team training is shown in detail, including the cost reduction that hospitals would achieve. Concerning the local scenario, the highest score was observed in the 'clinical applicability' domain (44.4%) given that the evidence used for the development of the recommendations could also be found. Nevertheless, it is quite relevant to point out that it is not feasible to perform oral care every 2 h in the local context due to the lack of personnel in ICU. The domain with the lowest score was the one related to 'values and preferences' for both scenarios (ideal scenario – 20.8%, local scenario – 12.5%).[20]

The AGREE-REX scores for the SS presented by Vollman et al. showed a medium quality, with a value of 44.4% in the ideal scenario and 39.7% in the local scenario.[21] This SS has an impact on the users, as it stipulates the pre-requisite knowledge. Also, this SS mentions the suggested education schemes for both patients and their relatives, which can end up in the successful dissemination of the recommendations. In addition, they can be customized for patients form different subgroups. The latter justifies the highest score observed in the domain known as 'implementability' (75.0%). In the same way as discussed above for Sedwick et al., the SS by Vollman et al. has the main weaknesses in the domain of 'values and preferences, for the ideal and local scenarios, with a value around 4%. Although the SS involves the nurses by mentioning their pre-requisite knowledge, and the patients by suggesting education schemes, the values and preferences of these objective users and the population were not reported in the design of the recommendations.[21]

Discussion

Given that in-patients in ICU are in a critical health state that puts their life in danger, oral care is not part of the priorities. The oral issues are shadowed and underestimated because of other health complications concerning other integral health fields,[20] which might explain the lack of previous studies on the CPG and SS concerning this topic. To the best of our knowledge, the quality assessment of SS related to the oral health of ICU in-patients, as well as their recommendations, has not been reported in the literature yet.

The previously mentioned situation suggests a new problem. In cases where the in-patient overcomes their health difficulty and is discharged from ICU, they will have to deal with oral health issues in the long term, as during their stay in hospital, they could develop caries, gingivitis and periodontitis, among others.[22,23] However, within the assessed SS, one can find several practices involving oral care, which can, in turn, be complemented and improved to mitigate future oral health issues in ICU in-patients.[24]

The identified quality of the 13 recommendations found, related to oral care in ICU in-patients, was low. With regard to the SS objective, recommendations that are exclusively oriented to the prevention of oral health issues were not found. However, there were recommendations linked with oral care, but mainly intended to prevent ventilation-associated pneumonia (VAP).[25]

Additionally, the proposed recommendations lack of a rigorous methodological design, e.g., the different considerations depending on the population comorbidities,

the alignment between the practice scope and the objective users' actions to achieve a proper clinical applicability, the identification of the required resources, and the detection of possible barriers. Given that the aforementioned elements are missing, the implementation of the recommendations turns to be difficult.

Four possible roles, which oral health professionals can either directly or indirectly play in the oral care of ICU in-patients, have been determined.[23] First, the participation of the dentist in the design of the recommendations. The existence of some SS, which already have recommendations associated with oral care to avoid the development of respiratory infections, becomes an opportunity to complement such recommendations by considering possible oral complications. Second, the dentist actively executing the recommendations, i.e., the dentist being part of the ICU team. Some evidence has shown improvement in oral health and the prevention of respiratory infections when involving the dentist.[23] Third, the dentist performing the training of the CPG and SS users. For example, the nurses have been shown to perceive the ICU in-patients' oral care as the most difficult task to do, in addition being a low-priority intervention.[21] Thus, there is an undoubtable need for the ICU nurses to be trained by the dentist. Fourth, the dentist in the post-ICU care. Most of the patients who get discharged from ICU have to deal with health sequelae at the physical and mental level, known as post-ICU syndrome.[26] It is required to evaluate whether the knowledge-based and practice-based training of the dentists is adequate to cope with the challenges emerging during the post-ICU period.

Limitations

Some limitations must be considered. Since it is a systematic review, one should keep in mind the existing bias caused by the language, which was constrained to English and Spanish. Therefore, gray literature reports could have been omitted within the systematic review.

Conclusions

There exists a high risk to develop oral health complications in patients after having been discharged from ICU, even though oral care-related recommendations were followed. Oral care recommendations are not designed to prevent oral complications; instead, they are designed to prevent respiratory infections associated to ventilation.

This shortcoming presents an opportunity to complement the existing recommendations, broadening their purpose – not only focusing on infection prevention, but also on mitigating the potential long-term oral complications during the post-ICU period. It is concluded that the dentist plays a significant and essential role in improving CPG and SS for ICU in-patients. Additionally, it is crucial to consider the management and economic limitations, as well as the coverage of the health care system, when implementing these recommendations in the local context.

Registration and protocol

The present study is registered in PROSPERO under the ID CRD42021254982. It follows a rigorous methodology proposed by Johnston et al.,[13] and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[27]

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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